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Explanatory dictionary of key terms in toxicology, part II

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INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

CHEMISTRY AND HUMAN HEALTH DIVISION

EXPLANATORY DICTIONARY OF KEY TERMS IN TOXICOLOGY, PART II (IUPAC Recommendations 2008)

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Explanatory dictionary of key terms in toxicology, part II

(IUPAC Recommendations 2008)

Abstract: The objective of the Explanatory Dictionary of Key Terms in Toxicology is to give full explanations of the meaning and usage of toxicological terms chosen for their importance and complexity with regard to the merging of chemistry into 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 an international level and between disciplines. The explanatory comments should help to break down such barriers. This dictionary is a follow up and continuation of part I published in 2007. It consists of a collection of terms chosen from the IUPAC Glossary of Terms Used in Toxicology. These terms are organized under 19 main headings. The authors hope that this explanatory dictionary will be helpful to chemists, pharmacologists, toxicologists, risk assessors, regulators, medical practitioners, regulatory authorities, and everyone with an interest in the application of chemistry to solving toxicological problems. It should be of particular value to those involved in risk assessment and management.

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

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INTRODUCTION

Within the framework of IUPAC Division VII, Chemistry and Human Health, and its Subcommittee on Toxicology and Risk Assessment, this paper is part of a larger project to develop a reasonably comprehensive "Explanatory Dictionary of Terms Used in Toxicology" [1], which in turn is part of ongoing activities related to toxicology education. Following the preparation of the Glossary of Terms Used in Toxicology, 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 often unstated assumptions that are part of the concepts are explained in order to help communication between toxicologists, chemists, and members of other related disciplines.

Toxicology has grown rapidly over recent years and has been incorporated in ever-stronger laws designed to ensure safe production, use, and disposal of chemicals. Like many IUPAC bodies, Division VII, Chemistry and Human Health, is concerned to promote worldwide "regulation, standardization, or codification" in relevant areas of chemistry. In this context, lack of understanding of the terminology used in toxicology has been a problem for chemists who must implement measures for the safe use of chemicals.

This explanatory dictionary is compiled for all those who now find themselves requiring knowledge of toxicology, especially in communicating with regulators, to whom they must provide appropriate information, and with downstream users, whose use of chemicals

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they must guide. Special attention has been paid to the usage of toxicological terms in regulatory guidance documents.

The terms included in this explanatory dictionary have been chosen with regard to their frequent use in the current literature and their use in areas that have recently developed rapidly such as epigenetics (Section 8). The compilers have also included explanations of terms known to cause confusion even among experienced toxicologists. Such terms are especially difficult for the newcomer to toxicology.

The explanations have been compiled to show the relation of terms to each other and also to clarify apparently contradictory usages. All entries are introduced by the IUPAC approved definitions from the "Gold Book" [2, 3] or from the IUPAC Glossary of Terms used in Toxicology, 2nd ed. [4]. Where neither of these sources had appropriate definitions, reference was made to the most recent version of the Online Oxford English Dictionary [5]. Where differences in usage exist between related disciplines, these are pointed out in the relevant text. Commonly used abbreviations, acronyms and initialisms are listed in Annex 1. This is designed to be a self explanatory document and only a few key references are given in the text. However, Annex 2 provides a list of further reading for those who wish to learn more of any topic

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 their names 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. AEROBIC AND ANAEROBIC

IUPAC definitions

aerobic

Requiring dioxygen.

anaerobic

antonym aerobic

Not requiring dioxygen.

Explanatory comment

The term "aerobic" refers to the requirement of an organism for dioxygen (O_2), and therefore is based on metabolism. A description of our current knowledge of metabolism can be found in any good biochemistry textbook. One that is also accessible through the internet is Boyer's "Concepts in Biochemistry" [6]. Central to understanding aerobic metabolism is understanding the mechanism that higher organisms use to generate adenosine triphosphate (ATP) in the process of respiration. Respiration, in turn, refers to the oxidation of fuel molecules with dioxygen (with the exceptions noted below) as the ultimate electron acceptor and the derived energy stored in the terminal phosphate bond of ATP (oxidative phosphorylation). The breakdown of fuel molecules to yield metabolic energy in the absence of dioxygen is referred to as anaerobic metabolism, and is considered to be the antonym of aerobic metabolism. Here, a fuel molecule (very frequently glucose) is split into two moieties in a process called fermentation. One moiety then oxidizes the other and the energy released by the reaction is used for generating ATP. In higher organisms, this is typified by glycolysis, which produces ATP under conditions of relative dioxygen limitation, but also serves as an entry point to oxidative metabolism by providing pyruvate to the tricarboxylic acid (TCA) cycle.

Many cells can perform aerobic metabolism when dioxygen is present but survive by anaerobic metabolism when it is not. These are called facultative anaerobic cells. Cells that survive only by anaerobic metabolism, i.e., cells that do not have the capacity to utilize dioxygen as an oxidant and for whom dioxygen is therefore toxic, are called obligate anaerobes.

Biochemistry of aerobic metabolism

In the mitochondria of cells of higher animals, including humans, a cyclic series of enzyme reactions is central to the production of reducing equivalents for respiration. This is known variously as the TCA cycle, Krebs cycle, or citric acid cycle (Fig. 1). Various nutrients

(carbohydrates, amino acids and fatty acids) are metabolized by distinct pathways to generate 2-carbon acetate units that are coupled to Coenzyme A. The resultant acetyl coenzyme A reacts with the 4-carbon substrate oxaloacetate to produce the 6-carbon citrate. Sequential enzymatic conversion of citrate to isocitrate via cis-aconitate provides the substrate for isocitrate dehydrogenase, which converts isocitrate to 2-ketoglutarate with loss of CO_2. Loss of a second molecule of CO_2 converts 2-ketoglutarate to the 4-carbon succinate. Oxaloacetate is regenerated from succinate via the intermediates fumarate and malate, to begin the cycle again. One turn of the cycle releases 8 H atoms. These provide 4 pairs of electrons, which are recovered by the reduction of three molecules of nicotinamide adenine dinucleotide (NAD) to three molecules of $NADH_2$, and reduction of one molecule of flavin adenine dinucleotide (FAD) to $FADH_2$. If the source of acetyl coenzyme A is carbohydrate, two additional H atoms are released during conversion of pyruvate to acetate with production of an additional $NADH_2$. The electrons from $NADH_2$ and $FADH_2$ are passed down a reduction potential gradient known as the electron transport chain, consisting of a series of quinone- and cytochrome-containing proteins. These serve to split the electron pairs derived from $NADH_2$ or $FADH_2$ using the quinone/semiquinone and Fe(III)/Fe(II) redox couples, and achieve the stepwise 4-electron reduction of O_2 to $2H_2O$. Reduction of one molecule of dioxygen to $2H_2O$ by $2NADH_2$ is coupled in the electron transport chain to the production of 6 molecules of ATP from ADP. This process is called oxidative phosphorylation. ATP production is coupled to electron transfer at three sites in the electron transport chain where the drop in potential is large enough to pump a pair of protons outward across the mitochondrial membrane, creating an electrochemical gradient or protonmotive force. Their return through a proton channel in an ATPase provides the energy to reverse the ATPase activity and act as ATP synthase. Toxic substances that allow the electron transport chain to continue with the reduction of dioxygen, but prevent the formation of ATP and dissipate the energy as heat instead, are called uncoupling agents. Examples include 2,4-dinitrophenol (DNP), carbonyl cyanide p-[trifluoromethoxy]-phenyl-hydrazone (FCCP), and carbonyl cyanide m-chloro phenyl hydrazone (CCCP).

Operation of the electron transport chain is one of the major sources of production of reactive oxygen species (ROS) in respiring cells. Increased flux of glucose through glycolysis and ultimately of the resulting pyruvate through the TCA cycle feeds an abundance of $NADH_2$ into the electron transport chain, and experimental hyperglycemia leads to an increase in ROS that are thought to account for many of the chronic changes in diabetes. Overexpression of the uncoupling agent uncoupling protein-1 (UCP-1) dissipates the trans-mitochondrial membrane

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proton gradient required for operation of ATP synthase, and decreases many of the biochemical changes associated with the microvascular complications of diabetes.

Glycolysis and fermentation

As noted above, cells that survive in the absence of dioxygen break down a nutrient molecule into fragments in a process called fermentation. One fragment then serves as the electron acceptor in the oxidation of another, and this oxidation is coupled to the formation of ATP. The most important nutrient for anaerobic metabolism is glucose. Because fermentation results in incomplete breakdown whereas respiration degrades glucose to CO_2 and H_2O, the amount of energy released in respiration is much greater. Early life evolved under anaerobic conditions, but when O_2 became available in the environment, some organisms adapted to its use. Because of the greater efficiency of respiration, organisms today that can use either respiration or fermentation generally prefer respiration when dioxygen is available. Yeast and bacteria can be classified according to their fermentation products of glucose. For example, some break down glucose to ethanol and CO_2, others to ethanol and acetic acid, yet others to acetone or butanol. The fermentation of glucose to pyruvate is called glycolysis (Fig. 2). This is the form of anaerobic metabolism that occurs in higher animals.

