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IUPAC EXPLANATORY DICTIONARY OF KEY TERMS IN TOXICOLOGY

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Abstract

The objective of the **Explanatory Dictionary of Concepts 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 international level and between disciplines. The explanatory definitions should help to break down such barriers. The dictionary consists of about 68 terms chosen from the IUPAC

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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. Thus, it is expected that there will be a wide audience for this document.

IUPAC For Peer Review Only
IUPAC Provisional Recommendations

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Preface

Within the framework of IUPAC Division VII, Chemistry and Human Health, the project to develop a "Explanatory Dictionary of Terms used 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 (Registration, Evaluation and Authorisation of Chemicals) program. Like many IUPAC bodies, Division VII 'Chemistry and Human Health' is concerned to promote world-wide "regulation, standardization, or codification" in relevant areas of chemistry. In this context, lack of understanding of the terminology used in toxicology has constituted a problem in the relationship of chemistry to toxicology and its consequences for regulation of 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 'Gold Book' [1, 2] and from the glossaries of terms in toxicology and toxicokinetics published in Pure and Applied Chemistry [3, 4]. 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.

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1. ABSORPTION

IUPAC Definitions

absorption (general)

1. 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. [2, 4]

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 of or all its energy. [4]

3. in biology

Penetration of a substance into an organism by various processes, some specialised, 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, gastro-intestinal tract, or skin. [4]

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*. [4]

Explanatory Definition

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, alpha, beta or neutron particles, or as high energy electromagnetic waves such as X-rays or gamma radiation. Radiation may be ionizing or nonionizing. Ionizing radiation is particle radiation in which an individual particle (for example, a photon, an alpha particle or a beta particle) carries enough energy to ionize an atom or molecule (that is, to completely remove an electron from its orbit).

1
2
3 Alpha particles are a highly ionizing form of particle radiation which has low penetration.
4 Each particle consists of two protons and two neutrons bound together into a particle
5 identical to a helium nucleus; hence, the alpha particle can be written as He^{2+} . A thin sheet of
6 paper may prevent uptake of alpha particles. Alpha particles are also stopped by the skin
7 where they may nevertheless cause malignant transformation. Radionuclides that release
8 alpha particles are also dangerous if they are taken into the body by inhalation (breathing in)
9 or ingestion (eating and drinking) as discussed further below. The health adverse effects
10 caused by radon are explained by alpha-particles that are absorbed in the lung and thus an
11 "internal" radiation source is developed inside the body. Thus, indoor radon exposure leads
12 to an increase in lung cancer. Exposure from radon in drinking water is also of concern.
13 Many countries have set exposure limit recommendations.
14
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16

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18 Beta particles are high-energy electrons. There are two forms of beta decay, β^- and β^+ , which
19 respectively give rise to the electron and the positron. The depth to which beta particles can
20 penetrate the body depends on their energy. High-energy beta particles (several MeV) may
21 penetrate a cm or so of tissue, although most are absorbed in the first few mm. As a result,
22 beta emitters outside the body are hazardous only to surface tissues such as the skin or the
23 lens of the eye. When beta emitters are taken into the body, they irradiate internal tissues and
24 become a more serious hazard.
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29 As for toxic chemicals, the effect of radiation depends on the amount received. Thus, amount
30 of radiation received is referred to as a dose, and the measurement of doses is known as
31 dosimetry. Also, as with toxic chemicals, what is important is not so much the total dose to
32 the whole system as the dose per kg body weight. Thus in radiation protection
33 measurements, the unit of absorbed dose is specified in terms of the amount of energy in
34 joules deposited by radiation in 1 kg of body tissue. This unit is the gray, abbreviated Gy,
35 named in honour of the British physicist, Louis Gray. The gray is a large dose and for most
36 normal situations we use the milligray (abbreviated mGy) and the microgray (abbreviated
37 μGy). Absorbed dose is given the symbol D (measured in grays).
38
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42 The gray describes the physical effect of the incident radiation (i.e., the amount of energy
43 deposited per kg), but it tells us nothing about the biological consequences of such energy
44 deposition in tissue. Studies have shown that alpha and neutron radiation cause greater
45 biological damage for a given energy deposition per kg of tissue than gamma radiation does.
46 One gray of alpha or neutron radiation is more harmful than one gray of gamma radiation.
47
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51 Quality factors are used to compare the biological effects of different types of radiation. For
52 example, fast neutron radiation is considered to be more damaging than X-rays or gamma
53 radiation. This leads to the idea that fast neutron radiation is of higher "quality", because a
54 lower absorbed dose produces equivalent biological effects. This is expressed in terms of the
55 quality factor (Q). The Q of a certain type of radiation is related to the density of the ion
56 tracks it leaves behind it in tissue; the closer together the ion pairs, the higher the Q.
57
58

59
60 The Q values for the various types of radiation are 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, minutes or hours.

Table 1 The quality factors for the various types of radiation

<i>Radiation</i>	<i>Energy</i>	<i>Q</i>
gamma	All	1
beta	All	1
neutrons	Slow	5
neutrons	Fast	20
alpha	All	20

The absorbed radiation dose, when multiplied by the Q of the radiation delivering the dose, will give us a measure of the biological effect of the dose. This is known as the equivalent dose. Equivalent dose is given the symbol H. The unit of H is the sievert (Sv). 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 one gray of X or gamma rays. In practice, the millisievert (mSv) and microsievert (μ Sv) are the units in common use. Equivalent dose, quality factor and absorbed dose are related by the expression following immediately below.

$$H \text{ (Sv)} = D \text{ (Gy)} \times Q$$

Most of the instruments used to measure radiation doses or dose rates display the values in mSv or μ Sv. The collective dose that to which a population is exposed is measured in "man-sieverts" (man.Sv). 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 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 (dW) absorbed by (dissipated in) an incremental mass (dm) contained in a volume element (dV) 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)

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2
3 In toxicology we are mainly concerned with absorption as defined in 3 and 4 above. In other
4 words, we are concerned with the processes by which a chemical crosses the various
5 membrane barriers of a living organism and especially those processes by which a chemical
6 is taken up from environmental media including food and other ingested material such as
7 drinking water, liquid refreshments, and medication of all kinds. Sometimes it can be
8 discussed whether the toxic effect caused by radioactivity or chemicals is worse or vice
9 versa. This means that which effect develops first the irradiation or the toxicant. Absorption
10 is the first step in the series of processes analysed in the study of toxicokinetics. The others
11 are distribution (including storage), metabolism and excretion, usually referred to by the
12 acronym ADME.
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17 The main barrier to uptake is the phospholipid bilayer that forms the core of biological
18 membranes. This prevents passive diffusion of water and water-soluble molecules but
19 permits passive diffusion of fat-soluble molecules. Passive diffusion is driven by the
20 electrochemical gradient of the substances involved. Fat-soluble substances move down their
21 gradient of chemical activity (proportional to concentration) and/or the gradient of electrical
22 charge (positive or negative).
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27 Chemicals (foods, medicines, drugs of abuse, industrial chemicals, and environmental
28 chemicals) can enter the human body by various routes following ingestion, inhalation,
29 injection (intravenous, subcutaneous, intramuscular), skin application, use of suppositories,
30 and uptake through mucous membranes of the eye, oral or nasal cavities).
31
32

33
34 Except for injection directly into the blood stream, chemicals must pass through a complex
35 system of cell membranes before they can enter the bloodstream. For example, chemicals
36 that enter the digestive tract in solution or after solubilisation may be absorbed by the cells
37 lining the small intestine and then transferred through the cell to the other side (the
38 transcellular route) where they cross the endothelial barrier into the blood stream. Some
39 chemicals may also pass between the epithelial cells of the intestine by what is called the
40 paracellular route. Such transport is restricted by the junctions between cells and this
41 provides selectivity for the chemicals that can use this route. Chemicals that are inhaled must
42 pass through the alveolar cells to the adjacent capillary cells and through them to the blood
43 stream.
44
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48 **Figure 1. Transcellular and paracellular routes of absorption from the intestine**

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50
51 As chemicals pass into and out of cells, they must cross the cell membrane. The membrane
52 defines the shape of the cell, controls the chemistry of the cell interior by regulating passage
53 of substances into and out of the cell and acts as a transducer for extracellular chemical
54 regulators such as hormones. The cell membrane consists mainly of phospholipid and
55 protein in the form of a lipid bilayer. Two phospholipid layers face each other inside the
56 membrane with the more water soluble parts of the phospholipid molecule (phosphate
57 groups) facing the aqueous media inside the cell (cytoplasm) and outside the cell
58 (extracellular fluid). The resultant structure is termed the fluid mosaic model and the fluidity
59 is crucial to its function. The membrane proteins provide some rigidity to the structure and
60 some may act as transporters. Some 'float' in the membrane, binding to external substances

1
2
3 and diffusing with them from one side of the membrane to the other. Others may traverse the
4 membrane structurally and, on binding to a substance, change shape so that the substance is
5 transported across the membrane. This process may be associated with input of energy from
6 nucleoside triphosphate breakdown, permitting transfer against an electrochemical gradient,
7 referred to as active transport (see below).
8
9

10 11 **Figure 2. The Fluid-Mosaic Cell Membrane**

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15 The most fundamental mechanism for transport of either foreign chemicals or ordinary ions
16 through the cell membrane is passive diffusion. The driving force for passive diffusion of a
17 chemical is based on the difference between the concentration (or better chemical activity) of
18 the chemical (or chemicals of the same electrical charge if ionized) on the outside of the cell
19 and that on the inside of the cell. This is properly called the electrochemical gradient. The
20 greater the difference in the relevant electrochemical activity between the outside and the
21 inside of the cell, the greater the diffusion of the chemical down the resultant gradient, in or
22 out. Since the membrane barrier to chemical movement is mainly lipid, the ability of a
23 chemical to diffuse across the membrane is largely dependent on its lipophilicity, solubility
24 in lipid. This is measured in terms of its octanol-water partition coefficient, the ratio of the
25 concentration in octanol to that in water after the substance is mixed thoroughly with both
26 and they are allowed to come to equilibrium.
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31 Because they are not lipid soluble, charged molecules do not readily diffuse across the
32 plasma membrane. The pH of the fluid surrounding the cell is important in this respect
33 because it influences ionization and hence molecular charge. Weak acids in their un-ionized
34 form may be lipid-soluble and will diffuse across membranes quite easily. The degree of
35 ionization of a molecule at different pHs is dependent on its pKa, i.e. the pH at which 50%
36 of the chemical is ionized and 50% is un-ionized. This is important in the gut. In the human
37 and general carnivore stomach lumen, the pH may be as low as 1.5-2.0. In other species, e.
38 g. in rats, the pH may be much higher, e.g. pH 4. However, the pH inside the small intestine
39 is about 7.0-8.0. Thus, the ratio of ionized to un-ionized chemical differs for any chemical in
40 these two environments depending on its pKa and the amount of ionisable chemicals
41 absorbed from these two sites is different. This property may be used in designing drug
42 molecules to ensure preferential absorption from the stomach by giving it the appropriate
43 pKa.
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49 Water-soluble biomolecules and other chemicals may be transported across the membrane
50 with the aid of carrier proteins to which they become bound (see also *carrier*). One
51 possibility is facilitated diffusion in which the molecules move down the electrochemical
52 gradient exactly as happens with normal diffusion, although the process may be limited by
53 the availability of carrier molecules and the kinetics of the binding/unbinding reaction
54 between carrier and chemical. It is also possible for chemicals to compete for the binding
55 sites on carrier proteins. A particularly important carrier is multidrug resistance carrier which
56 has been demonstrated to transport a wide range of xenobiotics conjugated to glutathione,
57 glucuronide, or sulphate as well as unmodified anionic compounds such as the antifolate
58 agent, methotrexate [5].
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5 Chemicals may also cross the cell membrane by diffusion through water-filled membrane
6 pores. This diffusion is dependent on the size of the pore and the molecular size and shape of
7 the chemical.
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11 Some inorganic ions, such as sodium and potassium, and many drugs, move through the cell
12 membrane by a process called 'active transport'. This process moves substances against the
13 electrochemical gradient and requires input of energy, usually in the form of adenosine
14 triphosphate (ATP). Thus, active transport absorption by any cell will reflect its metabolic
15 activity and, in some circumstances, may stress this so much as to have adverse effects. A
16 good example of active transport, the sodium pump is illustrated in Figure 3.
17

18 19 20 **Figure 3. Reaction cycle of the sodium pump (Na⁺/K⁺-ATPase)**

21
22
23 ATPase binds Na⁺ and ATP in the E₁ conformational state (step 1) and is phosphorylated at
24 an aspartate residue by the -phosphate of ATP. This leads to the occlusion of three Na⁺ ions
25 (step 2) and then to their release to the extracellular side (step 3). This new conformational
26 state (E₂-P) binds K⁺ with high affinity (step 4). Binding of K⁺ leads to dephosphorylation of
27 the enzyme and to the occlusion of two K⁺ cations (step 5). K⁺ is then released to the cytosol
28 after ATP binds to the enzyme with low affinity (step 6). The dashed box highlights the
29 electrogenic steps of the catalytic cycle.
30
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34 Finally, the membrane can engulf the chemical, form a vesicle, and transport it across the
35 membrane to the inside of the cell. This process is called endocytosis and is particularly
36 important for particulates. The same process can occur in reverse and then is known as
37 exocytosis. It is energy dependent and may result in the transport of a mixture of chemicals
38 because, although it is usually induced by a specific process, it may take unspecifically
39 enclosed substances in to the cell. This process has been suggested to be more active in the
40 neonate.
41
42

43 44 **Figure 4. Endocytosis and exocytosis**

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46
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48 Once the chemical has entered the bloodstream or the lymph, it is distributed to organs
49 distant from the site of absorption. Initially, simply because of the electrochemical gradients
50 between the blood or lymph and the organs that it perfuses, many chemicals will tend to
51 leave the blood (or lymph) passively and enter the surrounding cells (uptake). The pH of the
52 blood (or lymph), pH 7.4, will determine the ionization state of polar organic chemicals and
53 this will influence passage through the cell membranes of the cells separating the blood (or
54 lymph) from the organs.
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56

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59
60 Anatomical and physiological factors may affect the movement of a chemical around the
body. For example, the cells bounding the capillaries in the brain have tight junctions
impeding 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

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3 large molecules from entering the brain. This structure constitutes what is known as the
4 "blood-brain barrier."
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8 The placenta is an organ that permits nutrients to pass from the mother's bloodstream to that
9 of her fetus but does not allow the passage of all chemicals. The maternal blood and the fetal
10 blood do not have direct contact. Generally, molecules with a molecular mass greater than
11 1000 Da have difficulty entering the fetal blood supply. The placenta has the ability to
12 metabolize chemicals and the derivatives may be responsible for effects on the embryo and
13 fetus.
14

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16
17 Other factors to be considered in relation to the availability of chemicals to cells in the
18 human body are chemical affinity and the resultant long-term incorporation into tissues such
19 as fatty tissue and bone. Chemicals that are lipophilic have an affinity for and tend to get
20 absorbed by and to accumulate in fat cells, from which they are released very slowly under
21 normal circumstances. Such chemicals may cause no problem for years but the fat cells can
22 break down quite rapidly during pregnancy or illness or in old age, and any stored lipophilic
23 xenobiotics may then flood the blood stream and cause illness as a result of uptake of toxic
24 doses into susceptible organs particular into the critical organ. The same effect occurs in
25 birds and wild animal species during the winter when food supplies are short.
26
27

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29
30 Affinity for proteins in the blood may also be important in determining availability of
31 chemicals to susceptible tissues. In particular, some chemicals may become strongly bound
32 to plasma proteins (such as albumin) and the rate of release from such binding will
33 determine how long the chemical is available to exert its biochemical and physiological
34 effects. Serum albumin is important in the transport of bile pigments such as bilirubin. Some
35 drugs such as sulfisoxazole and ceftriaxone can compete for bilirubin-binding sites on the
36 albumin molecule causing it to be released and deposited in tissues where it causes damage.
37 This is just one example of many hundreds of clinical importance.
38
39

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41
42 For some inorganic species, such as fluoride ions, lead ions and strontium ions,
43 incorporation into bone may occur. The elements may stay there for long periods of time. As
44 bone slowly renews itself or is partly broken down during pregnancy, illness, or old age,
45 these chemicals may be released. If this occurs during pregnancy, resultant toxicity can
46 affect both the mother and her child. Similar toxic effects may be seen in the sick and the
47 elderly. Since these effects may occur some time after exposure to the chemicals, diagnosis
48 of the cause depends upon their detection in blood or urine samples.
49
50

51
52 For some toxic substances it is of interest to evaluate if the adverse effect i.e the toxicity
53 caused by the substance attention should be made from radioactivity or to the chemical
54 effect. Thus radioactivity versus chemical toxicity should be considered. To illustrate such
55 statements the chemical effect eg ^{203}Hg towards Hg , $^{109}\text{Cd}/\text{Cd}$, and cesium against
56 radioactive cesium. Radioactive isotopes have been emitted after nuclear power plant
57 disasters and after fallout they can give rise to serious health effects caused by the
58 radioactivity.
59
60

2. ACUTE and CHRONIC

1. 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. [4]

2. In clinical medicine, sudden and severe, having a rapid onset. [4]

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. [4]

acute exposure

Exposure of short duration. [4]

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.). [4]

2. Ability of a substance to cause *adverse effects* within a short time of dosing or *exposure*. [4]

Antonym: *chronic toxicity*

Explanatory Definition

acute (in toxicology)

1
2
3 Acute in toxicology is a word that is used in combination with exposure, toxicity, and effect.
4 Acute exposure is a single or very short lasting dosing by any route. Talking about acute
5 toxicity addresses adverse effects (see explanatory definition below) - harmful effects, i. e.
6 unwanted negative effects that occur immediately after or within a short time after
7 administration of a single dose of a substance, or following short exposure or concurrently
8 with continuous exposure, or recurrently following shortly after multiple doses. "Short"
9 implies a time of 24 hours or less. Some effects considered to be acute can occur up to as
10 long as after 96 hours after exposure. Uremia can be an acute effect but it takes almost 96
11 hours to see such an outcome. In toxicity testing it is most important to be aware of this in
12 order not to draw any false conclusions from animal studies with agents giving rise to such
13 an acute effect. Acute effects occur or develop rapidly after a single exposure. However,
14 acute effects can also appear immediately after, or during, repeated or prolonged exposure.
15
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19
20 Historically, an important aspect of acute toxicity has been the identification of the lethal
21 dose or exposure that kills an organism after a short exposure or a single dose. This has been
22 established by a test exposing selected organisms to a series of increasing dose levels until a
23 dose is reached at which all the organisms die. For regulatory purposes, in order to permit
24 extrapolation to humans, it is usually performed with at least two mammalian species. From
25 such tests the LD₅₀ for the test species has been derived and used for the classification of the
26 toxicity of chemicals to humans. Such tests involve killing large numbers of animals to
27 obtain a toxicity classification based on lethality and which tells us nothing about sublethal
28 effects such as immunotoxicity or teratogenicity. This situation was clearly unsatisfactory
29 and so acute toxicity testing is now designed in such a way as to obtain the maximum of
30 information about all aspects of acute toxicity using the minimum number of animals.
31
32
33

34
35 In Europe, classification of new chemicals for toxicity is no longer based on the LD₅₀. The
36 tests used for this purpose are based on survival rather than on lethality. For example, the
37 method of fixed dose testing is usually limited to a maximum dose of 500 mg per kg body
38 weight. If 5 males and 5 females exposed to a dose of this magnitude survive with no
39 evidence of toxicity, the chemical tested need not be classified as toxic. Toxicity
40 classifications based on this approach can provide a similar classification of toxicity to the
41 old LD₅₀ system but with a huge reduction in the number of animals used and in animal
42 suffering compared to the traditional LD₅₀ tests. Another approach to achieve the reduction
43 in numbers of animals used is the up and down procedure which produces a value
44 approximating to the LD₅₀. This procedure uses sequential dosing together with
45 sophisticated computational methods. It provides a point estimate of the LD₅₀ while
46 achieving significant reductions in animal use.
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51 Since chronic toxicity testing (see below) is expensive and labour intensive, there is a great
52 need to replace it where possible with shorter term predictive acute tests and early
53 identification of biomarkers of toxicity. This has been possible to some extent in relation to
54 carcinogenicity. In the past a cancer study was designed to expose animals to the toxicant
55 and to follow the animals during their lifetime. Each animal upon death was examined for
56 occurrence and localisation of tumors in the body. Since it is expensive to maintain animals
57 over long periods, the need for new tools to identify carcinogens is clear. Many cancers start
58 with mutations or chromosome damage and this can be assessed with short term tests such as
59 the Ames test or the host mediated (legator) test. The Ames test is based on reversal of a
60 point mutation in a *Salmonella* strain which makes it unable to synthesise the amino-acid

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3 histidine. Back mutation can be detected by growth of the bacteria in a histamine depleted
4 medium. Rat liver microsomes are included in the test medium in order to simulate the
5 metabolic activation of organic compounds that may take place in the intact animal. The host
6 mediated test looks for chromosome changes *in vitro* and (or) *in vivo*, including chromosome
7 breaks and sister chromatid exchanges, in microbial cells introduced (e.g. by intravenous
8 injection) into a host animal. The host animal receives the test compound orally and
9 therefore acts as a source of chemical metabolism, distribution and excretion. Another whole
10 animal test involves looking for the production of micronuclei in animals exposed to
11 possible carcinogens. The micronucleus test is less sensitive than bacterial tests but is a more
12 realistic measure of likely chromosomal damage in mammals at risk.
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17 It is also possible to test quickly for mutagenicity and the possibility of associated
18 carcinogenicity by adding suspect substances to cell cultures and looking for chromosome
19 damage and cell transformation. Another approach to carcinogenicity testing is to apply the
20 substances to tissues in culture and or to syngenic transplants and similarly assess the
21 changes that occur.
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25 While the acute tests for mutagenicity give a quick indication of the mutagenic potential of
26 substances tested, it must be emphasized that the effects observed may not necessarily
27 extrapolate to the intact organism. The Ames test bacterial strains have been selected for the
28 absence of DNA repair mechanisms so that they are much more sensitive to mutagenicity
29 than any normal organism. Cultured cells and tissues lose differentiated properties and are
30 abnormal in this way. Dedifferentiated cells tend to divide more rapidly than normal and this
31 may facilitate chromosomal damage. With regard to predicting carcinogenicity, not all
32 mutations lead to cancer nor are all cancers the result of mutations. Thus, while these acute
33 tests may indicate the possibility of carcinogenicity, they are not sufficient to prove it and
34 can be regarded only as screening tests to select substances for further study in this regard.
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2. 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. [4]
2. In clinical medicine, long established or long lasting. [4]

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. [4]

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. [4]

Antonym: *acute exposure*

Synonym: *long-term exposure*

chronic toxicity

1. *Adverse effects* following *chronic exposure*. [4]
2. Effects which persist over a long period of time whether or not they occur immediately upon exposure or are delayed. [4]

Antonym: *acute toxicity*

Explanatory Definition

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

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3 progressive loss of organ function, for example increasing liver damage following regular
4 ingestion of ethanol. For humans, a particularly serious example of chronic toxicity may be
5 the gradual loss of brain cells due, for example, to excessive exposure to ethanol or other
6 neurotoxic agents. Brain cells do not divide and cannot be replaced once they are lost.
7 Because we have a large reserve capacity of such cells, their gradual loss may not be
8 apparent but this added to the normal loss associated with aging may result in premature
9 dementia and related adverse effects.
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14 For the toxicologist, a particular problem arises when the dose or exposure is low or the
15 effect develops a very long time after exposure as may happen with cancer. In these
16 circumstances, it is very difficult to attribute a cause to the delayed effect. It is also difficult
17 to test substances for such effects. Cancer in humans may take up to 40 years to develop
18 after exposure to a carcinogen. Our normal test animals, rats and mice have life spans of
19 about 2 years and 18 months respectively. In order to cause malignant tumours within such a
20 short time, very large doses of suspect carcinogens must be applied. Thus, test doses are
21 much higher than those to which humans may ever be exposed and may therefore
22 overwhelm metabolic defence mechanisms that work well within likely human exposure
23 ranges.
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28 Subchronic (sometimes subacute) toxicity refers to the adverse effects observed when
29 animals are administered a toxicant over a period of time, as a result of repeated daily dosing
30 of a chemical, or exposure to the chemical, for a significant part of an organism's lifespan
31 (usually not exceeding 10%). Observations of acute and subchronic toxicity indicate what
32 the critical (target) organ and the critical effect are. With experimental animals, the
33 subchronic period of exposure may range from a few days to 6 months. The terms
34 'subchronic' and 'subacute' suffer from many variations in their usage and are best avoided.
35 It is better to replace them by giving precise definition of the times of administration and
36 observation. Subchronic testing has usually been limited to 90 days. Chronic toxicity testing
37 should be over the lifetime of the organism which means 1.5-2 years in the case of the
38 mouse or the rat.
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43 Chronic toxicity testing in rodent and nonrodent species identifies not only general toxicity
44 but also aspects of mutagenicity, carcinogenicity and reproductive toxicity (in the rat and the
45 rabbit) including specific effects on the reproductive organs, teratogenicity and reproductive
46 toxicity. Some strains of, e.g., mice have different frequencies of naturally occurring effects.
47 Most chronic studies are carried out with at least two animal species, usually rats and a
48 nonrodent species such as dogs or primates. For cancer testing it is important to choose a
49 species known to have a low frequency of tumours. For example, the Syrian Golden hamster
50 has low background frequency of tumours in the trachea and lung. Currently there is great
51 activity in developing alternatives to chronic animal testing, e.g., the use of stem cells, tissue
52 culture, and *in silico* methods.
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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. [4]

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/LD₅₀) or *concentration* (1/LC₅₀). [4]

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). [3, 4] 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:

$$n(\text{TEQ}) = \sum_{i=B}^N f_i n_i \quad [4]$$

Explanatory Definition

adverse effect in toxicology

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3 Living organisms have evolved to adapt to a range of environmental conditions and to
4 change in response to environmental changes, including chemical changes. Such changes in
5 organisms in response to the environment constitute effects. These effects may be beneficial
6 (as perhaps with essential nutrients), neutral, or harmful. Harmful effects of a substance in
7 relation to dose define its toxicity. Extreme damage is easy to identify but the toxicologist
8 wants to define the earliest signs of harm and this is not so easy. If the effect resulting from
9 exposure to a potentially toxic substance is small, it may be within the normal range of
10 physiological variation required for life to adapt and cause no harm. In that case, it is not an
11 adverse effect. On the other hand, it may have a small effect which causes no immediate
12 harm but may contribute to harm in future if the organism lives long enough. For example,
13 lead may replace calcium in bone with no immediate effect but may accumulate there with
14 time to cause harm during illness, pregnancy or old age. Thus, the apparently clear definition
15 of an adverse effect may become difficult to apply in practice.
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21 Once an adverse effect has been identified, it is important to know whether it is reversible or
22 irreversible. It may be possible for an organism to recover completely from a reversible
23 effect but irreversible effects can accumulate with time and repeated exposures.
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27 In assessing the consequences of adverse effects, the organ most affected, the critical organ,
28 is a key factor, together with the dose-effect relationship. If one knows the initiating reaction
29 for the adverse effect, this may help not only to assess the likely outcome but also to suggest
30 treatment for alleviating the effect, for example by blocking the active site for the toxicant
31 on a receptor molecule.
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34 35 *Distinguishing between adverse and nonadverse effects*

36 The simplest definition of an adverse, or 'abnormal' effect experimentally is a measured
37 effect that is outside the 'normal' range. The 'normal' range is usually defined on the basis
38 of values observed in a group of presumably healthy individuals, and expressed statistically
39 as a range representing the 95% confidence limits of the mean or, if the mean has been
40 determined on the basis of a very large sample, the 95% limits will be equal to $\mu \pm 1.96$
41 sigma where μ (mu) and sigma are the population values of the mean and the standard
42 deviation, respectively. An individual with a measured value outside this range may be either
43 genuinely 'abnormal' or one of a small group of 'normal' individuals who have extreme
44 values. This distinction between 'normal' and 'abnormal' values based on statistical
45 considerations may be used as a criterion for adverse effects, if the exposed population
46 consists of adult, generally healthy individuals, subject to periodical medical examination,
47 such as workers. In these circumstances, departures from 'normal' values associated with a
48 given exposure can be considered as adverse effects, if the observed changes are:
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51 (a) statistically significant ($P < 0.05$) in comparison with a control group, and outside the
52 limits ($\mu \pm 2\sigma$) of generally accepted 'normal' values;