Glycolysis follows a regulated enzymatic pathway simpler than that of respiration. Overall, 6-carbon glucose is split into two 3-carbon molecules of lactic acid, which does not represent a redox reaction. However, before cleavage, glucose undergoes sequential phosphorylation and isomerization reactions and then is converted by aldolase into the two 3-carbon moieties, dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. Glyceraldehyde-3-phosphate undergoes a multistep conversion to pyruvate, which then acts as the electron acceptor for oxidation of additional glyceraldehyde-3-phosphate, itself being reduced to lactate by lactate dehydrogenase. The oxidation of glyceraldehyde-3-phosphate by pyruvate is coupled to the generation of two molecules of ATP from ADP. Because dihydroxyacetone phosphate and glyceraldehyde-3-phosphate are interconvertible by the enzyme triose phosphate isomerase, both 3-carbon fragments of glucose are ultimately converted into pyruvate. Two ATP molecules are required for the initial hexose phosphorylation reactions, and the subsequent 3-carbon moieties yield two ATP each, for a net production of two ATP per glucose. The fate of pyruvate depends on the metabolic context of the cell (Fig. 2). In anaerobic glycolysis, it is converted to lactate. In alcoholic fermentation it is converted to ethanol with release of CO_2. Under aerobic conditions, it is decarboxylated and coupled to Coenzyme A (CoA) with the release of CO_2 and generation of NADH from NAD^+.

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The resultant acetyl CoA enters the TCA cycle via reaction with oxaloacetate, while the NADH ultimately transfers its electrons to O_2 through the mitochondrial electron transport chain.

Physiological considerations

In an aerobic cell, lactate is further oxidized to CO_2 and H_2O, and pyruvate is shuttled into the TCA cycle. Human cells can survive varying periods of anoxia depending on their glucose or glycogen stores, and their complement of glycolytic enzymes. The availability of dioxygen can also affect the pattern of drug metabolism. Whereas Phase I metabolism in the mammalian liver and other drug-metabolizing organs is typified by oxidation carried out by enzymes such as the cytochrome P450 and flavin-containing monooxygenases, monoamine oxidase, and peroxidases, reductive Phase I metabolism in other situations involves enzymes such as reduced cytochrome P450 and NADPH-cytochrome P450 reductase.

Skeletal muscle has a high capacity for anaerobic metabolism, allowing prolonged periods of exercise. During exertion, the quantity of glucose metabolized in glycolysis may exceed the capacity of the oxygen supply to support mitochondrial reoxidation of NADH. Under these circumstances, a debt of lactic acid is built up from conversion of pyruvate. This lactic acid must be metabolized during recovery. Some is oxidized in muscle, while some diffuses out of the muscle cell and is converted to glucose in the liver or oxidized in heart and other tissues. In contrast to skeletal muscle, cardiac muscle is abundant in mitochondria and requires a constant supply of dioxygen. It has limited capacity for anaerobic metabolism, oxidizing its own pyruvate as well as oxidizing lactate released from other tissues. The enzyme lactate dehydrogenase is a critical determinant of this difference between cardiac and skeletal muscle, which catalyzes the interconversion of lactate and pyruvate. The tetrameric enzyme is made up of any combination of the isoenzyme subunits H (abundant in heart) and M (abundant in muscle). The H subunit operates much more effectively with lactate as a substrate, compared to pyruvate, and is inhibited by high concentrations of pyruvate. Thus, in tissue rich in H subunits, such as heart, pyruvate is preferentially utilized by reactions other than that catalyzed by lactate dehydrogenase, and the oxidation of lactate to pyruvate is facilitated. The M subunit is not inhibited by pyruvate, and converts pyruvate to lactate when means of oxidizing NADH and pyruvate are compromised.

Microbiology

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The grouping of bacteria according to the effects of dioxygen on their metabolism and growth is important for their isolation and identification, as well as for understanding pathogenesis. One classification distinguishes among

a) Obligate aerobes that require dioxygen and lack capacity for significant fermentation. Examples include the tubercle bacillus and some spore-forming bacilli.
b) Obligate anaerobes that grow only in the absence of dioxygen. While dioxygen inhibits some of the enzymes required by these organisms for fermentation, it is also lethal to many. This is largely due to the lethal effect of superoxide in organisms lacking superoxide dismutase. Examples include *Clostridia* and *Propionobacter*.
c) Aerotolerant anaerobes, which grow in the presence or absence of dioxygen, but retain fermentative metabolism in both circumstances. These include most lactic acid bacteria.
d) Facultative organisms, which grow in the presence or absence of dioxygen, usually preferring the more efficient aerobic metabolism when dioxygen is present. These include enterobacteria and many yeasts.

While animal physiologists think of respiration as requiring dioxygen, microbiologists distinguish fermentation from respiration by determining whether the ultimate electron acceptor is organic (fermentation) or inorganic (respiration). Although most respiring bacteria use dioxygen as the acceptor, anaerobic respirers exist and include those that use nitrate (denitrifiers), sulfate (*Desulfovibrio*), or carbon dioxide (methane bacteria and some *Clostridium* sp.) as alternative oxidizing agents.

Anaerobic infections are caused by anaerobic bacteria, and occur in low dioxygen environments such as deep wounds and internal organs. They are frequently of mixed bacterial species and are characterized by abscess formation, foul smelling exudates, and necrotic tissue destruction. Anaerobes normally colonize bodily regions of low dioxygen exposure, including the mouth (dental infections are commonly anaerobic), the gastrointestinal tract, and the vagina (a danger in septic abortion). They cause infection when normal tissue barriers are damaged by injury, including surgical procedures. They are also found in soil and decaying vegetation. Common diseases caused by anaerobic *Clostridium* bacteria are gas gangrene (*C. perfringens*), tetanus (*C. tetani*), and botulism (*C. botulinum*). Because their growth is limited by dioxygen, infectious colonies are generally slow-growing and therefore difficult to treat.

2. APOPTOSIS AND OTHER MODES OF CELL DEATH

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IUPAC definition

apopto/sis n., **tic** adj.

Active process of programmed cell death, requiring metabolic energy, often characterized by fragmentation of DNA, and cell deletion without associated inflammation.

necro/sis n., **/tic** adj.

Sum of morphological changes resulting from cell death by lysis and (or) enzymatic degradation, usually accompanied by inflammationand affecting groups of cells in a tissue.

Explanatory comment

A number of years ago it was recognized that cells may die by means other than a simple irreversible response to injury, ultimately leading to membrane rupture (i.e., necrosis), such as hepatic necrosis induced by trichloromethane or acute tubular necrosis in the kidney caused by mercury. The alternative appeared to follow a sequential program of events leading to a systematic disassembly of the cell without the release of proteolytic and proinflammatory contents. The process was termed apoptosis (Gr. *apo,* from, *ptosis*, to fall; hence, falling off) by Andrew H. Wyllie in 1972. There is no consensus as to whether the 'a' is long or soft, or the second 'p' silent, and several pronunciations are in common use.

Apoptosis

In contrast to necrosis, occurring in response to acute injury such as hypoxia or exposure to toxic substances, apoptosis is considered to occur in physiological circumstances such as atrophy or at certain stages of development, or in selected pathological circumstances. It is now also known to play a key role in balancing a stable cell population in a tissue in a triad of stem cell differentiation, cell proliferation, and apoptotic cell loss. For many years the recognition of apoptosis was by visual criteria. Apoptosis involves individual cells, undergoing shrinkage and formation of apoptotic bodies, whereas in necrosis groups of cells are involved in swelling and tissue disruption. Phagocytosis of apoptotic bodies is in contrast to the inflammation and subsequent regeneration or fibrosis consequent upon necrosis. In apoptosis, organelles generally remain intact whereas necrosis is characterized by swelling of the mitochondria and endoplasmic reticulum. Especially characteristic changes are seen in the nucleus, where condensation of chromatin in apoptosis is accompanied by internucleosomal breaks (karyorrhexis) and a characteristic pattern of bands of multiples of approximately 200 base pairs on agarose gel electrophoresis ("DNA laddering"). In contrast, random DNA breaks precede loss of DNA (karyolysis) in necrosis.

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Subsequently a family of proteases was discovered that was responsible for the controlled cleavage of a variety of substrates during the apoptotic program. These have in common an active center cysteine thiolate that cleaves at aspartic residues. Called caspases (for Cysteine ASPartate proteASES), these include upstream initiator caspases that cleave and activate downstream effector caspases, which in turn cleave other protein substrates. About 14 caspases are currently known in humans, and for a time caspase activation was a hallmark of apoptosis.

As more biochemical detail emerged, two biochemical pathways to apoptosis were distinguished, termed extrinsic and intrinsic. The extrinsic pathway responds to extracellular triggers such as tumor necrosis factor • and Fas ligand, which acts at so-called death-ligand receptors to activate caspase-8. The intrinsic pathway is mediated by destabilization of the mitochondrial membrane to release cytochrome c, which acts with caspase-9 to form the apoptosome. The pathways converge at the effector caspase-3. Both caspase-8 and the apoptosome cleave procaspase-3 to active caspase-3.

DNA damage is one trigger of the intrinsic pathway, with the tumor suppressor p53 mediating the signal between DNA damage and mitochondrial destabilization. The Bcl-2 proteins are a family of proteins that act at the mitochondrial membrane to either stabilize (anti-apoptotic, e.g. Bcl-2, Bcl-X_L) or destabilize (pro-apoptotic, e.g., Bax, Bak) the mitochondrial membrane. A key determinant of apoptosis is the balance in expression between pro- and anti-apoptotic Bcl-2 family proteins. Caspase-8 can cleave the Bcl-like protein Bid to a truncated form, tBid, which is pro-apoptotic, thus providing cross-talk between the extrinsic and mitochondrial-mediated (intrinsic) pathways.

Necrosis

Necrosis itself spans a range from disorganized destruction of the cell to a more highly organized or programmed necrosis. While it may arise as a result of a metabolic catastrophe, it may also progress through steps that involve signaling pathways, the active suppression of caspase activity and other apoptotic signals, and even require ATP. In 1999 Lemasters introduced the term “necrapoptosis” to refer to a state bridged between necrosis and apoptosis. This was an important recognition that cell death could not be described adequately by two discrete terms. It also placed an important focus on bioenergetics in cell death and survival, as explained further below.