53 (b) statistically significant ($P < 0.05$) in comparison with a control group, but within the
54 range of generally accepted 'normal' values, provided such changes persist for a
55 considerable time after the cessation of exposure; and

56 (c) statistically significant ($P < 0.05$) in comparison with a control group, but within the
57 'normal' range, provided statistically significant departures from the generally accepted
58 'normal' values become manifest under functional or biochemical stress.
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5 The preceding statistical considerations will not be appropriate if the available data are
6 nonparametric, i.e. not consistent with a Gaussian (normal) distribution. For nonparametric
7 data, the median replaces the mean as a measure of the central value. Two of the simplest
8 nonparametric procedures are the sign test and median test. The *sign test* can be used with
9 paired data to test the hypothesis that differences are equally likely to be positive or
10 negative, (or, equivalently, that the median difference is 0). The *median test* is used to test
11 whether two samples are drawn from populations with the same median. The median of the
12 combined data set is calculated and each original observation is classified according to its
13 original sample (A or B) and whether it is less than or greater than the overall median. The
14 chi-square test for homogeneity of proportions in the resulting 2-by-2 table tests whether the
15 population medians are equal. The major disadvantage of nonparametric techniques is clear
16 from its name. Because the procedures are *nonparametric*, there are no parameters to
17 describe and it becomes more difficult to make quantitative statements about the actual
18 difference between populations. For example, when the sign test says two treatments are
19 different, there is no confidence interval and the test doesn't say by how much the exposures
20 differ. However, it is sometimes possible with the right software to compute estimates (and
21 even confidence intervals!) for medians and differences between medians. The second
22 disadvantage of nonparametric procedures is that they throw away information. The sign test
23 uses only the signs of the observations. Ranks preserve information about the order of the
24 data but discard the actual values. Because information is discarded, nonparametric
25 procedures can never be as powerful (able to detect existing differences) as their parametric
26 counterparts when parametric tests can be used.
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33 The statistical definition of adverse effects is likely to be inappropriate for a population
34 which includes groups that may be specially sensitive to environmental factors, particularly
35 the very young, the very old, those affected with disease, and those exposed to other toxic
36 materials or stresses. For such a population, which is the norm for humans, it is practically
37 impossible to define 'normal' values, and any observable biological change may be
38 considered as an adverse effect under some circumstances. Thus, it is important to establish
39 criteria for adverse effects based on biological considerations as well as on statistically
40 significant differences with respect to an unexposed population (control group).
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45 There are no generally agreed biological criteria and so ultimately the decision on what is an
46 adverse effect tends to depend on experience and expert judgment. It may nevertheless be
47 useful to give examples of such criteria, illustrating at the same time the difficulties in
48 applying these criteria. One approach is to try to define which effects are nonadverse and to
49 eliminate them from further consideration. Nonadverse effects have been defined negatively
50 as the absence of changes in morphology, growth, development, and life span. In addition,
51 nonadverse effects are those which do not result in impairment of the capacity to compensate
52 for additional stress. Nonadverse effects should be reversible following the end of exposure
53 without any detectable reduction in the ability of the organism to maintain homeostasis, and
54 should not enhance its susceptibility to the harmful effects of other environmental
55 influences. Thus, in contrast, adverse effects may be defined as changes that:
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59 1. Follow single, intermittent or continued exposure and that result in loss of functional
60 capacity (as determined by anatomical, physiological, and biochemical or behavioural
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 'nonadverse' 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 behavioural changes, accompanying 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

Statistical lower confidence limit 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 %. [4]

benchmark dose

Statistical lower confidence limit 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 %. [4]

Figure 5. Dose-response curve showing BMC and BMCL (BMC 10% lower confidence limit).

Explanatory Definition

benchmark concentration and 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 LD₅₀ determination. The aim is to be able to define a concentration between NOAEL and 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 no-observed-adverse-effect-level (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.

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3 Determination of the BMC or BMD requires quantitative analysis of the data relating level
4 of exposure to the effects of a chemical on animal or human health. The aim of the data
5 analysis is to determine, as accurately as possible, the relationship between a given exposure
6 and the likelihood of its producing a defined harmful effect as measured by the response (the
7 percentage of a test population showing the defined effect). The statistical uncertainty to
8 which any data is invariably subject is incorporated into the calculations. The dose-response
9 relationship obtained is plotted graphically and used to calculate the BMC or BMD: this is
10 the concentration or dose which corresponds to a chosen statistical percentage likelihood of
11 health impairment in the exposed population - for instance, 5 per cent or 10 per cent. The
12 BMC or BMD is divided by an uncertainty factor to yield a health-based recommended
13 permissible exposure limit, chosen with the aim of protecting the whole population at risk.
14 Software for calculating the BMD is available on the internet (see next paragraph).
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20 The BMC or BMD method takes account of research data uncertainties which are largely
21 ignored in the NOAEL method and, while the NOAEL is by definition one of the
22 experimental doses applied, the BMD is a quantity derived from all the available
23 experimental values. The BMD method can also give information about the risks associated
24 with exposure exceeding the health-based recommended exposure limits because it is related
25 to a statistically modelled dose response curve.
26

27 The BMC or BMD method requires three choices to be made:
28

- 29 1. The statistical likelihood of an effect occurring in the test population that would be used
30 in the determination of the BMC or BMD;
- 31 2. The dividing line between an effect considered to be tolerable and one considered to be
32 harmful;
- 33 3. The choice of a model function with which to describe the relationship between dose and
34 response
35

36 With current methodology, these choices have to be made and justified on a substance-by-
37 substance basis. Derivation of uncertainty factors used to derive health-based permissible
38 exposure limits is still *ad hoc*.
39

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

52 Following hazard characterization and selection of appropriate effect endpoints for use in
53 dose-response assessment, studies appropriate for modelling and BMC or BMD analysis
54 should be evaluated. All studies that show a graded monotonic response with concentration
55 or dose are likely to be useful for BMC or BMD analysis. The minimum data set for
56 calculating a BMD should show a significant dose-related trend in the selected effect
57 endpoint(s). It is preferable to have studies with one or more doses near the level of the
58 Benchmark response (BMR), usually 5 or 10 %, in order to give a better estimate of the
59 BMC or BMD, and thus, a smaller confidence interval. Studies in which all of the
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3 concentration or dose levels show changes compared to the control values (unsuitable for
4 NOAEL determination) can be used for BMC or BMD estimation, provided the lowest
5 response level is reasonably close to the BMR.
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9 There are at least three types of endpoint data which may be available from toxicity testing:
10 quantal (dichotomous), continuous, or categorical. A quantal (dichotomous) response may be
11 reported as either the presence or absence of an effect, a continuous response may be
12 reported as an actual measurement, or as a comparison (absolute change from control or
13 relative change from control). In the case of continuous data, the number of subjects, mean
14 of the response variable, and a measure of response variability (e.g., standard deviation (SD),
15 standard error (SE), or variance) are needed for each group. For categorical data, the
16 responses in the treatment groups are often characterized in terms of the severity of effect
17 (e.g., mild, moderate, or severe histological change).
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21 Selection of endpoints should not be limited to only the one with the lowest concentration
22 for giving rise to the lowest observed adverse effect a kind of minimum value for the lowest
23 observed adverse effect level (LOAEL). In general, endpoints that have been judged to be
24 appropriate and relevant to the exposure should be modelled if their LOAEL is up to 10-fold
25 above the lowest LOAEL. This will help ensure that no endpoints with the potential to have
26 the lowest BMDL are excluded from the analysis on the basis of the value of the LOAEL or
27 NOAEL. Selected endpoints from different studies that are likely to be used in the dose-
28 response assessment should all be modelled, especially if different uncertainty factors may
29 be used for different studies and endpoints. As indicated above, the selection of the most
30 appropriate BMCs (BMDs) and/or NOAELs (if some endpoints cannot be modelled) to be
31 used for determination of health based permissible exposure levels will be a matter of
32 scientific judgement.
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38 *Selection of the benchmark response (BMR) value*

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40 Calculation of a BMD is directly determined by the selection of the BMR, the increase in the
41 incidence of a given adverse effect in a population subjected to exposure to a toxicant. For
42 quantal effects such as cancer or mortality, an excess incidence of 10% is usually the default
43 BMR, since the 10% increase in detectable response in a given population is at or near the
44 limit of sensitivity in most cancer bioassays and in some noncancer bioassays as well. If a
45 study has greater than usual sensitivity, then a lower BMR may be used, although the ED₁₀
46 and LED₁₀ should always be presented for comparison purposes.
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51 For continuous data, if there is an accepted level of change in the endpoint that is considered
52 to be biologically significant, that amount of change should be selected as the BMR.
53 Otherwise, if individual data are available and a decision can be made about what individual
54 levels should be considered adverse, the data can be "dichotomized" based on that cut-off
55 value, and the BMR can be set as above for quantal data. Alternatively, in the absence of any
56 other idea of what level of response to consider adverse, a change in the mean equal to one
57 control SD from the control mean can be used. The control SD can be computed including
58 historical control data, but the control mean must be from data concurrent with the
59 treatments being considered. Regardless of which method of defining the BMR is used for a
60 continuous dataset, the effective dose corresponding to one control SD from the control

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3 mean response, as would be calculated for the latter definition, should always be presented
4 for comparison purposes.
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8 *Choice of the model to use in computing the BMD*

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10 The goal of the mathematical modelling in BMD computation is to fit a model to dose-
11 response data that describes the data set, especially at the lower end of the observable dose-
12 response range. In practice, this involves first selecting a family or families of models for
13 further consideration, based on characteristics of the data and experimental design, and
14 fitting the models using one of a few established methods. Subsequently, a lower bound on
15 dose is calculated at the BMR. USEPA guidance on BMC and BMD calculation
16 recommends that 0.1 be used to compute the critical value for goodness of fit, instead of the
17 more conventional values of 0.05 or 0.01, and that a graphical display of model fit be
18 examined as well. For comparison of models and selection of the model to use for BMDL
19 computation, the USEPA recommends the use of Akaike's Information Criterion (AIC), see
20 the website referred to below.
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25 *Computation of the confidence limit for the BMD (BMDL)*

26 The USEPA benchmark dose guidance document (available at the USEPA website
27 <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=20871>) discusses the computation of
28 the confidence limit for the BMD, the fact that the method by which the confidence limit is
29 obtained is typically related to the data type, and the manner in which the BMD is estimated
30 from the chosen model. Details for approaches to CI computation specific to particular data
31 types (quantal, clustered, continuous, multiple outcomes) are provided in the USEPA
32 document.
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37 *Advantages of the benchmark dose (concentration) method*

38 The advantages of using the BMD approach are many. First, all the experimental data are
39 utilized to construct the dose-response curve. Second, the variability and uncertainty are
40 taken into account by incorporating standard deviations of means, and third, the method
41 represents a single methodology which can be applied to cancer and non-cancer endpoints. It
42 may also be possible to use fewer animals in testing.
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5. BIOMARKERS

IUPAC Definitions

biomarker

Indicator signalling an event or condition in a biological system or *sample* and giving a measure of *exposure*, effect, or susceptibility. [4]

Note: Such an indicator may be a measurable chemical, biochemical, physiological, behavioural 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. [4]

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. [4]

biomarker of susceptibility

Biomarker of an inherent or acquired ability of an organism to respond to *exposure* to a specific substance. [4]

Explanatory Definition

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. Non-invasive 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

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3 about exposure through all routes including those of non-occupational exposure. An example
4 where this is important is the combination of occupational exposure to lead with exposure to
5 lead through hobbies (such as in soldering, shooting, or glazing with lead) and with
6 environmental exposure to leaded gasoline. Another example is occupational exposure to
7 solvents combined with exposure to solvents at home during painting or while engaged in
8 hobbies involving paint and glue.
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12 Biomarkers related directly to exposure can be classified into two groups: a) biomarkers of
13 exposure (biological monitoring), b) biomarkers of effect (biological effect monitoring).
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17 *Use of biomarkers of exposure in monitoring individual dose of toxicants (biological*
18 *monitoring)*
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20 To assess internal dose and body burden, the amounts of toxicants of concern or their
21 metabolites and/or derivatives in cells, tissues, body fluids or excreta are measured. In
22 addition or alternatively, an indirect biomarker of exposure may be determined such as
23 cytogenetic change or reversible physiological change in exposed individuals. For example,
24 if lead effects on haem synthesis are detected, they may be used in addition to measurement
25 of lead in blood as a measure of internal dose (integrated in time) for this particular effect. In
26 the case of cadmium in blood, there is no effect on the blood and thus cadmium in blood has
27 to be used exclusively as a measure of internal dose. An early effect of cadmium is on the
28 kidney, causing the leakage of proteins into urine. A good dose measure to relate to this
29 effect is the concentration of cadmium in urine, adjusted for urinary dilution. This measure
30 also gives an indication of the body burden.
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35 For assessment of internal exposure and dose, sampling technique must be precisely
36 controlled since it can profoundly affect results. Data for substances which disappear rapidly
37 from the blood cannot be interpreted unless there is a documented standard time at which
38 samples are taken. A standard time for sampling is especially essential if the half life of the
39 toxicant of concern in the body is short. Contamination is the major source of errors when
40 analysing many substances, especially metals such as nickel, chromium and cadmium.
41 Contamination can come from the air, from the skin and sweat, sample containers, and
42 anticoagulants (for blood samples). The risk of contamination from skin, clothes, and hair, as
43 well as from the air at the workplace is particularly great when collecting urine samples.
44 Precipitation and adsorption are problems when collecting and storing urine samples. Certain
45 chemicals, for example aluminium and volatile organic compounds, are adsorbed on glass
46 and plastic.
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51 The most common sample materials are anticoagulated whole blood, serum or plasma, urine,
52 and exhaled air. Saliva, sweat, hair, and nails may also be used in biological monitoring for
53 certain substances. Urine samples are frequently used as urine is easy to collect in large
54 amounts. Variations in liquid intake and fluid loss (e.g., in a warm working environment
55 where a lot of fluid is lost through sweat) result in large variations in concentrations of
56 substances in urine. This variation is often corrected using the creatinine concentration of the
57 urine or by measuring the urinary 24 hr volume output. Relative density of urine can also be
58 used for such corrections. Because of the differences between men and women, results for
59 each gender should be reported separately.
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5 *Biomarkers of effect (biological effect monitoring)*
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7 Biomarkers of effect are measurable biochemical, physiological or other alterations within
8 an organism that can be recognised as associated with an established or potential health
9 impairment or disease. Biomarkers of effect are often not specific for a certain substance but
10 sometimes may be sufficiently specific to be used as surrogate measures of exposure and of
11 dose. This will certainly be true if it is known that only one of the possible causes of a given
12 effect can be involved in a given exposure situation. However, in some circumstances,
13 single biomarkers of effect may be useful in blanket monitoring of multiple exposure, where
14 those exposures have a common effect. If multiple biomarkers are used, and one or more
15 markers is positive, additional markers or environmental monitoring can be used to
16 determine the substance(s) causing the effect.
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21 Examples of biomarkers of effect are:

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

IUPAC Definition

biotransformation

Chemical conversion of a substance that is mediated by living organisms or enzyme preparations derived therefrom. [4]

Explanatory definition

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, micro-organisms are often employed in waste management and environmental clean-up i.e. 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 context, biotransformation can be exploited to facilitate delivery to a site of effect, and activation at that site. Dopamine, used in the treatment of Parkinson 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.

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3 Biotransformation is an extremely important concept in toxicology, as it often involves
4 systems that have evolved to detoxify xenobiotics. However, initial steps in detoxification
5 may increase toxicity. An example would be epoxidation of an aromatic hydrocarbon, with
6 the intent of increasing its solubility for excretion or creating functionality for conjugation,
7 but in the process creating reactive chemical species that can damage biomolecules like
8 lipids, proteins and DNA by creating oxygen-centred radicals and forming adducts.
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12 The above processes are the basis for a classification of reactions of drug metabolism into
13 Phase I and Phase II. Phase I refers to the chemical modification of a substance by processes
14 such as hydroxylation, oxidation, reduction, or chlorination. This introduces functionality for
15 subsequent further metabolism. Phase II refers to conjugation of the Phase I products,
16 usually to enhance their hydrophobicity and facilitate urinary or biliary excretion. Whereas
17 Phase I processes increase hydrophilicity and add functionality at the risk of increased toxic
18 potential, those of Phase II almost invariably decrease toxicity.
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21 22 23 *Phase I Metabolism*

24 Phase I metabolism includes a variety of reactions that modify the core structure of the
25 xenobiotic. These include oxidations by mixed function oxidases (MFO), reductions,
26 hydrolyses, hydrations and isomerisms. The MFO cytochrome P₄₅₀ (P450) is so important
27 for Phase I oxidations that it is usual to consider oxidations by P450 separately from others.
28 Non-CytP450 oxidations are catalyzed by enzymes such as alcohol dehydrogenase, xanthine
29 oxidase, amine oxidases, and aromatases; they require oxygen. Reductive biotransformation
30 includes reactions that are generally inhibited by oxygen and require NADPH. Substrates
31 include azo- and nitro-compounds, halogenated compounds, nitrogen heterocycles, and
32 epoxides. A number of enzymes effect hydrolysis, most significantly the esterases, while
33 hydration without hydrolytic cleavage is the domain of less abundant hydratases. Simple
34 reactions such as cyclization, isomerization, dimerization, transamidation, and
35 decarboxylation are also considered Phase I.
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41 The P450 family of enzymes use NADPH and molecular oxygen to hydroxylate many
42 different substrates. These are heme-containing enzymes of about 45-55 kDa embedded in
43 the membrane of the endoplasmic reticulum. The *P450* genes are classified into families and
44 subfamilies, and well over 30 human P450 proteins are uniquely identified. Some of these
45 are primarily important for drug metabolism (e.g., P450s 2E1, 2D6, 2C9, 3A4); others
46 function in sterol synthesis (e.g., P450s 11A1, 21A2). Genetic variation in P450 isoforms is
47 associated with differing rates of drug metabolism (e.g., P450 2D6 variants give rise to
48 different rates of debrisoquine metabolism), and potentially susceptibility to some
49 carcinogens.
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54 55 *Phase II Metabolism*

56 Phase II metabolism acts upon the products of Phase I to render them even less toxic and
57 more water-soluble by conjugation reactions, enhancing urinary and biliary excretion. These
58 reactions include addition of glucuronic acid (glucuronidation), glycosylation, sulfation,
59 methylation, and acetylation. Conjugation of many substances (generally strong electrophiles
60 produced by Phase I metabolism) to glutathione represents a major route of detoxification.
This may occur spontaneously, or be catalysed by glutathione-S-transferases. The

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3 glutathione (Gly-Cys-Glu) thioconjugate may be excreted directly through the bile or urine,
4 or may become a substrate for sequential attack by glutamyl transpeptidase and a second
5 peptidase that results in release of the cysteine conjugate of the xenobiotic. This occurs
6 particularly in liver and kidney. Conjugations with fatty acids or cholesterol esters also
7 occur, as with the conjugation of cannabinoids to stearic and palmitic acids. Glucuronidation
8 of bilirubin is important in preventing build-up of this potentially toxic metabolite. In
9 general, conjugations require an 'activated' intermediate, either of the xenobiotic itself, or
10 involving UDP-glucuronic acid (for glucuronidation), S-adenosyl methionine (for
11 methylation), acetyl coenzyme-A (for acetylation), or phospho-adenosine phosphosulfate
12 (for sulfation). Though not generally commonly used, the term Phase III metabolism refers
13 to metabolism of conjugates arising from products of Phase II. An example would be the
14 further metabolism of glutathione conjugates in the gut.
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20 Among the most important sites of biotransformation from the perspective of toxicokinetics
21 are the liver and the lung, although the gastro-intestinal tract, kidney, and erythrocytes are
22 also important in xenobiotic metabolism. The liver plays a central role in biotransformation
23 and detoxification of xenobiotics; indeed that is one of its major roles. The 'first pass effect'
24 refers to the fact that oral exposure to a substance absorbed in the gut will first meet the liver
25 through the portal circulation, and undergo Phase I processing. Most of the enzymes of
26 Phase I and Phase II metabolism were initially identified in liver tissue. Phase I oxidative
27 enzymes in liver and other cells are almost exclusively in the endoplasmic reticulum,
28 whereas Phase II enzymes, including the glutathione-S-transferases, are cytosolic: an
29 exception is glucuronosyl transferase, which is in the endoplasmic reticulum. There are
30 effective transfer systems for sulfate, glucuronic acid, glutathione, and glycine conjugates in
31 liver cell membranes. These permit uptake of conjugates into liver cells and their secretion
32 into bile. Similar transfer systems are found in kidney cell membranes.
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38 The lung is the portal of entry for inhaled substances and has its own defensive barrier.
39 Pulmonary alveolar macrophages are adapted to antioxidant defence. Paradoxically, the very
40 defences of lung tissue result in metabolic activation of many compounds. This is the
41 consequence of a metabolically active, aerobic tissue. Many toxicants first enter the body
42 into the circulation. The erythrocyte, as the major circulating cell, protects itself by
43 detoxification through biotransformation. Erythrocytes lack nuclei and reproductive
44 capacity, nevertheless serve a unique role in detoxification. They exist in a high O₂ and high
45 iron environment, and must withstand this environment of high oxidative stress. Iron,
46 haemoglobin, and oxygen (oxidative stress) participate in oxidative biotransformation
47 reactions in the erythrocyte. Glutathione conjugates are actively transported outward across
48 the red cell membrane. A major protective enzyme against oxidative stress in erythrocytes is
49 glutathione peroxidase. Characteristic biotransformation reactions in erythrocytes are
50 glutathione transferase and N-oxidations of some substrates.
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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. [4]
2. Person who is heterozygous, that is 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. [3]

Explanatory Definition

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 in the Glossary 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 difference 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

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3 displacement into a phase of interest. Albumin is often used as a “carrier protein” because of
4 its ready abundance and mixed hydrophobic and hydrophilic nature.
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8 A final meaning of the term “carrier” in biology is used to describe a molecule or molecular
9 complex that ‘carries’ a substance across a biological barrier. Here the term “transporter” is
10 often substituted, though this may be a more specific term if it is intended to imply active
11 transport requiring energy, as in the case of ATP hydrolysis, coupled transport, or
12 conformationally coupled processes. Such transport frequently refers to delivery across a cell
13 membrane. See also “1. Absorption” above.
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8. COMPARTMENT

IUPAC Definitions

compartment

Conceptualised part of the body (organs, tissues, cells, or fluids) considered as an independent system for purposes of modelling and assessment of *distribution* and *clearance* of a substance. [4]

compartmental analysis

Mathematical process leading to a model of transport of a substance in terms of *compartments* and *rate constants*, usually taking the form $C = Ae^{-\alpha t} + Be^{-\beta t} \dots$ where each exponential term represents one compartment. C is the substance *concentration*; A, B, \dots are proportionality constants; α, β, \dots are rate constants; and t is time. [4]

physiologically-based pharmacokinetic modelling, PBPK

Mathematical modelling of kinetic behaviour of a substance, based on measured physiological parameters. [4]

synonym *toxicologically-based pharmacokinetic modelling*

Explanatory Definition

compartment

A compartment as used in toxico- or pharmaco-kinetics 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 differential 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 behaviour, then, is the exponential rate of decay of a substance in the blood, i.e. its disappearance from the blood.

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5 If compartments can be considered as hypothetical spaces between which substances move
6 according to defined kinetic principles, we can introduce the idea of compartmental analysis
7 as the mathematics that describes this movement. Complex multicompartment models can be
8 employed to model complicated behaviour, but the general principles are well illustrated by
9 considering the cases of one- and two-compartment systems. Surprisingly, the intricate
10 biology that determines the full toxicokinetics of a substance is often modelled very well by
11 these simple compartmental descriptions based on first order kinetics.
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15 The simplest one-compartment model depicts the whole body as a single homogeneous unit.
16 If a substance distributes rapidly throughout the body, this might be a rather accurate model,
17 and the plasma might be the compartment of choice to describe the substance's behaviour,
18 because of its accessibility for sampling. Note that the criterion of homogeneity does not
19 mean that the substance is assumed to be uniformly distributed throughout the body; rather,
20 kinetic homogeneity means that the rate of the substance's appearance in, or disappearance
21 from, the plasma well represents its rate of appearance or disappearance in any other part of
22 the body. Important concepts are the maximum concentration reached in the compartment
23 (C_{\max}) and the time at which it occurs (t_{\max}). The integration of the curve of concentration of
24 the substance vs. time, from zero to infinity (area under the curve), depends upon the total
25 amount of the substance that has entered the compartment, and is useful for describing dose
26 or exposure. The rate, K , of addition to, or elimination from, a compartment is the sum of
27 individual rate constants (k_i) of the various processes. In the example of an infused drug, X ,
28 it is common to write the elimination rate as $dX/dt = k_0 - KX$, where k_0 is the rate of infusion.
29 Often in pharmacokinetics one uses the Laplace transform to solve this linear differential
30 equation and eliminate the time variable, yielding equations more easily manipulated by
31 simple algebra. For instance, with the Laplace operator, s , we write $s \cdot X(\text{bar}) = (k_0/s) -$
32 $K \cdot X(\text{bar})$, and the relationship $X(\text{bar}) = k_0/K \cdot [1 - \exp(-Kt)]$ follows, reminding us of the
33 simple exponential decay.
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40 Two-compartment (and higher order) models are useful for describing exchange between
41 pools. In the simplest sense, a pair of exchange rate constants, k_{12}/k_{21} , describe the flux
42 between compartments, and an elimination rate constant, k_{10} , accounts for removal of the
43 substance from a single compartment. As additional compartments and multiple sites of
44 uptake and/or elimination come into play, the arithmetic becomes more complicated, but the
45 underlying principles, and their utility, remain the same.
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49 The description of transfer of a substance among compartments facilitates modeling of
50 kinetic behaviour, so-called pharmacokinetic or PK modeling. The resulting models are
51 descriptive, giving a concise mathematical description of empirical data, for instance.
52 Predictive models, on the other hand, accommodate non-linear aspects of ADME (see **1.**
53 **ABSORPTION**) that are not well handled by classical compartment models. PBPK is one of
54 the most successful among predictive models. Proposed mechanistic aspects of
55 physiological phenomena are incorporated, and rigid assumptions, e.g. of steady state or first
56 order kinetics, are avoided. For instance, successful PBPK (PBTk) models of lead,
57 chromium, and methyl mercury in humans have been developed that take into account
58 perfusion rates of various tissues where redox reactions, lipophilic partitioning, or selective
59 ligation can occur.
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9. DETERMINISTIC (NONSTOCHASTIC) and STOCHASTIC

IUPAC Definition

deterministic effect, deterministic process

Phenomenon committed to a particular outcome determined by fundamental physical principles. [4]

stochastic

Pertaining to or arising from chance and hence obeying the laws of probability. [4]

stochastic effect, stochastic process

Phenomenon pertaining to or arising from chance, and hence obeying the laws of probability. [4]

Explanatory Definition

Deterministic (nonstochastic) 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 'permissible exposure levels' which underlie all regulatory activities to protect people and their environment from harm that chemicals may cause.

Deterministic effects give the classical S-shaped dose-effect and dose-response curves which represent the Gaussian statistical variability of interaction between toxicant and receptor and between individuals in a population. If the population has subgroups of markedly different susceptibility to the toxicant, more than one such curve may be apparent in the test data but this situation can only be identified if very large populations are studied.

Stochastic effects, sometimes referred to as quantal effects, are those which are assumed to be all or none, either occurring or not occurring, and for which no threshold of exposure can be defined below which an effect is not expected. For such effects, the probability of its occurrence and not its severity is related directly to increasing dose. Stochastic (or pseudo-stochastic) effects include death, mutagenicity, genotoxic carcinogenicity, aspects of teratogenicity, and immunologically mediated effects. 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, 'permissible exposure levels' are set with reference to a calculated low probability of the adverse effect occurring. This probability for humans is usually set at 1 in a hundred

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3 thousand or at 1 in a million. To some extent, this is based on the practicality that an increase
4 in disease rate (see below) of this order is too low to be detected by current techniques of
5 epidemiological monitoring.
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9 A problem with stochastic effects is that the shape of the dose-response curve is uncertain in
10 at least three ways. First, the curves derived from animal trials are usually based on no more
11 than two central data points (out of perhaps four doses used). Second, there is an inherent
12 statistical uncertainty if an extrapolation is made to those low dose levels which are likely
13 for normal human exposure and therefore of regulatory importance. Third, the curves are
14 extrapolated to low doses using debatable mathematical models, usually chosen for
15 precautionary purposes to give the highest estimate of probability of effect.
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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. [4]

Explanatory Definition

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 and, in relation to prescription drugs, the general public often thinks in terms of numbers of tablets or teaspoons or other simple measures.