Early in the Third Millennium, we began to view cell death as a spectrum of mechanisms from apoptosis to necrosis. It was recognized that some cells that looked as if they

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were dying in apoptosis were not activating caspases, and thus the term caspase-independent apoptosis was introduced. It is still not precisely clear to what mechanism(s) this refers. One approach entertained for a short time was to refer to cell death as apoptosis, apoptosis-like, necrosis-like, or necrosis. The term late apoptosis has been used, implying, perhaps, a bridging state between late apoptosis-like and early necrosis-like death, but these terms lack mechanistic meaning.

Necrapoptosis

Necrapoptosis was considered to represent a state where the cell was poised between states of adequate energy and energy depletion. Its historical significance lies in this recognition of the role of bioenergetics in determining the pattern of cell death. However, we can now speak of ATP-independent apoptosis and ATP-dependent necrosis. Thus, while many processes of classical apoptosis (caspase cleavage, apoptosome formation, various translocation events) are ATP-dependent, ATP depletion can also trigger apoptosis. On the other hand, circumstances have been described where necrotic death cannot proceed without ATP hydrolysis to maintain expression of certain ion channels to allow transmembrane ion fluxes. It is also now established that ATP depletion, leading to increased ADP/ATP ratios, and ultimately increased concentration of AMP, activates AMP kinase. AMP kinase inhibits the target-of-rapamycin (TOR) protein, thus favouring death by autophagy. These bioenergetic events are not clearly understood at present.

Autophagy

Before we come to a contemporary, though surely-to-be short-lived classification of cell death, we should also consider the concept of autophagy. Upon nutrient deprivation, cells have the capacity to sequester their own contents in vacuoles, digest the contents, and survive for a limited time by adaptive autodigestion to maintain ATP levels. Of course this can also become a mechanism of cell death. Interestingly, pan-caspase inhibition alone is sufficient to divert some cells to autophagy, suggesting a role of caspases in survival, and inhibition of autophagy (e.g., by disruption of Atg genes) can initiate apoptosis. Autophagy is now known to be under the control of at least 31 genes, referred to as Atg1-Atg31.

Classification of cell death mechanisms

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One fairly satisfactory contemporary classification of cell death mechanisms derives from terminology related to neuronal cell death, and considers five distinct alternatives on a biochemical basis.

1. Apoptosis. Type I cell death or nuclear cell death. Morphological hallmarks of apoptosis, with or without caspase activation. This is subdivided into caspase-dependent and caspase-independent apoptosis.

2. Autophagy. Type II cell death. Non-apoptotic and characterized by autophagic vacuoles, ultimately fusing with lysosomes to degrade vacuolar contents.

3. Programmed necrosis. Type III programmed cell death. Also called parapoptosis, or cytosolic death. Vacuolization, but no lysosomal involvement.

4. Non-apoptotic, non caspase-dependent death with nuclear shrinkage, dependent upon PARP (poly-ADP ribose polymerase) activation, PAR polymer accumulation, and AIF (apoptosis-inducing factor) release from mitochondria. It has been called Parthanatos, and may be neuronal-specific.

5. A distinct Ca^{2+}-dependent mode of cell death - programmed, in that it is mediated by regulated activation of cathepsins and calpains, and is suppressed by calreticulin.

One of the most interesting challenges posed by this classification is to discern a mechanism for caspase-independent apoptosis that is distinctly apoptotic, yet also distinct from the other four mechanisms. Details of mechanisms 4 and 5 will be fascinating to learn.

3. BIOACCESSIBILITY AND BIOAVAILABILITY

IUPAC definitions

bioaccessibility

Potential for a substance to come in contact with a living organism and then interact with it. This may lead to absorption.

Note: A substance trapped inside an insoluble particle is not bioaccessible although substances on the surface of the same particle are accessible and may also be bioavailable. Bioaccessibility, like bioavailability, is a function of both chemical speciation and biological properties. Even surface-bound substances may not be accessible to organisms which require the substances to be in solution.

bioavailability (general)

biological availability

physiological availability

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Extent of absorption of a substance by a living organism compared to a standard system.

bioavailability (in toxico- or pharmacokinetics)

Ratio of the systemic exposure from extravascular (ev) exposure to that following intravenous (iv) exposure as described by the equation:

$F = A_{ev} D_{iv} / B_{iv} D_{ev}$

where F (fraction of dose absorbed) is a measure of the bioavailability, A and B are the areas under the (plasma) concentration time curve following extravascular and intravenous administration respectively, and D_{ev} and D_{iv} are the administered extravascular and intravenous doses.

Explanatory comment (see also 'Ecotoxicology')

Substances are biologically available if they can be taken up by living cells and organisms and can interact with 'target' molecules, including those on the cell surface. Thus, in the strictest sense of the term, it describes availability at the ultimate receptors. Measurement of the amount reaching the receptors is usually not possible. Hence, surrogate measurements are normally used. For humans, these may be levels found in blood or plasma. For plants, tissue concentrations may be used. For unicells such as protozoa and bacteria, the cell content may be appropriate.

Substances that are not bioavailable may still cause physical damage or may alter the bioavailability of other substances. Bioavailability of any element depends upon its chemical speciation (see Section 17 - Speciation), but for many elements the determinants are poorly understood. Whereas bioavailability of many small organic molecules (i.e., carbon species) can often be predicted or explained on the basis of their physicochemical properties, the bioavailability of chemical species of other elements is, in general, difficult to predict.

Before bioavailability becomes relevant, substances must be accessible to the living organism at risk. An extreme case would be where a substance occurs in a part of the globe where life is impossible. In such circumstances, the substance is not accessible to any life form. The cases which require careful consideration are those where living organisms occur, but where essential nutrients or toxicants are locked into physical or chemical compartments which inhibit or prevent contact with the living organisms. In such cases, bioaccessibility becomes the limiting factor that determines the possibility of exposure and resultant adequate nutrition or potential toxicity. This is particularly true in relation to substances contained in soils, sediments and other particulate matter. For particles, bioaccessibility requires consideration of the physical nature of the particle, of the distribution of substances of concern, and particularly

of whether they occur on the surface of the particles or not. Wherever a substance occurs in a particle, the chemical speciation of both the substance of concern and of the matrix and surface of the particle influences both accessibility and availability of the substance (see below). Figure 3 illustrates these considerations in the context of a unicellular organism living in an aqueous medium. More complex diagrams may be developed from this for multicellular organisms and other environments. Environmental factors may restrict or facilitate accessibility, as may many aspects of the biology of the organism, from behaviour, through physiology to fundamental molecular properties of receptors.

Determinants of bioaccessibility and bioavailability

Metallic elements in non-ionic and uncombined form are mostly not bioaccessible or bioavailable. Mercury vapor is a notable exception. Mercury vaporizes under normal environmental conditions and dissolves in cell membranes because the vapour is lipid-soluble. In the case of mercury, the cationic forms, mercuric and mercurous ions, are much less able to pass into or through biological membranes. On the other hand, mercuric chloride exists in seawater in an unionized lipid soluble form which can be absorbed readily by living organisms. Further, mercuric chloride can be converted by various organisms to methylmercury chloride which is readily absorbed by living organisms because of its lipid solubility and which is sufficiently stable to bioaccumulate and to biomagnify in food webs.

In general, metallic elements that form hydrated ions readily upon solution in water, are accessible to living cells through this medium. This reflects their solubility products. Elements that form insoluble precipitates in water may not be bioaccessible except to organisms which can solubilise them after phagocytosis or some similar activity. Apart from this, in general, the hydrolysis of metallic elements in water in the presence of dioxygen determine the bioavailability of the elements. For example, iron is found in natural aerobic waters in very small amounts because the redox potential restricts it to the Fe^{3+} state that precipitates as insoluble ferric hydroxide. Thus, iron cannot be found in natural aerobic waters as a soluble divalent cation, e.g., Fe^{2+}, or as a soluble anion such as FeO_4^{2-}. Hydrated ions may be bioaccessible but may not be bioavailable if there is no mechanism for their uptake. Absence of an uptake mechanism may reflect the size of the hydrated ion. Thus, a knowledge of relevant chemical speciation and the uptake mechanisms applicable to the organisms at risk is essential for any risk assessment of exposure to metallic elements. It should also be noted that metallic elements are not always bioavailable as hydrated cations. For example, chromium is

bioavailable largely as the anion chromate. Unfortunately, the bioavailable form is often given in the scientific literature as Cr^{6+} and this has led to some confusion.

A substance may be complexed by inorganic and organic ligands, or adsorbed onto or bound within particles. The bioavailability of complexed ions varies with the nature of the complex. For example, aluminum complexed with citrate is more easily absorbed from solution than aluminum complexed with hydroxide, and is therefore more bioavailable. The absorption and bioavailability of many divalent ions is reduced by complexation with phytic acid but may be enhanced by complexation with some chelating agents such as ethylene diamine tetra-acetic acid (EDTA). Often aqueous complexes exist in an equilibrium state that may fluctuate considerably with environmental conditions. Such conditions may change quite rapidly, sometimes with dramatic consequences for the affected ecosystem.

In addition to being affected by complexation, metallic elements and ions derived from them can cycle between oxidation states of varying bioavailability, e.g., ferrous ions are generally more bioavailable than ferric ions. Complexation and redox cycling are often associated with large differences in reactivity, kinetic lability, solubility, and volatility because of resultant changes in chemical speciation. Thus, timing of exposure in relation to environmental conditions is an important factor to consider. In particular, chemical changes resulting from the contrasting effects of photosynthesis in the daytime (oxygen release, carbon dioxide fixation, increase in pH) and respiration at night (carbon dioxide release, oxygen uptake, decrease in pH) can cause major differences in water chemistry and hence in bioaccessibility and availability of both nutrients and toxicants.