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, behaviour 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 minimise 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 behaviour, 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, minimising the internal dose associated with a given lead ion concentration.

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3 To determine the internal dose of a given chemical, analysis of tissues and body fluids can
4 be carried out. In determining the internal dose, analysis is aimed at measuring amounts of
5 the substance itself, and (or) of its metabolites. Since the internal dose is defined as the total
6 amount of a substance absorbed, measurement of the substance of concern should be
7 repeated over the full period of exposure and the measurement results should be integrated
8 over this time.
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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. [4]

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. [4]

Explanatory Definition

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 is useful, as done in the IUPAC 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 LD₅₀ 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*. [4]

elimination (in toxicology)

Disappearance of a substance from an organism or a part thereof, by processes of *metabolism*, secretion, or *excretion*. [4]

See also *clearance*

elimination rate

Differential with respect to time of the *concentration* or amount of a substance in the body, or a part thereof, resulting from *elimination*. [4]

Explanatory Definition

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 differential 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 a bit 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

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3 instance that so many millilitres of blood were cleared of the substance in a minute. This is
4 quite different from an elimination rate where the expression is dc/dt . We do not mean that
5 the substance is completely eliminated from the reference volume of blood in a given time.
6 That is, we do not focus on one millilitre of blood and say that c drops to zero. Rather, if the
7 concentration drops by one half we would say that this has removed enough substance to
8 clear one half of the blood volume.
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12 One reason it is useful to express clearance in this way is that it relates to the total volume of
13 the compartment, e.g., blood. So if elimination is due to the functioning of a particular organ,
14 for example, this gives us an indication of the organ's functional capacity. It also allows for
15 consideration of changes in blood volume, for instance due to transfusion or haemorrhage.
16 This means of expression is also mathematically useful in compartment models.
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20 A note is added to the Glossary entry for clearance that total clearance of a substance is the
21 sum of individual clearances from each eliminating organ or tissue. This might seem
22 intuitively obvious from conservation of mass. But it reminds us that (i) we may not always
23 be able to identify all sources of elimination, (ii) in multicompartiment models subtraction
24 from one compartment may result in addition to another, and (iii) this relation holds whether
25 concentration of substance or volume of compartment is being divided by time.
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29 Clearance as commonly used in pulmonary toxicology has a somewhat different nuance.
30 When a substance is inhaled and deposits on the epithelial surface of the bronchi,
31 bronchioles or alveoli, clearance is expressed as disappearance from this surface. The value
32 is only qualitative, for two reasons: (i) We cannot easily measure the surface area, so use
33 lung mass or volume instead, but of course the surface to volume ratio is not known. (ii) We
34 cannot really measure surface concentrations. The clearance will be derived by considering
35 the amount of surface deposition that could arise from a certain exposure, and the time it
36 takes to drop to a presumed value of zero, determined by analytical detection, with inherent
37 detection limits, following a procedure such as lavage. Because we define clearance in
38 pulmonary toxicology on the basis of tissue surface, elimination may be either mechanical,
39 through mucociliary transport and (or) the cough reflex, or by cellular uptake. When the
40 substance is taken up by the cells, it may remain in the lung tissue or be eliminated by
41 metabolism or absorption into the body, but it has been cleared from the lining surface of the
42 respiratory tissues.
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49 Clearance in renal toxicology has a somewhat specialized meaning, reflecting the unique
50 physiology of the kidney. Renal clearance is quantitative. While it is often more useful to
51 know the effect of removal a substance from the body fluids than to know the composition
52 of the urine, plasma clearance frequently depends primarily on renal clearance, and the latter
53 is an important measure of renal function. A substance with a molecular mass of less than
54 about 65 kDa will be filtered from human plasma at the renal glomerulus, and then
55 reabsorbed (or not) to varying degrees during subsequent passage through the remainder of
56 the nephron. For a substance that is removed from plasma exclusively by urinary excretion,
57 its concentration in urine divided by its concentration in plasma will equal its plasma
58 clearance divided by the urinary rate of flow. With units as examples.
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$$\begin{aligned} & [\text{Plasma clearance (ml/min)}] / [\text{Urine flow (ml/min)}] \\ & = [\text{Concentration in urine (mol}\cdot\text{L}^{-1})] / [\text{Concentration in plasma (mol}\cdot\text{L}^{-1})] \end{aligned}$$

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 GFR = 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. [4]

Synonym: *half time*

half time, $t_{1/2}$

See synonym: *half life*.

Explanatory Definition

Half life in toxicology is either radioactive or biological. Radio-active half life is the time taken for half the number of atoms in a radio-active 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 radio-activity in that organism are small because the radionuclide will probably be excreted before radio-active decay occurs. However, 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 radio-active daughter isotopes. In this situation, every case must be considered individually and no generalisations 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

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3 consequences and to take steps to minimize them. Different organs have different half-lives
4 for the presence of the same substance. For example, methylmercury has been reported to
5 have a half life of about 50 days in the liver and 150 days in the brain. Thus, the tendency for
6 accumulation in the brain is greater and so is the risk of brain damage. Since the overall half
7 life for methylmercury in the human body is only 70 days, it is clear that there is a danger of
8 underestimating risk if only total body half life is assessed.
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12 An increasingly important aspect of half life determination is the half life for disappearance
13 from the natural environment, especially of manmade toxicants. Persistence in the natural
14 environment is seen as a substance property which must eventually lead to problems simply
15 because all substances are toxic at a given concentration. The toxic concentration will
16 eventually be reached for even the least toxic substance if it persists sufficiently long and is
17 constantly being added to the environment. This concept underlies the definition of
18 persistent organic pollutants (POPs), which are defined as persistent if;
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- 21 1. There is the potential for long-range transboundary atmospheric transport: necessary
22 evidence includes a half life in air of > 2 days;
 - 23 2. There is aquatic persistence: half life in water > 2 months;
 - 24 3. There is persistence in soils: half life in soil > 6 months; or
 - 25 4. There is persistence in sediments: half life in sediments > 6 months.
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31 There is a problem in applying these criteria in that laboratory tests to establish half-lives for
32 organic pollutants cannot simulate all the environmental possibilities contributing either to
33 breakdown or stabilization of compounds. In particular, since most organic breakdown in the
34 natural environment is due to micro-organisms, the presence or absence of appropriate
35 micro-organisms is often the determining factor in half life assessment and so there may be a
36 large difference between laboratory estimates and environmental behaviour simply because
37 of variability in this factor.
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41 Further problems arise in assessing half-lives of inorganic compounds in the natural
42 environment. While 'mineralization', breakdown of organic compounds to carbon dioxide
43 and water is considered the 'harmless' end of their natural life, no similar criterion is
44 available for inorganics. Historically, most inorganics containing metallic elements have
45 been defined toxicologically in relation to their content of the metallic element. Thus,
46 toxicologists have talked loosely of the toxicity of chromium or nickel when in fact most of
47 the toxicological effects relate to specific forms of these metals such as chromate anions or
48 nickel tetracarbonyl. In consequence, regulatory authorities have regulated for levels of the
49 element in environmental media, sometimes defining a relevant oxidation state as with
50 chromium(VI). Thus, since elements are persistent by definition, all inorganic elements
51 regulated in this way automatically become persistent inorganic pollutants (PIPS). However,
52 where testing has been done, most simple elements in the pure elemental state show low
53 toxicity, mainly because they are not bio-available. Thus, there is a need for a better
54 approach to regulation of inorganic pollutants incorporating an appropriate method for
55 determining environmental half life of those compounds which are genuinely toxic.
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3 Substances with a short biological half life may nevertheless accumulate if a small amount
4 becomes tightly bound or the organism makes more receptor molecules, even if most is
5 cleared from the body rapidly. There is also the possibility that substances with a short
6 biological half life may have cumulative toxic effects. This is the most difficult situation for
7 the toxicologist to interpret but it may be quite common in long lived organisms such as
8 human beings. Thus, care must be taken to understand the toxicokinetic metabolism of any
9 substance before regarding it as relatively harmless on the basis of a short half life.
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14 Similar reservations must be applied to consideration of substances with a short half life in
15 environmental media. Half life is measured in relation to the bulk of the chemical present
16 and it may be that a small fraction persists in association with a component of the
17 environment which is small in quantity but ecologically important or essential for a species
18 which has colonized a unique niche. Again, interpretation of half life for risk assessment
19 must be cautious and based on as full as possible an understanding of environmental
20 chemistry and ecology.
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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. [4]

Explanatory Definition

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 Figure 6). Factors affecting toxicity may include metabolism, dose-effect and dose-response relationships, and variations in target susceptibility, amongst others.

FIGURE 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

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3 made of these substances along all the available relevant information. Usually the relevant
4 information will be available in the form of Material Safety Data Sheets (MSDS). In many
5 countries there is a legal requirement that MSDS must be provided by chemical producers or
6 suppliers. It is also a normal legal requirement that chemicals should be labelled in such a
7 manner that hazardous properties are clearly identified. In the European Union, appropriate
8 'risk' and 'safety' phrases must be included on the label in the language of the country
9 where the chemicals are to be used. These phrases are allocated by an international expert
10 group involving representation from the International Programme on Chemical Safety. The
11 phrases are also to be found with other evaluated hazard information on the International
12 Chemical Safety Cards produced by IPCS and available on the INCHEM web site and the
13 U.S. National Institute of Occupational Safety and Health (NIOSH) web site.
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18 *Hazard characterization*

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

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34 This is the third step in the process of hazard assessment aiming at the determination of the
35 qualitative and quantitative relationship between exposure to a hazard and the resultant
36 adverse effects under the defined conditions of exposure which may give concern. As in
37 hazard characterisation, it is important to define and include any attendant uncertainties. In
38 risk assessment, assessment of probability of exposure is related to hazard evaluation and a
39 level of exposure likely to produce a significant level of harm (see 'Risk').
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43 *hazard quotient (HQ)*

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

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

IUPAC Definitions

additive effect

Consequence which follows exposure to two or more physico-chemical 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. [4]

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. [4]

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. [4]

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. [4]

Explanatory definition:

Terms related to interaction

When an organism is exposed to two or more substances that produce a particular physiological effect, these substances may interact, or not. 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

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3 measurements made upon exposure to the substances together, compared to the sum of the
4 individual exposures. With this reasoning, several points should be made:
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7 1. Additivity (or lack thereof) refers only to what can be measured. Therefore, it refers only
8 to specific effects. Two substances that may be additive with respect to a certain effect, may
9 be non-additive with respect to other effects. For example, two drugs might be strictly
10 additive with respect to an effect on blood pressure, but have non-additive effects on liver
11 function. So-called drug-drug interactions often refer to non-additivity with respect to side
12 effects.
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- 14 2. Additivity may occur at some concentration ratios and not others. Because any substance
15 may be non-toxic at some levels and toxic at others (Paracelsus – it is only a question of the
16 dose), non-additive effects might be observed only when one component reaches a critical,
17 threshold concentration. For instance, two anti-cancer drugs might have additive
18 effectiveness, until one reached a threshold concentration for suppressing angiogenesis, at
19 which point the effectiveness of the other might increase based on an ability to target
20 hypoxic tissue.
21
- 22 3. Additivity, in the strictest sense, is in general probably not the norm. The complexity of
23 biological systems is such that multiple effects will probably occur with any bioactive agent,
24 and overlap with the effects of a second agent will produce candidate effects for non-
25 additivity.
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30 So, when two or more substances are related through a common toxic, therapeutic, or other
31 biological effect, yet their effect is non-additive with respect to a measured parameter, this
32 interaction is described by several different terms. In the case where the effect of one
33 substance is diminished by the presence of a second, the situation is fairly straight forward.
34 The Glossary defines *antagonism* as the “Combined effect of two or more factors which is
35 smaller than the solitary effect of any one of those factors.” A straightforward addendum in
36 the Glossary is that “in bioassays, the term *antagonism* may be used when a specified effect
37 is produced by exposure to either of two factors but not by exposure to both together.” In
38 this case it is not necessary to specify which of the two factors is decreasing the activity of
39 the other; both are assumed to have a certain activity, which when they are present together
40 is less than additive. This distinguishes *antagonism* from inhibition, where one substance
41 may or may not elicit an effect common with another, but is nevertheless capable of
42 antagonizing that response. When exposure is to more than two substances i. e. to mixtures,
43 outcomes are often quite difficult to predict.
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48 Perhaps a more challenging distinction is between potentiation and synergism, situations
49 where the measured effect of two or more agents is greater than that attributed to either
50 alone. The Glossary entries list *potentiation* as “Dependent action in which a substance or
51 physical agent at a concentration or dose that does not itself have an adverse effect enhances
52 the harm done by another substance or physical agent”, and *synergism* as “(in toxicology)
53 Pharmacological or toxicological interaction in which the combined biological effect of two
54 or more substances is greater than expected on the basis of the simple summation of the
55 toxicity of each of the individual substances”. In essence, potentiation refers to an effect of
56 substance A to increase the effect of B, when A itself does not cause the same effect as B,
57 whereas synergism means that A and B share a common effect, which is greater than
58 additive when both are present.
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3 Maintaining this distinction between potentiation and synergy may not be very useful. One
4 argument would be that if B has no influence on an effect that is elicited by A, but when
5 present increases the effect of A, then it would be said to potentiate the effect of A. On the
6 other hand, if B has an effect in common with A, and both when present together give an
7 effect that is greater than the sum produced by both alone, then we would call that
8 synergism. But this distinction is not straightforward, because it is often experimentally
9 difficult to determine whether the very effect measured may be elicited by one substance
10 only in the presence of the other. Suppose that A and B do not interact but elicit a common
11 effect, E, with contributions from isolated exposures of E_A and E_B , respectively, such that
12 when given together $E_{AB} = E_A + E_B$ (additivity). Now suppose, on the other hand, that B can
13 influence the effect of A (E_A), so that this effect of A has a 'pure' component, E_{Aa} , and a
14 component dependent on the presence of B, E_{Ab} . Then $E_A = E_{Aa} + E_{Ab}$. Ignoring for the
15 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
16 additivity. If $E_{Ab} < 0$, we have antagonism. But if $E_{AB} > E_A + E_B$, is this because $E_{Ab} > 0$ with
17 $E_B = 0$ (potentiation) or with $E_B > 0$ (synergy)? The decision may be difficult
18 experimentally, when one considers that A may also have an influence on E_B (i.e., $E_{AB} =$
19 $E_{Aa} + E_{Bb} + E_{Ab} + E_{Ba}$). Even if B has no effect in isolation, E_B may be non-zero only in the
20 presence of A.
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27 Finally, it should be noted that some textbooks of pharmacology recommend against the use
28 of potentiation, referring to any increase above additivity ($E_{AB} > E_A + E_B$) as synergism,
29 regardless of whether either of E_A or E_B is zero.
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16. LOCAL EFFECT and SYSTEMIC EFFECT

IUPAC Definitions

local effect

Change occurring at the site of contact between an organism and a *toxicant*. [4]

systemic

Relating to the body as a whole. [4]

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. [4]

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

Explanatory Definitions

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 blood stream.

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 taken up by any other route and enters into the general blood circulation and transported to various organs throughout the body giving rise to effects on

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3 these organs. Once taken up in an organ, redistribution can occur by a release to the blood
4 stream and the agent can be taken up in an other organ. This is seen for many metals like for
5 instance cadmium and lead. Most substances which are not highly reactive at body surfaces
6 tend to produce systemic effects. Some substances produce both serious local and systemic
7 effects. Tetra-ethyl lead produces effects on skin at the site of absorption which means that
8 there is a local effect and then, after absorption, on the central nervous system and other
9 organs. Another example is phenol.
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14 In general, while systemic toxicity may produce effects throughout the body, the major
15 effects are on only one or more organs. These organs are referred to as target organs.
16 However, the first organ that develops an adverse effect is called the critical organ for that
17 particular toxicant. Many times it is discussed whether the damage is reversible or
18 irreversible. Although target organs show the most serious toxic effects, they are not
19 necessarily the sites of highest accumulation of the toxicant. For example, lead is
20 concentrated in bone but its most serious effects are probably those on the brain. Common
21 target organs are the brain and central nervous system, the circulatory system, the blood and
22 haemopoietic system, the liver, kidney, lung, and skin. Systemic effects often have target
23 organ-specific names, for example - neurotoxic (affecting the central nervous system),
24 cardiotoxic (affecting the heart), hepatotoxic (affecting the liver), or nephrotoxic (affecting
25 the kidney).
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30 Increasing dose may permit a substance which causes a local effect to enter the affected
31 organism and cause a systemic effect on a particular target organ. Increasing dose further
32 will increase the number of target organs and effects until the whole organism is affected if it
33 is not already dead.
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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. [4]

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.

After [35]

rate constant, k

Proportionality that relates the *rate* of a chemical reaction to some function of reactant concentrations. [4]

Explanatory Definition

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 Gold Book of IUPAC defines rate as a derived quantity in which time is a denominator quantity, adding that the rate of x is dx/dt . 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 inter-compartment 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

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3 clearance, the glomerular filtration rate (GFR) is an important concept. It refers to the
4 volume of serum ultrafiltrate cleared of a substance by passage through the glomerular
5 capillaries, again per unit time. Clearance from a compartment may reflect more generally
6 metabolism, excretion, or transfer to another compartment. For instance, the rate of
7 clearance of a reactive organic intermediate would contribute to the clearance of the
8 substance. But, we also speak of a rate of biotransformation. This concept is closest to
9 chemical terminology, where the biotransformation usually reflects an enzymatically
10 catalyzed phase I or phase II reaction. The rate here is a clear chemical rate constant, where
11 the mechanism is known or presumed.
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16 The somewhat different use of rate in epidemiology requires some explanation. We often
17 think of a rate as a change with time, and the concept of a rate as a proportionality factor is
18 not, perhaps, intuitively obvious. In its first use, the term is closely connected with the
19 chemical (arithmetical) concept of the rate constant. When we add the qualification that “the
20 numerator is contained within the denominator”, we move out of the strict dimension of time
21 to allow a rate or proportionality in the denominator.
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25 Use of the word “frequency” in the definition of the epidemiological sense of rate again
26 introduces the concept of time – though in a different sense – as here frequency involves a
27 certain number of occurrences in a fixed period of time. We would speak of the number of
28 events (e.g., deaths) from a particular cause, based on the whole population, as a rate. Here
29 the denominator would logically be a number of people, and the resulting rate would be a
30 dimensionless proportionality. The rate, then, is the number of affected or identified
31 individuals normalized to the whole population. We define incidence as the number of new
32 individuals succumbing to a particular event or illness in a period of time. Prevalence is the
33 number of events existing per unit population at a given time.
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38 So, in epidemiological terms, both incidence and prevalence refer to time as a base, and thus
39 are rates, though they express somewhat different concepts. Incidence is the number of new
40 cases, e.g., of individuals falling ill, normalized by population, in a period of time, whereas
41 prevalence is defined as the number of incidences of a disease or other events existing at a
42 given point in time. Rates are affected by environmental and physiological conditions. In
43 conclusion, knowledge of rates of exchange among body compartments is the key to
44 producing effective toxicokinetic models. Knowledge of rates in epidemiology as
45 proportions is central to developing environmental models that are needed for risk
46 assessment.
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18. REACTIVE OXYGEN SPECIES

IUPAC Definition

reactive oxygen species, ROS

Intermediates in the reduction of molecular O₂ to water. [4]

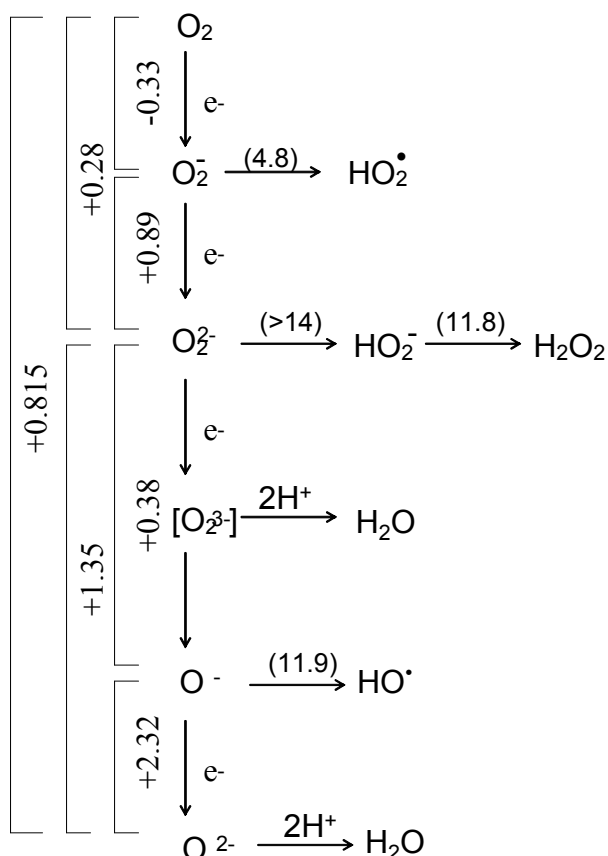
Examples: superoxide O₂^{•-}, hydrogen peroxide H₂O₂, and hydroxyl HO[•].

Explanatory Definition

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 O₂ 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 Figure 7. Molecular oxygen (O₂) exists in a double-bonded triplet ground state with an excess of two π-bonding electrons. Acceptance of the first electron into an anti-bonding orbital reduces the bond strength and creates the radical anion species superoxide (O₂^{•-}). A second electron, also accepted into a π antibonding orbital, further reduces the bond strength, and produces the singly bonded peroxide species (O₂²⁻). Because the pK_as of this species are >14 and 11.8, it exists as H₂O₂ under biological conditions. The next electron, also accepted into an antibonding orbital, breaks the single bond, and the resulting HO[•] is reduced to hydroxide and water. The one-electron redox potentials shown in Figure 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[•].

Figure 7. Four-electron stepwise reduction of molecular oxygen to water. Numbers in parentheses are pK values for protonation of the intermediates. Redox potentials for various reductions are shown to the left in volts at pH7.



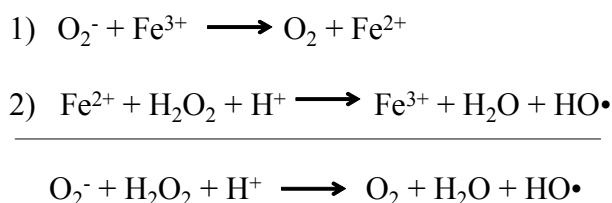
The traditional descriptor 'reactive' is somewhat misleading; for a radical, superoxide is relatively stable and persists to diffuse into the extracellular space. For instance, reperfusion injury (see below) can be diminished by scavenging superoxide from the extracellular space. The HO^\bullet radical, 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 ($\text{HOOH} \rightarrow 2\text{HO}^\bullet$) or reductive heterolytic ($\text{HOOH} + e^- \rightarrow \text{HO}^\bullet + \text{HO}^-$) 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 (X-ray or ultraviolet, for example) can permit radiolysis of water to yield $\text{HO}^\bullet + \text{H}^\bullet$. 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 (Figure 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.

Figure 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

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3 indirect effects such as the formation of unnatural disulfide bonds in an oxidizing
4 atmosphere, to direct poisoning of enzymes through attack of essential residues. Oxidation
5 of many proteins also targets them for proteosomal degradation. Nucleic acid mutations can
6 lead to short and long term effects. Short term effects include changes in gene expression
7 and cell phenotype that may be overcome with rapid activation of DNA repair mechanisms.
8 Longer term effects include either apoptosis or the potential for malignant transformation if
9 genetic defects are not repaired.
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14 ROS are rarely discussed without reference to the many defence mechanisms that have
15 evolved to protect cellular structures against them. These include i) enzymes such as
16 superoxide dismutase and catalase, that eliminate superoxide and peroxide, respectively, ii)
17 antioxidants such as ascorbate and vitamins E and A, iii) the glutathione/glutathione
18 peroxidase system, and iv) proteins that sequester potentially Fenton-active metals, such as
19 ferritin for iron or metallothionein for copper. Despite these defences, excess production of
20 ROS is harmful. It is a major contributor to long term tissue damage in diseases such as
21 hemochromatosis, where excess accumulation of iron results in increased Fenton activity.
22 Another process in which overproduction of ROS causes extensive tissue damage is
23 reperfusion injury. Following infarction of a tissue, restoration of perfusion with oxygenated
24 blood results in a rapid production of ROS, notably superoxide, and tissue damage is
25 exacerbated. Damage following a myocardial infarction, for example, can be decreased with
26 radical scavengers, antioxidants, metal chelators, superoxide dismutase, and catalase.
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32 Though generally thought of as harmful in the field of toxicology, ROS are an essential part
33 of normal cell function; they serve as rapid, diffusible signalling molecules. For example,
34 basal levels of H_2O_2 are required for signalling by the platelet-derived growth factor. And
35 generation of superoxide at the surface of activated neutrophils is necessary for efficient
36 killing of invading bacteria. Patients with genetic defects in the superoxide generating
37 cytochrome b_{558} machinery develop constrictive granulomas, especially in the lungs (chronic
38 granulomatous disease) as a result of repeated and persistent infections.
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42 The term “oxygen-centred radical” should be avoided. Simple molecular radical species
43 containing nitrogen are also of biological importance, and include the NO^- , NO^* , and NO^+
44 species. Without distinction of where electron density is centred, these are sometimes
45 referred to as reactive nitrogen species. They are important in regulating vascular physiology
46 by eliciting smooth muscle relaxation, and their metabolism includes protein
47 thionitrosylation and formation of peroxynitrite.
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19. RISK

IUPAC definition

risk

1. Probability of *adverse effects* caused under specified circumstances by an agent in an organism, a population or an ecological system. [4]
2. Expected frequency of occurrence of a harmful event arising from such an *exposure*. [4]

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*. [4]

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. [4]

Explanatory definition

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 $\text{risk} = (\text{probability of unwanted event}) \cdot (\text{severity of event})$. 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

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3 the establishment of *dose-effect* and *dose-response* relationships in likely *target* individuals
4 and populations. The process includes four steps: hazard identification, dose–response
5 assessment, exposure assessment, and risk characterization.
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9 **Figure 9. Risk assessment concept diagram**

10 **hazard identification**

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12 Already described under “hazard” above but the other concepts in the diagram are defined as
13 below.
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17 **dose–response assessment**

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19 Second of four steps in risk assessment, consisting of the analysis of the relationship
20 between the total amount of an agent absorbed by each of a group of organisms and the
21 changes developed in the group in reaction to the agent, and inferences derived from such an
22 analysis with respect to the entire population.
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27 It should be noted that dose-response assessment always involves extrapolation of results
28 from an experimental or observational group (a sample) to an entire population. Thus, there
29 is a degree of uncertainty resulting from this procedure. Such uncertainty should be
30 determined statistically and clearly stated to inform subsequent management decisions.
31
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34 **exposure assessment**

35 Third step in the process of *risk assessment*

36
37 1. quantitative and qualitative analysis of the presence of an agent (including its derivatives)
38 that may be present in a given environment and the inference of the possible consequences it
39 may have for a given population of particular concern. Exposure often changes with time
40 and so a calculated mean exposure over a given time interval may be used as the basis for
41 risk assessment. In this case, special attention may need to be given to the possible
42 occurrence of short term peaks of extreme exposure.
43
44

45 2. determination, using a range of different techniques, of the amount of a chemical,
46 physical, or biological agent that could be present in a given medium and the fate of such
47 agent under a number of potential circumstances, and the inference of possible consequences
48 for a hypothetical system that could be affected by this agent. This determination may be
49 based on theoretical considerations and use computer modelling to predict movement and
50 distribution of a substance within one or more environmental compartments at risk.
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54 **risk characterization**

55 *Preferred definition:*

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

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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: It is 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.