In the simplest consideration of bioavailability, uptake into cells may be driven by diffusion or another form of electrochemical gradient. The concentration-of-substance or the charge that drive uptake through the plasma membrane will each be those in the vicinity of the membrane. Both may be dependent upon biotransformation and (or) localization within cells or cell compartments. Uptake is also affected by components of the exposure medium, such as the presence of similar chemical species that may compete for uptake sites. Bioavailability of an organic compound is often determined by its presence in solution in a surrounding aqueous medium and is dependent upon its hydrophobicity (lipophilicity). For ionizable organics, hydrophobicity is dependent on pH. For example, organic acids become unionized, and thus more hydrophobic, at acid pH. It should be noted that dependence of total uptake simply on the aqueous concentration of the dissolved organic molecules of concern ceases to apply if the concentration of dissolved organics is kept constant by continuing dissolution from a large fraction sorbed to particulate matter. In such circumstances, uptake is continous, perhaps at a

slow rate, and large amounts of such organics may accumulate in exposed organisms with passage of time. Thus, time becomes the main determinant of total uptake.

Whatever the biologically available form of a substance in solution, it may be derived from other chemical species, and the thermodynamic equilibrium for production of an available solute or the rate of its release from a bound form may be the limiting factor for its uptake by living cells, i.e., transfer from the external medium to a biological receptor on or in the cell. Knowing which chemical species determine the rate and amount of uptake by living organisms of a substance of concern is essential for risk assessment. Thus, in order to determine the relevant chemical species for risk assessment of a substance, three questions must be answered:

1. What is the mechanism of uptake of each chemical species of the substance?
2. Which chemical species determine(s) the rate of uptake and excretion of the substance by the cell?
3. How do chemical species interact in the uptake and excretion processes? Interactions may occur outside the organism, where species may interact directly or compete for transport sites, or inside, where they may compete for binding sites that regulate transport systems.

Bioaccessibility and bioavailability in relation to particulate matter

Any substance must come in contact with a living organism before it can interact with it and be absorbed. Thus, a substance trapped inside an insoluble particle is not bioaccessible although substances on the surface of the same particle may be bioaccessible. Bioaccessible substances are not necessarily bioavailable. Substances on the surface of particles may be accessible to some organisms following phagocytosis but may not be accessible to organisms which require the substances to be in aqueous solution or which lack appropriate surface receptors. Thus, bioaccessibility, like bioavailability, is a function of both chemical speciation and biological properties.

In some cases, bioaccessibility will be the limiting factor which determines whether a substance is or is not toxic. This is particularly important in relation to substances in soils, sediments, aerosols and other particulate matter to which humans may be exposed.

Bioaccessibility and bioavailability through a food chain

Organisms can be placed in a chain of dependence, known as a food chain, with several different trophic levels (levels at which organisms feed). Such a chain (Fig. 4) starts with plants or other primary producers absorbing light, carbon and other nutrients, and passing nutrients and organic molecules with their inherent chemical energy to higher trophic levels of

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herbivores and carnivores. Each trophic level produces waste material as excretory products and dead matter, and carbon dioxide from respiration. Decomposer organisms such as bacteria and fungi break down the waste products, releasing nutrients back to the environment where they are available for re-use. Thus, nutrients cycle between organisms and the environment. This is part of the general biogeochemical cycle that applies to all the elements used by living organisms. Integrity of biogeochemical cycles must be maintained in order to ensure continuing biological productivity.

Food chain bioaccessibility and bioavailability varies between elements depending upon the environmental chemical species, the chemical species produced by the organisms involved, how they are stored, and the sinks for different chemical species in the environment. Thus, mercuric sulfate, which is water-insoluble, tends to stay wherever it enters the environment. On the other hand, methylmercury chloride, which is chemically stable and lipid soluble, is readily taken up by living organisms and passes from prey to predator until the ultimate predators in a food chain accumulate it to high levels, a process known as biomagnification. It is usually assumed that proportions of chemical species remain roughly constant in different compartments but this may not be true, especially in the aquatic environment. The aquatic environment is affected by changes from photosynthesis to respiration from day to night, with resultant changes in carbonates and pH. It is also affected by intermittent pollution incidents, or, in estuaries, by tidal flow, with resultant fluctuations in salinity and sediment disturbance.

Some organisms accumulate certain elements and compounds from the environment (bioconcentration, e.g., metals in plant tissues), and from their prey (bioaccumulation, e.g., dioxins in predatory organisms), causing them to have very high body loads relative to outside concentrations. Because of losses of organic matter owing to respiration, each successive trophic level in a predator-prey relationship usually has a lower biomass, defined as the mass of living material in a given area at one time, or a lower productivity than the levels below it. The body concentration of bioaccumulated substances passed up the food chain can therefore increase through the process of biomagnification, sometimes resulting in toxic doses to organisms higher up the chain.

All ecosystems have two types of food chain – the grazing food chain based directly on plant photosynthesis within the system, and the detritus food chain based on consumption of organic detritus by detritivores (organisms feeding on detritus) that are eaten by carnivores. Normally the detritus chain is based on the waste products of the system's own resident

organisms. An estuarine ecosystem differs from most others in its great reliance on imported waste from other systems.

Bioaccessibility and bioavailability through a food web

A food web is a more holistic concept than a food chain. A simplified soil food web is given in Figure 5. Even with such a simple web, there can be a complex pattern of flow of energy, carbon, nutrients and toxicants based on the feeding preferences of different species, as indicated by the lines on the diagram. These feeding preferences determine bioaccessibility of toxicants that may be found in only a few species. In such cases, bioaccessibility depends on the feeding preferences of predators on these species. For any given habitat there is a degree of stability such that the same assemblages of species are present in the food web in successive years, with the same dominant and rare species. Similarly, the same flow pathways remain important while others are less significant.

Bioavailability, routes of exposure, and absorption in humans

Bioavailability to humans varies with routes of exposure. This is discussed under the heading 'Absorption' in part I of this explanatory dictionary [1].

4. BIOLOGICAL MONITORING (BIOMONITORING)

IUPAC definition

biological monitoring

biological assessment of exposure

biomonitoring

Continuous or repeated measurement of any naturally occurring or synthetic chemical, including potentially toxic substances or their metabolites or biochemical effects in tissues, secreta, excreta, expired air or any combination of these in order to evaluate occupational or environmental exposure and health risk by comparison with appropriate reference values based on knowledge of the probable relationship between ambient exposure and resultant adverse health effects.

Explanatory comment

Because of the uncertainties involved in estimating environmental exposures, i.e., external exposure through an environmental medium and the resultant internal dose, there is an

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increasing trend towards assessing exposures of living organisms to chemicals in their environment by biomonitoring. This essentially refers to the analytical process of measuring the concentration of substances or their metabolites in blood, urine, breast milk, hair, and other biological samples taken from exposed organisms. Biomonitoring should, in theory, be a better measure of exposure than analysis of environmental media since it relates to the internal dose produced by external exposure of the organism being studied. However, this relationship is affected by many environmental and physiological factors. Interpreting biomonitoring data is, therefore, often difficult unless there is clear evidence that such factors can be ignored.

Conventional environmental exposure scenarios often use "worst case" assumptions. They have been designed to provide estimates of maximum possible external exposure which can be allowed while still protecting the organisms at risk from harm. Thus, such assessments are likely to overestimate actual exposures. Biomonitoring provides values that are a direct measure of the dose resulting from an individual's integrated exposures from multiple pathways and sources. However, biomonitoring cannot identify specific sources or pathways of exposure or the relative contributions from multiple sources.

Objectives of biomonitoring

Biomonitoring studies are used for the following purposes:

(a) to determine which chemicals are taken up by living organisms and at what concentrations;

(b) to determine the prevalence of organisms with exposures likely to cause toxicity;

(c) to establish reference concentrations;

(d) to assess the effectiveness of attempts to reduce exposure;

(e) to compare exposure levels in different groups;

(e) to track trends in exposure over time; and

(f) to set priorities for research.

Achieving these objectives is facilitated by the maintenance of specimen banks under appropriate conditions to prevent deterioration of samples. Specimens must be well documented and defined precisely in relation to their origin and to all factors that might affect interpretation of relevant data. This will permit re-investigation of specimens as analytical techniques improve and new substances of concern are identified.

Biomonitoring data may be used to determine whether individuals or a population are at an increased risk of experiencing adverse health effects associated with an exposure to a specific substance. Criteria for the evaluation of biomonitoring data in this context have mostly

been developed for exposures in the workplace. In the United States, the American Conference of Governmental Industrial Hygienists (ACGIH) [7] began developing biomonitoring-based reference values known as biological exposure indices (BEIs) in the early 1980s. The ACGIH defines the BEIs as representing the levels of determinants that are most likely to be observed in specimens collected from a healthy worker exposed to chemicals following inhalation exposure at the Threshold Limit Value (TLV). The Deutsche Forschungsgemeinschaft (DFG) in Germany [8] has also developed biological monitoring reference values called biological tolerance values (BATs), and the World Health Organization [9] has established similar values which they call biomonitoring action levels (BALs). BEIs, BATs and BALs all refer specifically to occupationally exposed populations and exposures in occupational scenarios only.

For the general population, health-based screening levels for biomonitoring data exist for very few substances (exceptions being lead, mercury, arsenic, cadmium, and ethanol). At present, almost all regulatory health-based toxicity screening criteria are based on an estimated intake level (mg/kg body mass/day) or a concentration in an environmental medium (air, water, soil, etc.) that corresponds to what is regarded by an expert group as an acceptable level of intake.

Analytical aspects of biomonitoring

The first paper on biomonitoring was published in 1927 [10], and presented the analysis of lead in the urine of exposed workers as a means of diagnosing lead-induced occupational disease. A well-designed sampling program and accurate chemical analysis are essential for correct interpretation of the analytical results obtained. Although analytical techniques have developed with time, analytical quality is a major concern for all biological monitoring programs, and trustworthy external quality control must be established before any program starts. Analyses should be performed by an accredited laboratory.