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3 Acceptance of risk is subjective and dependent upon perception. Different people and groups
4 of people may have very different perceptions and thus “acceptable risk” can have no
5 absolute definition, for example in terms of a certain level of probability.
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8 9 **risk management**

10 Decision-making process involving considerations of political, social, economic, and
11 technical factors with relevant risk assessment information relating to a hazard so as to
12 develop, analyze, and compare regulatory and nonregulatory options and to select and
13 implement the optimal decisions and actions for safety from that hazard.
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16 Essentially, risk management is the combination of three steps: risk evaluation, emission and
17 exposure control, and risk monitoring. These steps have been defined above.
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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*. [4]

structure-metabolism relationship, SMR

Association between the physicochemical and/or the structural properties of a substance and its metabolic behaviour. [4]

quantitative structure-activity relationships, QSAR

Quantitative structure-biological activity models derived using *regression analysis* and containing as parameters physicochemical constants, indicator variables, or theoretically calculated values. [4]

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 behaviour. [4]

Explanatory Definition

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 endpoints of concern. This includes prioritising 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

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3 consequently less accurate is any prediction. However, in drug development, animal testing
4 may be avoided for certain compounds for which SAR clearly indicates the potential for
5 serious adverse effects.
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9 Elucidation of SARs is best developed for organic compounds and is still poorly developed
10 for inorganic compounds. For organic compounds, identification of SARs requires
11 knowledge of the biological activities of defined chemical structures, of biological
12 interactions when structures occur in the same molecule, and the derivation of models which
13 can be used to relate total molecular structure to biological effects.
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17 Creation of models uses physicochemical data along with manual pattern recognition
18 methods, cluster analysis, and regression analysis. For meaningful models, data from a
19 substantial number of compounds with differing substituent combinations and well defined
20 biological effects are required. The main difficulty is in analysing the data in order to
21 identify particular structural fragments responsible for the production of a defined effect.
22 Even if such fragments are identified, the question remains as to whether these fragments are
23 sufficient in themselves to produce the effect, whether they are always necessary for this
24 effect, and whether the effect is modified by the molecular environment.
25
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29 SAR methods are available for organic molecules to predict genotoxicity, carcinogenesis,
30 dermal irritation and sensitisation, lethality, biological oxygen demand, and teratogenicity,
31 with varying degrees of accuracy. USEPA and USFDA use models for mutagenicity /
32 carcinogenicity to screen for possible problem compounds.
33
34

35 36 **QSAR**

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

IUPAC definitions with comments

biocide

Substance intended to kill living organisms. [3]

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, 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: Fibre, 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

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2
3 Product-type 18: Insecticides, acaricides and products to control other arthropods

4
5 Product-type 19: Repellents and attractants

6
7
8 MAIN GROUP 4: Other biocidal products

9
10 Product-type 20: Preservatives for food or feedstocks

11
12 Product-type 21: Antifouling products

13
14 Product-type 22: Embalming and taxidermist fluids

15
16 Product-type 23: Control of other vertebrates

17
18
19 **drug**

20 Any substance which when absorbed into a living organism may modify one or more of its
21 functions. [3]

22
23 *Comment:* The term is generally accepted for a substance taken for a therapeutic purpose, but
24 is also commonly used for substances of abuse. Just as any substance can be a toxicant, so
25 any substance can be a drug. The term carries with it the implication of use for medical
26 purposes, but also the potential for abuse to produce an effect desired by the abuser, but
27 which is ultimately harmful.
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31 **pesticide**

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33 Substance intended to kill pests: in common usage, any substance used for controlling,
34 preventing, or destroying animal, microbiological or plant pests. [1, 2]

35
36 *Comment:* As with most of the terms in this group, the definition can only be applied with
37 knowledge of the intended use of the substance. Almost any substance can be a pesticide if it
38 is used for that purpose. For example, acetone can be used to kill most insects but it is
39 unusual to use it for this purpose and so it is not normally classified as a pesticide. Similarly,
40 sodium chloride can kill most plants but is not classified as a pesticide. Usually pesticide is
41 regarded as an organic compound acting on the nervous system i.e., acts as a neurotoxic
42 substance. Thus, the term as used in practice is usually based on some official list produced
43 for regulatory purposes and has little scientific logic behind it. This may lead to careless use
44 by people who do not understand that many pesticides are not specific for the pests to which
45 they are applied but can harm people as well. This may be a particular problem with
46 herbicides, which are named as though they were specific for killing plants although they
47 may be – like paraquat – extremely toxic to humans.
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53 **poison** (in toxicology)

54 Substance that, taken into or formed within the organism, impairs the health of the organism
55 and may kill it. [4]

56
57 *Comment:* This word comes from the Greek *potein* to drink and hence has the same root as
58 the word 'potion'. When love potions were devised, their use to affect other people, often to
59 their harm, gradually led to the idea of poison as we use the word today (see 'venom'
60 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. [4]

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 [4]

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) LD₅₀ 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 LD₅₀ 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. [4]

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-defence or predation and usually delivered by a bite or sting. [3]

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. [4]

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. [4]

Explanatory Definition

Volume of distribution can be expressed as:

$$V_d \text{ (litres)} = \text{dose (mg)} / \text{plasma concentration (mg} \cdot \text{L}^{-1}\text{)}$$

$$\text{or} \quad = \text{dose (mol)} / \text{plasma concentration (mol} \cdot \text{L}^{-1}\text{)}$$

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 emphasises 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 Criteria: a statistical procedure that provides a measure of the goodness-of-fit of a dose-response model to a set of data. $AIC = -2 \times (LL - p)$, where LL is the log-likelihood at the maximum likelihood fit, and p is the degrees of freedom of the model (usually, the number of parameters estimated).
ADME	Absorption, distribution, metabolism, and excretion
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
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
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	<i>N</i> -acetyl-D-glycosaminidase
NAS	National Academy of Science

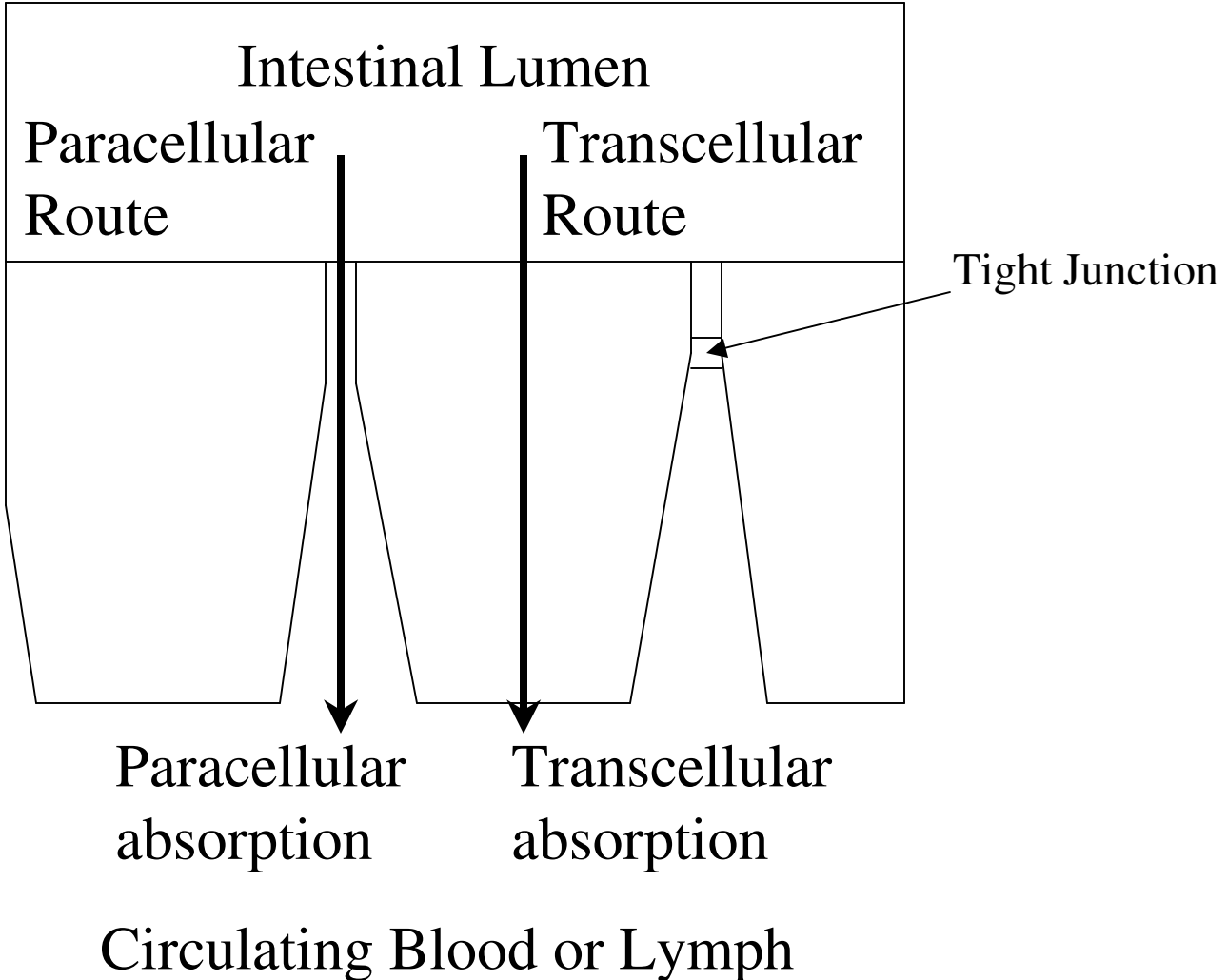
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3	NOAEL	No-Observed-Adverse-Effect-Level
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5	PBPK	Physiologically Based Pharmacokinetic Modelling
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7	PBPD	Physiologically Based Pharmacodynamic Modelling
8		
9	PBTK	Physiologically Based Toxicokinetic Modelling
10	PEL	Permissible Exposure Limit
11		
12	PIPS	Persistent Inorganic Pollutants
13		
14	PK	Pharmacokinetic
15	POPS	Persistent Organic Pollutants
16		
17	Q	Quality factor (radiation)
18		
19	QSAR	Quantitative Structure-Activity Relationship
20		
21	QSMR	Quantitative Structure-Metabolism Relationship
22	REACH	Registration, Evaluation and Authorisation of Chemicals
23		
24	RfC	Reference Concentration
25		
26	RfD	Reference Dose
27		
28	ROS	Reactive Oxygen Species
29		
30	SAR	Structure Activity Relationship
31	SAR	Specific (Standard) Absorption Rate
32	SD	Standard Deviation
33		
34	SMR	Structure-metabolism relationship
35		
36	SE	Standard Error
37	TEF	Toxicity Equivalency Factor
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39	TEQ	Toxicity equivalent
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41	UDP	Uridine Diphosphate
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43	USEPA	United States Environment Protection Agency
44	USFDA	United States Food and Drug Agency
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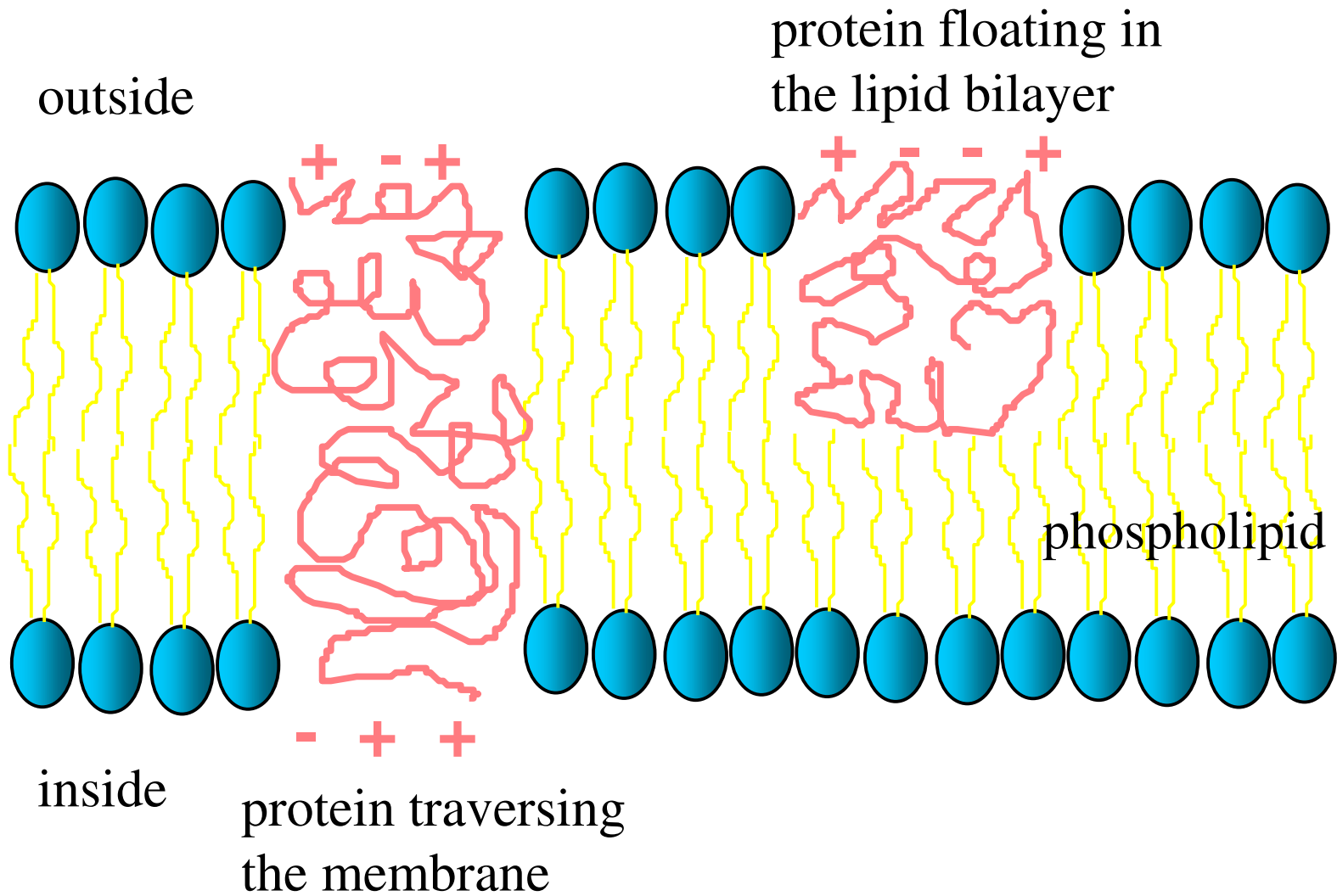
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IUPAC Explanatory Dictionary Figures 2005

John Duffus

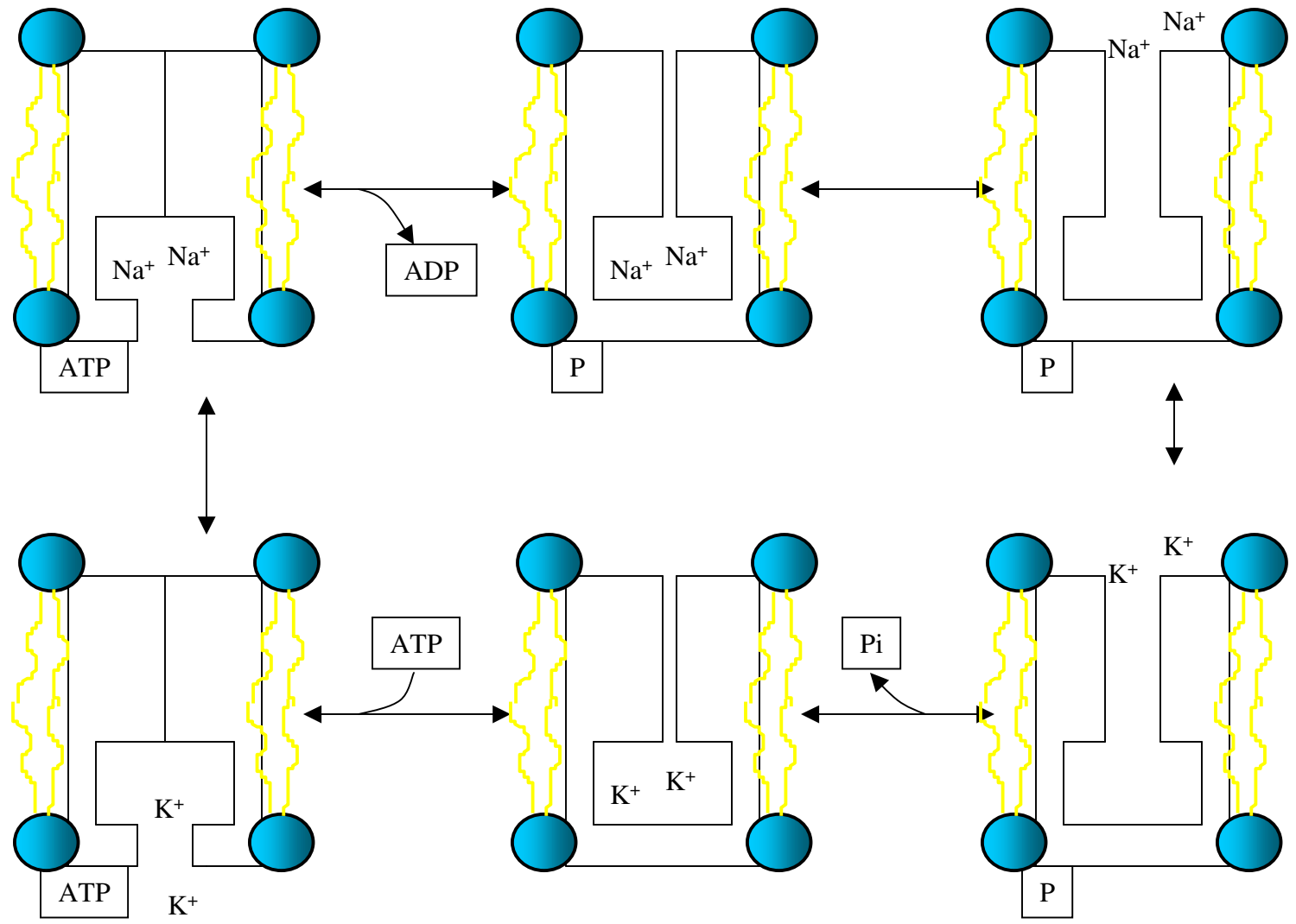
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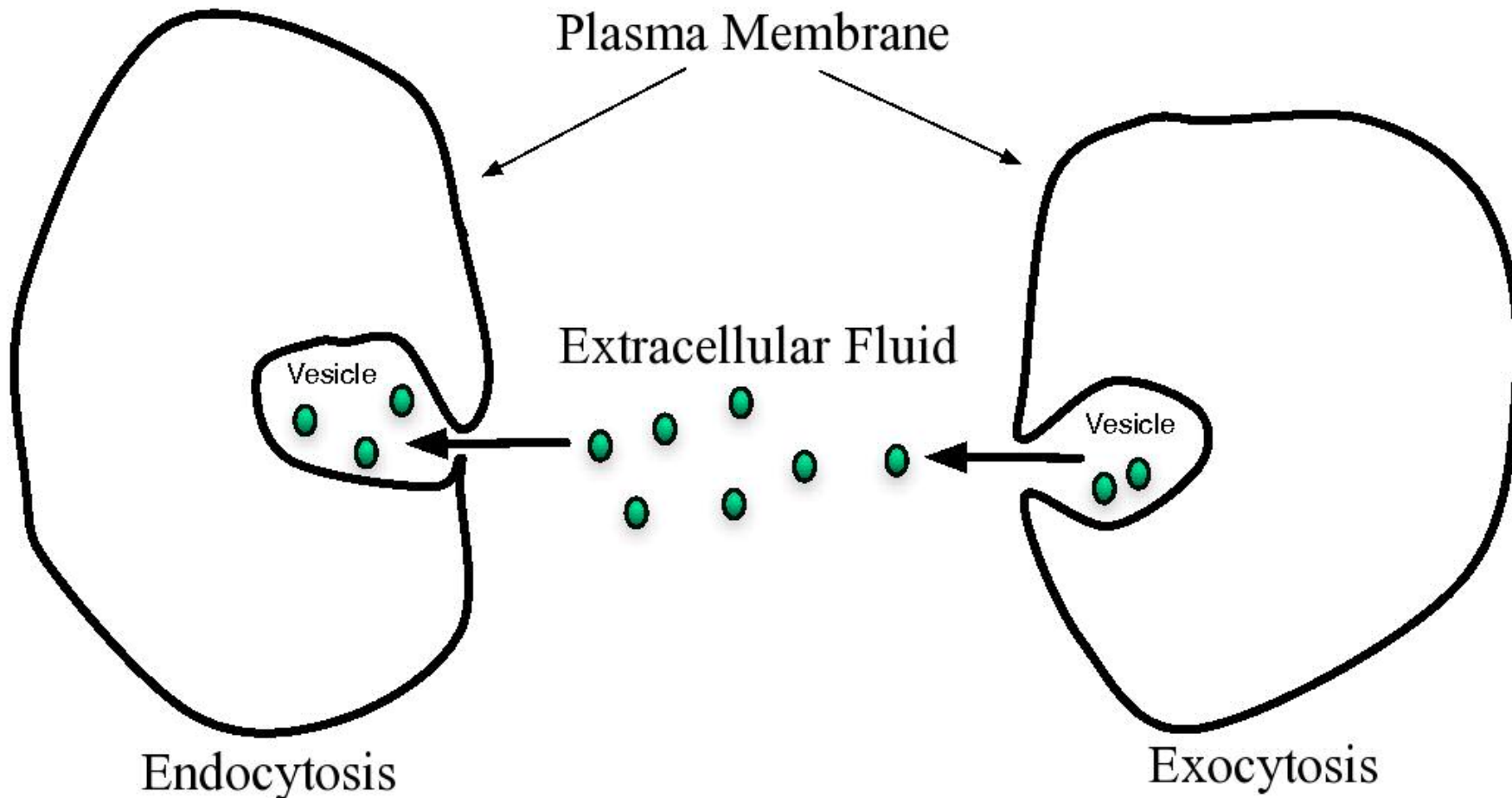


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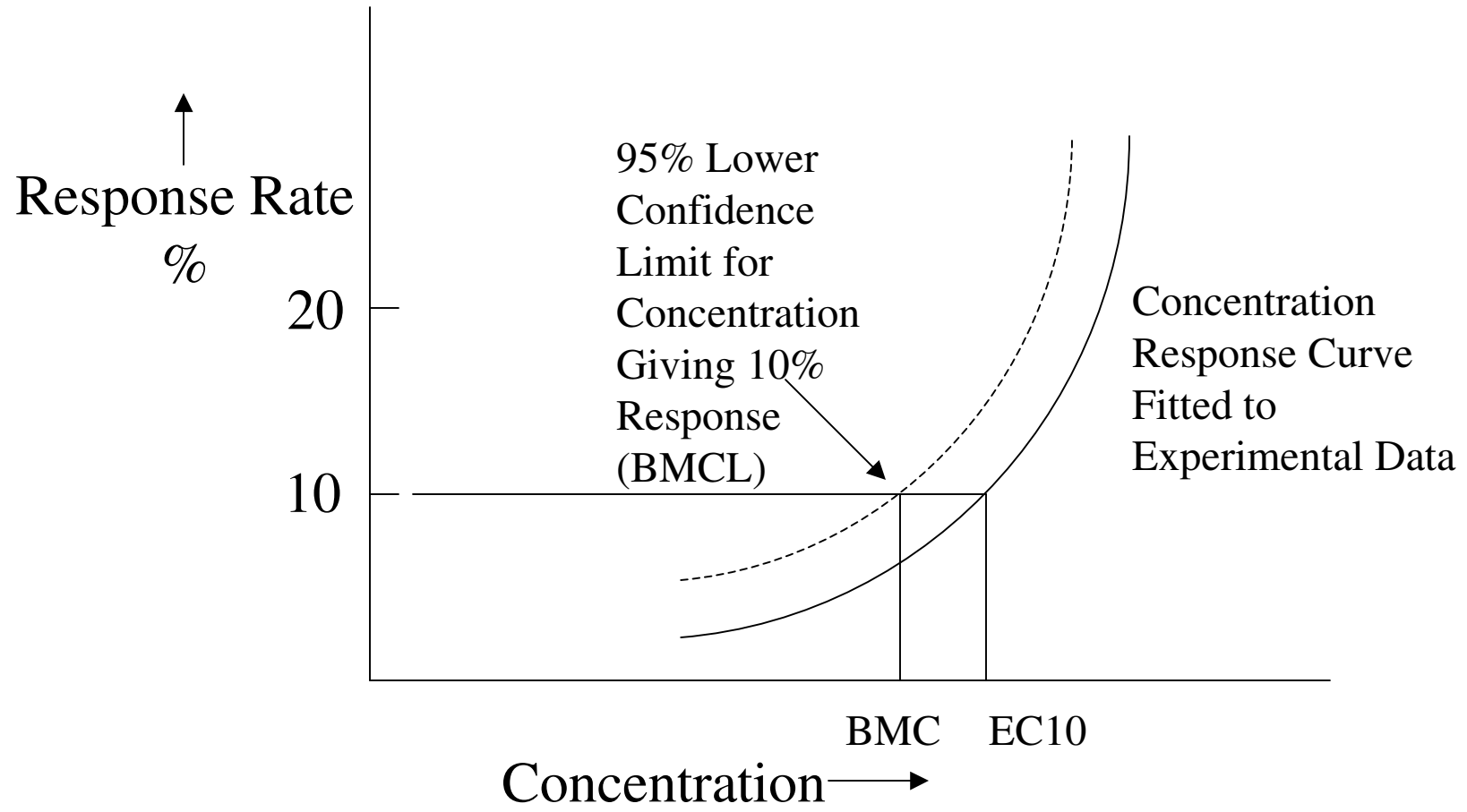
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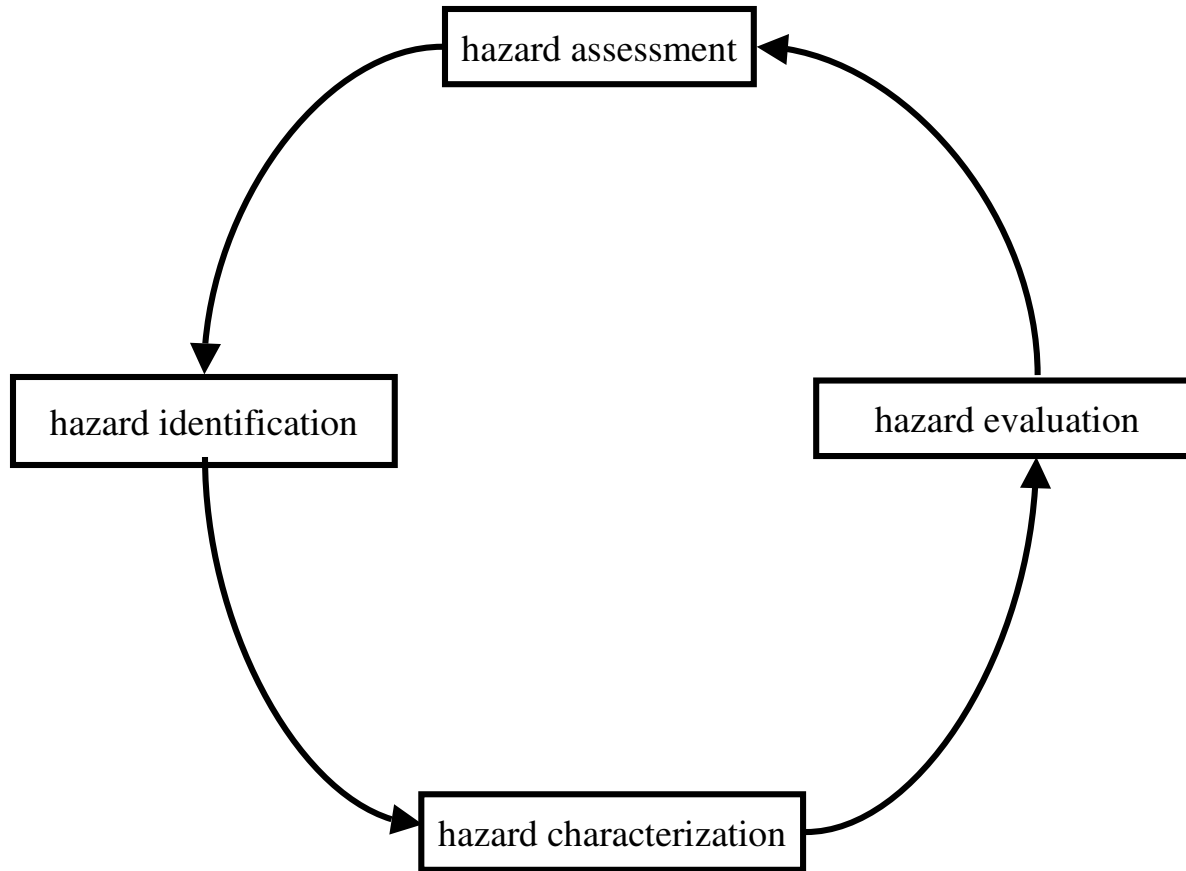


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