Biomonitoring of occupational exposure to chemical substances differs from normal clinical chemistry analyses in the dependence of the measured concentration of the chemical on toxicokinetics and exposure patterns. At work, the exposure is generally assumed to occur for a workshift period of 8 hours daily and may be limited to only short periods of time within the working hours. These assumptions vary with different regulatory authorities and must be known for biomonitoring data to be interpreted properly. Some chemicals or their metabolites have a very short half-life in the body, especially in blood, and thus the concentration drops very rapidly immediately after the exposure. Thus, the concentration measured may reflect the

time lapse between exposure and sample collection more than it reflects the original maximum concentration following exposure. This may lead to a distorted assessment of the exposure – and thus of the risk involved. The conclusion could even be that there was no exposure although exposure may have occurred, but, by the time the sample was collected, all of the substance of concern had disappeared from the blood.

Use of biomarkers in biomonitoring

Biomarkers are discussed in Part I of this explanatory dictionary [1]. Biomarkers of exposure may be used to identify exposed individuals or groups. This depends on comparing the results with established reference levels, or with biomonitoring action limits, if these have been defined. A biomarker level that occurs at a concentration above a reference level indicates that the individual monitored has been exposed to a greater extent than the reference population but is not of itself a measure of illhealth. However, it is a warning of a possible health hazard that requires further investigation. Biomarkers of exposure take no account of inter (or intra)-individual differences in the toxicodynamics of the chemical. Such differences may be identified by biomarkers of effect, bearing in mind that specificity of the relationship of the effect to the exposure of concern must be clearly established.

Biomarkers do not differentiate between sources of exposure and in order to decrease the risk from the chemical that is monitored, it may be necessary to consider (and to analyze) separately, whether the exposure occurred at work or at home. Biomarkers of exposure in humans usually reflect the amount of the chemical in the systemic circulation, and models have been developed to predict concentrations in other compartments in the body from such data. However, a major difficulty in the interpretation of biomonitoring data from humans relates to the concentration and effects of substances at the site of entry, e.g., effects on the lungs after exposure to particulates containing metallic elements such as nickel. Concentrations of nickel ions in the urine or the blood may reflect the concentrations in, or the health risks to, the lungs after exposure to such particles, but only inadequately since the nickel compounds most associated with lung cancers are largely insoluble. Nor do they indicate the exact chemical speciation of nickel in the lungs, which must be known to assess the risk of cancer developing since the risk varies hugely with the nickel species, nickel salts posing little if any risk, dependant, of course, on exposure concentration.

Biomarkers of effect have the intrinsic advantages over biomarkers of exposure in that they reflect differences in individual sensitivity to the chemical. Thus, e.g., in exposure to cadmium, assessment of excretion of low molecular mass proteins in urine may be used to

identify individuals who are exceptionally sensitive, and who develop adverse health effects at levels of exposure at which individuals with normal sensitivity remain healthy. However, cadmium-related renal toxicity is a well established example where such an advantage can be achieved. For cadmium-related renal toxicity, an increasing number of proteins and enzymes and expression of mRNA for a few proteins, e.g., metallothionein, have been identified to serve as biomarkers of effect and also to identify vulnerable groups in the population.

In regard to biomarkers of effect, the availability of highly sensitive analytical methods has provided insight into the formation, persistence, and repair of DNA adducts induced by a large number of chemicals. By understanding the molecular dose of such adducts in different cells and tissues, we have learned the metabolism and mode of action of a range of substances across species. In addition to DNA adducts, similar information has been obtained from studies of protein adducts and related chemical metabolites in urine and plasma. For example, much research has been conducted on the molecular dosimetry of aflatoxin in rats and humans, with measurements of DNA adducts, protein adducts, and urinary excretion of both adducts and metabolites. The earlier literature was reviewed by Busby and Wogan [11]. Aflatoxin B1 (AFB1) forms adducts at the N-7 position of guanine. These adducts can depurinate and be excreted in the urine, but they also form the ring-opened FAPY adduct, which is persistent and mutagenic [12] and therefore carcinogenic.

Chemical speciation in biomonitoring

If we ignore carbon and its derivatives, routine biomonitoring of all other elements has in the past been almost entirely dependent on the analysis of the total content of the element as a biomarker, without any consideration of the different chemical species in which the element may be present. For some elements, this straightforward approach may be sufficient. It is probably applicable where the key effect of the element on health is caused by its most common ionic form in aqueous media and the dose-response relationship between the total element concentration and any beneficial or adverse health effect is known. Thus, a reliable prediction of long-term health effects may generally be made from total lead in blood or total cadmium in blood or urine. However, if we are dealing with exposure to tetraethyl lead in air or to methylmercury in the diet, total elemental analysis will be seriously misleading for any health risk assessment. Any error will be compounded if there is concomitant exposure to different chemical species of the same element by different routes and under different circumstances, e.g., exposure to inorganic arsenic species in the workplace and organic forms in the diet.

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Toxicity is dependent on chemical speciation as much as on the organism at risk. Thus, chemical speciation analysis is essential for biomonitoring, whether of human beings or any other biological species. Chemical speciation analysis requires the development of three different approaches:

1) fractionation analysis (e.g., to separate organic and inorganic forms of arsenic);

2) speciation analysis, to precisely define individual chemical species;

3) analysis of the distribution of different species in tissues and organs (e.g., mercury in plasma, blood cells, and urine; chromate in erythrocytes and plasma).

If only the total concentration of an element is measured, exposure indices used for comparisons to assess health risks should relate this to defined chemical species to which the population has been exposed, and which have been characterized for their dose-response relationships to the health effect or effects of concern. At present such exposure indices are difficult to find and they will need to be developed for most common elements.

Biomonitoring equivalents

Most existing chemical risk assessment procedures rely on external exposure measurements to set exposure guidance values such as reference doses or tolerable daily intakes. However, based on metabolism and kinetics of the substance concerned, attempts have been made to establish what have been called biological monitoring guidance values referring to internal concentrations in humans, deduced for blood or urine samples. For example, human Health Guidance Values has been defined by the U.K. Health and Safety Executive (HSE) as levels of a substance or its metabolites in blood, or urine that is not associated with any adverse health effects [13]. Another type of biological monitoring guidance value defined by HSE is called a 'Benchmark Value'. This type of value is set when it would not be appropriate to set a Health Guidance Value - for example for substances that can cause cancer. The Benchmark Value is based on a survey of workplaces that are considered to have good control of exposure to the substance and it is the value found in 9 out of 10 samples in those workplaces. This type of guidance value can give no direct guide to the risk of ill-health. The benchmark guidance value just gives an indication of how well exposure is being controlled, and should be used as a trigger for further investigation. A similar approach has been adopted by the American Conference of Governmental Industrial Hygienists (ACGIH), which has established a series of recommended reference values for biomonitoring called the Biological Exposure Indices (BEI) [7]. BEI are defined as reference values intended as guidelines for the evaluation of potential health hazards in the practice of industrial hygiene. BEIs represent the levels of determinants

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that are most likely to be observed in specimens collected from a healthy worker who has been exposed to chemicals to the same extent as a worker with inhalation exposure to the TLV.

At present, only a few reference values are available to interpret the increasing amount of available human biomonitoring data. To solve this problem, the basic concept of Biomonitoring Equivalents (BEs) for the general population, i.e., biomonitored blood or urine concentrations of chemicals corresponding to existing exposure guidance values, was introduced by Hays et al. [14], integrating toxicokinetic data with existing chemical risk assessments to provide such reference values.

Some of the methods that can be used to develop BEs and reviewed by Hays et al. [15] are as follows:

1. Extrapolation from occupationally derived biomarker levels such as the Biological Exposure Indices (BEIs) set by the ACGIH [7];
2. Human PK studies and one-compartment steady-state models;
3. Multi-compartment and PBPK models;
4. Animal PK studies.

In general, the methods focus on blood as the medium of interest, although similar approaches can be used to convert to BEs for use with other sampled media. Interpretation of data for each medium requires consideration of issues specific to that medium. For example, urine is frequently sampled for metabolites from exposures to a wide range of drugs and chemicals, and appropriate methods for standardization of urinary output volumes must be considered.

A Biomonitoring Equivalents Expert Workshop was held in 2007 and this resulted in the production of detailed guidelines for the derivation of Biomonitoring Equivalents which were published in 2008 [14].

Ethical questions related to biomonitoring

The following questions should be considered in relation to any proposed biomonitoring project.

(a) Is biological monitoring justified by the predicted outcome(s)?

(b) Can the monitoring procedure cause any harm to participants?

(c) Have participants been fully and appropriately informed about the aim and methods of the biomonitoring program and the proposed use of any information gathered during the study?

(d) If the data indicate potential health problems for the participants in future, how should they be informed?

(e) Have the participants given informed assent or consent and been given the right to withdraw subsequently if they have any doubts?

(f) Are there possible health implications for any group to which the participant belongs?

(g) Are there systems in place for communication of results to participants?

(h) Will participants have access to their own data and have they been informed about relevant data protection including who has the right to know or not to know?

(i) If the data have commercial value, who will benefit from this?

Conclusions

Further development of biomonitoring will depend upon the following factors:

1) further development of inexpensive monitoring methods that make possible adequate biomonitoring of substances with a short half-life in the body;

2) derivation of exposure biomarker guidance values for defined elemental species which are serious risk factors;

3) identification of fully validated biomarkers of effect;

4) validation and application to effect-monitoring of the -omic technologies (See Section 9 - Genomics, Proteomics and Related Terms);

5) validation of the appropriateness of media and markers to be measured in proposed biomonitoring studies;

6) insistence on quality control at all stages of biomonitoring, especially relating to possible contamination of samples during collection;

7) improved definition of the stability (or lack thereof) of within-subject biomarkers to relevant exposures over time (especially in relation to the often unstated assumption that one measurement adequately reflects likely exposure during a critical period);

8) improved definition of between-subject variability in biomarkers and body burden after apparently similar exposures;

9) improved understanding of differences in the toxicokinetics of specific subgroups (e.g., relating to age and sex);

10) continuing development of the knowledge base relating to species differences in toxicokinetics and toxicodynamics.

5. CARCINOGENICITY

IUPAC definition

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carcinogenicity

Ability of an agent to induce malignant neoplasms, and thus cancer.

Amended from [4]

Explanatory comment

Cancer is a general term for a group of diseases characterized by the presence of dedifferentiated cells with a tendency to uncontrolled growth and invasion of other tissues. Uncontrolled growth of cells produces a swelling or a lump, usually referred to as a tumor. Tumors which grow slowly, are non-invasive, and are encapsulated, are said to be benign, while tumors releasing invasive cells are said to be malignant.

Tumors may be described as "neoplasms". Neoplasms are the result of neoplasia, new cell division leading to abnormal arrangement of cells that may result in the production of either benign or malignant tumors. However, some neoplasias do not result in tumors, e.g., neoplasias of cells in the blood system. Neoplasia must not be confused with hyperplasia, excessive multiplication of normal cells in the normal tissue arrangement.

Carcinogens, agents that cause cancer, may be chemical, physical, or biological. Some of them are organ-selective in their action, e.g., 1,2-dimethylhydrazine has been associated particularly with colon cancer in rats. Chemical agents may be so-called ultimate carcinogens, active without metabolism, or procarcinogens (proximate carcinogens), which must be metabolized into carcinogenic derivatives. Physical agents include ionizing radiation (which may be produced by the decay of radionuclides) and UV light. Biological agents include some types of bacteria and viruses. In general, anything that can cause chronic irritation and inflammation has the potential to be carcinogenic. Such agents include insoluble particulates. This property may not be absolutely dependent on the chemical nature of the particulates but may be, at least partly, a result of the physicochemical properties of surfaces. This may explain why chemically dissimilar particulates such as asbestos and crystalline silica can cause lung cancer.

Carcinogens as toxicants

Carcinogenicity is not related in any simple way to acute toxicity. Seldom does a single exposure to a potential carcinogen, even in a very large dose, cause cancer. Chronic exposure is usually required. Removal from exposure may permit recovery, e.g., repair of damage to DNA that could otherwise lead to cancer.

Mechanism of carcinogenesis

Carcinogenesis is a multistage process, involving a minimum of three stages: initiation, promotion, and malignant conversion as seen in Figure 6. Initiation is the first stage in which a single somatic cell undergoes non-lethal, but heritable mutation. Unlike its neighbors, the initiated cell can escape cell regulatory mechanisms restricting cell division.

Promotion, the second stage, occurs when the initiated cell is exposed to a tumor promoter, a substance that causes initiated cells to undergo clonal expansion. The signal to expand clonally may be the result of a direct effect of the tumor promoter on the initiated cell or of an indirect effect on the adjacent cells altering their interaction with the initiated cell. Tumor initiation and promotion together produce a relatively benign clonal expansion. An example of a promoter is the drug phenobarbital that promotes liver tumors.

Malignant conversion is the third stage of carcinogenesis. This process is generally slow, occurs over a long time, and is affected greatly by agents that alter growth rates (e.g., hormones, growth factors, vitamin A and the retinoids, vitamin D, folate, and calcium). It is thought to be caused by repeated rounds of division and further chromosome damage,in addition to that responsible for initiation. Progression to malignancy is probably the most complex of the three stages. Both acquired genetic and phenotypic changes occur, and cell division becomes rapid. Retinoids affect this stage, as do other inhibitors of growth (e.g., polyamine synthesis inhibitors) and antioxidants. As the tumor progresses, sensitivity to dietary compounds, inhibitors of growth, and enhancers of differentiation gradually disappears until the tumor becomes autonomous and controllable only by drastic intervention.

Genotoxic and non-genotoxic (epigenetic) carcinogens

Genotoxic carcinogens are agents which either directly or indirectly through their metabolites act directly on the DNA of cells. Genetic change (mutation) results either from chemical modification of the nucleotide sequence, from the introduction of errors when the DNA is transcribed (“read”), or from chromosome breakage during mitosis. There is postulated to be no threshold dose for genotoxic effects. In other words, it is assumed that even one molecule of a genotoxic carcinogen may react with DNA to cause a mutation which may lead to cancer.

Non-genotoxic (epigenetic) carcinogens do not modify the DNA nucleotide sequence causing mutations but act by other mechanisms, e.g., switching on cell division, enzyme stimulation or inhibition (which may modify DNA by adding to it, e.g., methyl groups), immuno-modulation, hormone balance changes, or a combination of these effects. (See Section 8 for a discussion of Epigenetics). Subsequent to these changes, mutations may occur,

reinforcing the malignant properties of resultant tumor cells. A threshold has been demonstrated for such nongenotoxic epigenetic effects. Ultimately, epigenetic mechanisms lead to the formation of malignant cells that contain mutations. However, the initial events in the process are not caused by mutation and this is why a threshold is observed. Epigenetic agents operate largely as promoters of cancer and usually require high and sustained exposures. For example, dietary fat can act as an enhancer of cancer induction at about 40% of total calories available, probably by enhancing availability of fat-soluble primary carcinogens. The effects of epigenetic agents, unlike those of genotoxic agents, are generally reversible. Accordingly, distinction between these two types of carcinogens is critical for informed risk assessment.

An important example of an epigenetic mechanism of carcinogenicity that has had consequences for carcinogenicity classification is peroxisome proliferation, recently reviewed by Melnick, Thayer, and Bucher [16]. Peroxisomes are subcellular structures that contain several oxidase enzymes. Agents that cause increases in their numbers are called peroxisome proliferators. Many peroxisome proliferators are rodent carcinogens, and the mode of action proposed for rodent liver tumor induction by peroxisome proliferators involves activation of the peroxisome proliferator-activated receptor (PPARα), which results in altered transcription rates of genes that regulate cell proliferation and apoptosis. However, this hypothesis has not been tested with experimental studies demonstrating consistent increases in liver tumor incidence as a direct function of the time-dependent induction of cell proliferation and suppression of apoptosis in rats and mice treated with peroxisome proliferators. A further problem with the hypothesis is that increases in cell proliferation are generally only a transient response that returns to control levels within about 2-4 weeks after initiation of continuous exposure, whereas tumor induction requires chronic exposure for most peroxisome proliferators. Peroxisome proliferation *per se* does not appear to be a causal event in liver carcinogenesis.

In spite of the above observations, there has been a general acceptance of the hypothesis that di(2-ethylhexyl)phthalate (DEHP) induces liver tumors in rats and mice by a non-DNA-reactive mechanism involving peroxisome proliferation. This mechanism is considered not to be relevant to humans because peroxisome proliferation has not been documented either in human hepatocyte cultures exposed to DEHP or in the liver of exposed nonhuman primates. Consequently, IARC downgraded the classification of di(2-ethylhexyl)phthalate (DEHP) from “possibly” to “not classifiable as to its carcinogenicity to humans” [17] (IARC 2000). Altered expression of cell growth and apoptosis genes by DEHP

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has not been demonstrated to be dependent on PPARα activation. In fact, the mechanism of tumor induction by DEHP is not known.

Available mechanistic data do not support the hypothesis that liver tumor induction in rats and mice occurs by a mechanism involving peroxisome proliferation that is not relevant to humans. A recent study has shown that dietary administration of DEHP induces liver tumors in mice lacking a functional PPARα gene [18]. This finding emphasizes the need to test mechanistic hypotheses that, if relied on, might lead to erroneous cancer risk classifications and inadequately protective public health decisions.

Carcinogenicity testing

The operational definition of a carcinogen is an agent having the ability to cause:

a) an increased incidence of tumors over the incidence observed in controls;

b) an occurrence of tumors earlier than seen in controls;

c) the development of tumor types different from those normally seen; and

d) an increased multiplicity of tumors in individual animals.

The conduct of carcinogenicity studies is very expensive in that:

(a) large numbers of animals are required;

(b) the duration should be almost the life-span of the animal (18 month for mice, 24 months for rats);

(c) animals need to be euthanized at intervals with particular attention being paid at necropsy to the morphological examination of tumors and the histological identification of cell hyperplasia, preneoplastic nodules, and whether tumors are benign or malignant.

Since carcinogenesis is a multistep process occurring over a prolonged period of time and progressing through a number of stages, only a few rodent carcinogenicity studies permit the study of mechanisms of action. The endpoint(s) of these studies is to consider one or more of the criteria set out above for a carcinogen without considering how and why the agent acts as it does.

Purely mechanistic studies can be conducted *in vivo* using special substrains of rodents having a predisposition toward developing high incidences of specific tumor types. These susceptible substrains of mice and (or) rats are already compromised genetically toward certain types of tumors (pulmonary, dermal, hepatic, breast, etc.), and chemical exposure for 90-120 days frequently causes an increased incidence and (or) earlier appearance of tumors than seen

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in control animals of the substrain (these latter having a higher incidence than seen in other strains). Most "normal" rodent strains will show a 1.0-5.0 percent incidence rate of tumors in the untreated state. The special substrains mentioned above may have a "normal" control incidence rate of 30 % that, following treatment with an appropriate carcinogen, may be as high as 60-90 %.

The International Agency for Research on Cancer (IARC)

Identifying carcinogens is essential for their regulation and for reduction of the incidence of cancer in populations at risk. This is the main activity of IARC. IARC convenes expert groups which assess the available evidence and, on the basis of this evidence, produces a classification of suspected carcinogens [19]. The evidence is subsequently published with the rationale of the classification in IARC Monographs [20].

6. ECOTOXICOLOGY

IUPAC definition

ecotoxicology

Study of the toxic effects of chemical and physical agents on all living organisms, especially on populations and communities within defined ecosystems; it includes transfer pathways of these agents and their interactions with the environment.

Explanatory comment

Toxicology originated as a science concerned mainly with the effects of toxicants on humans. Ecotoxicology was developed from toxicology in the late 20th century following the realization that pollution of the natural environment was having effects on other organisms, and that consequential effects were threatening human welfare.

Ecotoxicology can be considered at three levels. Firstly there is direct toxicity to an individual species; human toxicology is an example of this. Secondly there are toxic effects on inter-relationships between species, e.g., the effect of excess nutrients in causing eutrophication, algal blooms, release of toxins, and subsequent anoxia in affected waters. Finally, there is accumulation of toxicants by individual organisms and their movement between organisms and species through predator/prey relationships that result in biomagnification, e.g., the transfer of persistent organic pollutants like organochlorines from

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water to birds of prey, adversely affecting both their reproduction and tolerance of stress with a resultant decline in numbers. Underlying these considerations is the movement of potentially toxic substances through the natural environment and their physicochemical transformations in the different environmental media.

Ecotoxicology incorporates basic concepts of ecology, allowing the observed toxic effects to be interpreted, predicted, and prevented. Furthermore, ecotoxicology encompasses knowledge both of how organisms interact in nature with each other (the biotic environment) and of the physical and chemical aspects of the environment (the abiotic environment). In this, ecotoxicology differs from human toxicology that concentrates on effects of substances on individuals. Even when human populations are considered through epidemiology, the aim is normally to obtain knowledge of cause and effect to protect individuals at risk. In contrast, ecotoxicology is used to protect populations, species, and communities.

Ecosystems

There are many environmental conditions that must be satisfied for life on Earth to be possible but, quantitatively, there are two major requirements that all organisms have in order to sustain life. The first is a supply of carbon to form the organic molecules of which organisms are composed. The second is a supply of energy for the chemical reactions that keep the organisms alive and particularly for the biosynthetic processes that maintain their structure and function. Carbon is freely available in the environment as carbon dioxide in the air, as various inorganic forms, including carbonate and bicarbonate, and as organic carbon. With regard to organic carbon, organisms can be divided into two major groups, heterotrophs and autotrophs. Heterotrophs are organisms with a requirement for presynthesized organic molecules. Animals, fungi, and most bacteria are heterotrophs. Autotrophs are organisms that are independent of outside sources for organic food materials and manufacture their own organic material from inorganic sources. Green plants are autotrophs that use light energy to trap carbon and convert it to complex organic derivatives.

It is clear that toxicity to autotrophs must have an adverse effect on the heterotrophs that depend upon them, simply by depriving them of nutrition. On the other hand, some autotrophs, e.g., some algae, synthesize and release toxins. If such algae flourish, any heterotrophs that can eat them safely will grow in numbers while others may be eliminated by the toxins released. Hence, differential sensitivity to toxicants may result in changes in species balance that may destabilize an ecosystem. Such considerations of differential species

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sensitivity to environmental factors and of species interdependence are characteristic of ecotoxicology.

Energy and carbon

The number of autotroph species is relatively small but they are essential components of ecosystems because they make the organic compounds on which all living organisms depend. The majority of autotrophs are green plants. Fixation takes place by photosynthesis. The fundamental chemistry of this can be represented simply as follows:

$6CO_2 + 6H_2O + h\nu \bullet C_6H_{12}O_6 + 6O_2$

Photosynthesis is countered by respiration which may also be represented thus:

$C_6H_{12}O_6 + 6O_2 \bullet 6CO_2 + 6H_2O$ + chemical energy available for use in the cell

The energy released by respiration (a catabolic reaction and part of catabolism) is used for biosynthesis of components of the organism (anabolism) and for other life processes.

Only autotrophs make new organic matter while all organisms use or consume it. Fixation of carbon by autotrophs is called primary production. Subsequent incorporation of already-existing organic matter into heterotrophs is called secondary production. Thus, primary production by autotrophs must be sufficient to meet the needs of both autotrophs and heterotrophs. In a balanced ecosystem, there is a balance between production and catabolism. This means that total respiration should be balanced by photosynthesis in order to maintain the optimum carbon dioxide level in the atmosphere. It appears that, at the time of writing, this balance is no longer being maintained and that carbon dioxide is building up in the atmosphere and contributing to global warming. Cutting carbon dioxide production will help to correct this but attention must also be paid to maintaining autotrophs and their photosynthetic activity.

Other nutrients

In addition to energy and carbon, living organisms need at least 20 different elements in appropriate chemical species, either for physiological processes, biochemical reactions, or because they are components of particular compounds, e.g., nitrogen in proteins, iron in hemoglobin, magnesium in chlorophyll, etc. Plants absorb most elements, other than carbon, oxygen, and nitrogen in air, in selected chemical species from water, sediments, and soil. Appropriate chemical species of these elements are absorbed by heterotrophs in their diet. This is an important consideration in assessing bioavailability.

Some elements, e.g., nitrogen and phosphorus (principally as nitrates and phosphates, respectively), may occur in low concentrations in the environment compared with the amounts

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needed. Thus, availability of nitrogen and phosphorus may limit plant growth and primary production. If amounts of nitrate and phosphate are increased by their use as fertilizers in intensive farming, plant growth increases. Runoff of an excess of these nutrients into natural waters causes algal blooms that may release toxins. When they die, the algae are degraded by micro-organisms, often aerobically with depletion of oxygen that leads to anoxic conditions that kill fish.

Many elements may be at low environmental concentrations of bioavailable chemical species. However, this is not a problem if they are needed biologically only in small amounts. On the other hand, all elements which are essential for life may be toxic when bioavailable in relatively large quantities. Since bioavailability depends upon chemical speciation, analysis of total elemental concentration may give a false impression of both bioavailability and potential toxicity.

Environmental gradients

Any habitat has its own set of environmental conditions to which an organism must be tolerant if it is to occur there. Different species have different tolerances to physical and chemical environmental factors (abiotic factors), e.g., temperature, rainfall, or soil nutrient status. The range of abiotic factors tolerated along a gradient of such factors can be considered as the fundamental (theoretical) niche of the species. In practice, species usually occupy a narrower range of conditions than this - the realized niche. They do not occur at the extremities of the theoretical range because interactions with other organisms (biotic interactions) inhibit them. For example, a species will be best adapted to the environment near to the middle of its tolerance range. Towards the extremities of the tolerance range, the species will be under stress. It will not compete successfully there with better-adapted species, which are surviving near the middle of their tolerance ranges.

The ecosystem

An ecosystem consists of all the organisms in a particular place or habitat, their interrelationships with each other in terms of nutrient, carbon and energy flows, and in terms of biotic determinants of community composition, such as competition between species, the physical habitat, and the abiotic factors associated with it. These factors also play a role in determining community composition and in determining primary, and hence secondary production. Ecosystems can be quantified in terms of the fluxes of carbon, energy and

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nutrients, and the productivities of each trophic level. These properties can be quantitatively modeled using computers to enable predictions to be made about ecosystem performance.

The most important property of ecosystems is their 'dynamic stability' – their capacity to remain broadly the same over time in species composition and abundance, and in the magnitudes of processes, despite environmental variations. Although the climate fluctuates from year to year, the structure of an ecosystem tends to be stable within limits, and should be sustainable if the climatic fluctuations continue within their established limits. Any long period of global warming or cooling will cause continuing change until a new stable climatic situation is attained. One characteristic of dynamic stability is the maintenance over time of mean population sizes. In many insects reproduction occurs every year and the life span is one year or less. There can be fluctuations of several orders of magnitude in population size over several years but they fluctuate around a mean value. This may result from density-dependent factors, i.e., environmental factors whose intensity or effect depends on the population density. For example, at high density food may run short giving a population crash, while at low density the abundance of food may allow population size to increase, thus fluctuating about a mean over a period of years.

Ecosystem stability is not rigid. Systems change naturally. For example, on a short time scale, winter and summer aspects of a community in a temperate climate are very different. On a longer time scale there is ecological succession where one community naturally replaces another on an area of land or water, usually as a result of the modification of the habitat conditions by the organisms that are replaced so that the habitat is no longer suitable for their own survival. Thus, survivability is the basis of natural selection of species appropriate to the habitat conditions and hence of the evolution of species. This is generally explained on the basis of what has been called *r* / *k* selection theory. The terms, *r* and *k*, are derived from the Verhulst equation of population dynamics [21]:

$$\frac{dN}{dt} = rN(1-\frac{N}{k})$$

where *r* is the growth rate of the population (*N*), and *k* is the carrying capacity of its local environmental setting. Typically, *r*-selected species exploit empty niches, and produce many offspring, each of which has a relatively low probability of surviving to adulthood. Organisms with *r*-selected traits range from bacteria and diatoms, through insects and weeds, to various cephalopods and mammals, especially small rodents. In contrast, *k*-selected species are strong

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competitors in crowded niches, and invest more heavily in fewer offspring, each of which has a relatively high probability of surviving to adulthood. Organisms with *k*-selected traits include large organisms such as elephants, humans and whales, but smaller organisms, such as Arctic terns, may also use this "strategy" successfully,. In the scientific literature, *r*-selected species are occasionally referred to as “opportunistic”, while *k*-selected species may be described as “climax” or “equilibrium” species.

From the above considerations, it may be seen that mature stable ecosystems must be characterized by a preponderance of *k*-strategists - the species that succeed by having a very precise adaptation to their environment. Earlier stages in a succession may have a greater proportion of *r*-strategists, the opportunists - organisms with wide environmental tolerance that do not survive so well in stable habitats when competing with more precisely adapted *k*-species. In stressful environments, caused either by human intervention or by naturally harsh conditions, tolerance to abiotic factors becomes a greater determinant of community composition than biotic interactions, and *r*-strategists predominate.

The above description of the concept of an ecosystem stresses the ability of such systems to remain stable within limits in various ways. Loss of this stability may be the most serious effect of potential toxicants on ecosystems at risk. Loss of stability may be associated with a temporary increase in species numbers and diversity. Thus, the common assumption that high species numbers and diversity is characteristic of a healthy ecosystem may not always be true. Thus, management of ecosystems to maintain species balance may be more important than seeking to increase biodiversity.

Objective of ecotoxicology

The objective of ecotoxicology is to define the concentration of chemicals at which organisms in the environment will be affected and to suggest how this concentration can be avoided in the environment by appropriate management. To study the possibility that a chemical is toxic, ecotoxicologists usually start with single species tests and progress to more tests on higher ecological levels. This process is referred to as the "tiered testing”. As testing takes in more of the complexity of ecological relationships, the resulting data become more relevant. However, it takes much more time and resources to get these data and thus only those substances used in the very large amounts are tested at the highest tier, chronic testing in a model ecosystem.

For a population to flourish in its environment, individuals must survive to a size and age sufficient to permit reproduction. Effects of exposure to a potential environmental contaminant on survival, growth and reproduction are therefore the main concern of

ecotoxicologists. In general, for organics, structure-activity relationship information will be considered before proceeding to tiered toxicity testing. Subsequently, the resultant toxicity data will be combined with environmental fate data in order to provide a basis for deciding what, if any, regulation and management is required. This process is called environmental risk assessment.

Environmental risk assessment

The first step in environmental risk assessment is the calculation of the predicted environmental concentration (PEC). In calculating this, both short-term exposures, such as accidental spillage, which may result in high concentrations in the environment for a relatively short time, and long-term exposures that are the result of continuing discharges, resulting in continuous and perhaps increasing environmental exposure, must be considered. Exposure assessment requires knowledge of the biodegradation profile of the substances entering the environment and the extent to which they may be removed by wastewater treatment.

The second step in environmental risk assessment is calculation of the predicted-no-effect-concentration (PNEC). This is the concentration that is believed to cause no adverse effect to organisms in the environment at risk. This concentration is calculated from toxicity tests on species relevant to each environmental compartment. For the aqueous environment, indicator species are typically a freshwater fish, a freshwater invertebrate and freshwater green algae. For sediments and soils, they are sediment dwelling organisms, earthworms and terrestrial plants. For air, the indicator species might be birds or insects of importance such as bees.

The PEC / PNEC ratio is used as an indicator of risk and is called the Risk Quotient (RQ) or Risk Index (RI). A substance is judged to be environmentally acceptable if the PNEC is higher than the PEC, i.e., if this value is less than 1 (PNEC > PEC). A simple environmental risk assessment may be completed in a few weeks or months, but comprehensive assessments for substances produced in large amounts may take years to complete. Even after an environmental risk assessment is completed, especially for high production volume (HPV) chemicals, it may be decided to monitor the concentrations of substances of concern to confirm the accuracy of the PNECs and PECs.

7. ENDOCRINE MODIFICATION

IUPAC definition

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endocrine disruptor

endocrine modifier

Exogenous chemical that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, its progeny or (sub)populations.

Explanatory comment

In 1962, Rachel Carson pointed out in her book 'Silent Spring' [22] that synthetic chemicals, such as dioxins, dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCBs), were affecting the fertility, reproductive success and behavior of wild animals. This implied that there was interference with their endocrine systems.

Evidence for similar human health problems came from the work of Herbst, Ulfelder and Poskanzer [23]who observed that treatment of women during the 1950s and 1960s with diethylstilbesterol (DES), a synthetic estrogen agonist, had resulted in a marked increase in vaginal cancer among their daughters. This was supported by further studies by other workers and these led to a book entitled 'Estrogens in the Environment' edited by John McLachlan [24]. It may be noted that the effects of DES may be an example of the epigenetic effects described in section 8 of this paper.

In 1993, Richard Sharpe in the UK and Nils Skakkebaek in Denmark reported epidemiological studies apparently showing decreasing sperm count and sperm motility among men who were born after the Second World War in Denmark and Scotland. In a hypothesis paper in the Lancet [25], they advanced the hypothesis that environmental chemicals could be a cause for this decline in male fertility. Since then, increased rates of testicular cancer, undescended testes, and hypospadias have been attributed to endocrine disruption by synthetic environmental agents. Other examples of endocrine disruption (or modification) may be decreasing age at menarche, decreasing male-to-female sex ratio at birth, and various congenital malformations. The ultimate result could be a reduction in reproductive potential. It has been suggested that the observed increased incidence of neurobehavioral disorders and cancer of organs under hormonal influence may also be a consequence of exposure to endocrine disrupting chemicals. Even the current increasing incidence of obesity has been suggested to result from the ability of some endocrine disruptor (ED) substances to trigger fat-cell activity. However, the epidemiological data for effects of EDs have been questioned and no environmental ED has been clearly identified as a cause of possible effects.

Endocrine disruption in wildlife

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In 1991, an interdisciplinary conference considered the proposition that synthetic chemicals released into the environment could affect endocrine systems and alter the reproduction, development, and physiology of wildlife and humans. During the conference, the terms 'endocrine disruption' and 'endocrine disruptor' were coined. The proceedings of the conference were published as a book, "Chemically-Induced Alterations in Sexual Development: The Wildlife / Human Connection" (1992) [26]. The conclusions were that some environmental chemicals, described as 'endocrine disruptors', may act as hormone agonists or antagonists, or may interfere with hormone synthesis, and thus disrupt endocrine networks in animals and humans. It was postulated that embryonic and young organisms might be particularly sensitive to such disruptions. This includes adolescent organisms during sexual maturation, a process which depends on the correct levels and timing of hormone production. Thus, exposing the embryo, fetus, or even the maturing child or adult, to endocrine disruptors at biologically significant doses could potentially cause many diseases, ranging from cancer or physical malformations to immunological and neurological disorders and infertility.

Compared with humans, there is much more evidence that wildlife has been affected adversely by exposures to EDs, starting with the evidence of poor reproduction of DDT and PCB contaminated birds, cited by Rachel Carson, and caused by eggshell thinning and abnormal behavior. Subsequently there has been evidence of feminization of alligators exposed to DDT and dicofol in contaminated lake water, and of feminization of fish exposed to human female hormones and residues from hormonal drugs. Even more striking was the imposex effect of tributyltin oxide on dog whelks, causing females to develop male genital apparatus, making them sterile after exposure to concentrations barely detectable in seawater.

The strength of the evidence for endocrine disruption in wildlife may reflect the fact that many studies have been conducted in areas where the levels of environmental chemicals are high (e.g., where there are point source discharges, such as occur in the Great Lakes and the Baltic Sea). These studies have mostly concentrated on animals inhabiting aquatic ecosystems, which bioaccumulate certain EDs. In contrast with human studies, it has also been possible to experiment with the animal species of concern under both laboratory and field conditions. However, there are problems in determining the full range of potential effects of EDs on wildlife because of the large number of potential target species, differences in physiological mechanisms, and lack of detailed knowledge of endocrine function in many species.

Chemical structure of endocrine disruptors

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The chemical structure of potential EDs varies enormously. Examples of such chemicals are synthetic and natural hormones, plant estrogens, some pesticides, some persistent organic pollutants (POPs), bisphenol A, various metal species, and certain phthalate esters. Both phthalates and bisphenol A are high-production volume chemicals. In general, these substances have estrogenic effects although some phthalates studied are anti-androgenic and some may have anti-thyroid action [27]. Polybrominated diphenylethers (PBDEs) which are used as flame retardants have also been reported to interfere with thyroid metabolism.

Bisphenol A is of particular concern because it is a structural analogue of diethylstilbestrol (DES). It is widely used as an antioxidant in plastics and as an inhibitor in polymerization of polyvinyl chlorides. Bisphenol A has been associated with developmental toxicity, carcinogenic effects, and possibly neurotoxicity, in addition to its estrogenic effect. It is found in plastic tubes and syringes and in pacifiers for babies. It leaches from plastic bottles. Some countries have banned selling baby products that might contain bisphenol A because of its perceived potential to cause harm to children.

Endocrine disruptors may affect the gonadal-pituitary axis and modify the metabolism and (or) synthesis of estradiol and testosterone. Examples are the reductase inhibitors that block production of dihydrotestosterone. Such inhibitors are used as drugs, but are also found in herbs such as saw palmetto. Several chemicals are estrogen mimics that bind to the high-affinity estrogen-binding protein called the estrogen receptor (ER). These chemicals include drugs, natural products, and manufactured chemicals. It has been postulated that such estrogen mimics may be involved in causing breast cancer, uterine cancer, and developmental defects. There is also concern about herbal remedies that are marketed for “female” and “male” health. These preparations are physiologically active and modify the target endocrine system.

Substituted phenols such as bisphenol A, discussed above, and the longer chain nonyl- and octyl-phenols are widespread in the environment but it should be emphasized that they are many times weaker than the active human hormone estradiol in standard assays. Several derivatives of the pesticides DDT and methoxychlor are active as estrogens and (or) anti-androgens through mechanisms that involve the respective estrogen or androgen receptors. Some natural products, such as genistein from soy, exhibit remarkable estrogenic activity.

Conclusions

ED research has tended to focus on compounds that persist and bioaccumulate in exposed organisms. Considering the variations in the endocrine system during development and aging, and in response to environmental conditions, there is a need to pay more attention to the

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consequences of timing, frequency, and duration of exposure to potential ED substances. It must also be noted that there is a wide range of possible mechanisms for endocrine disruption depending on the substances involved and the animal species that is exposed. Endocrine disruption itself is not a precisely defined toxicological endpoint and subsidiary endpoints must be identified. These may range from interaction with molecular receptors up to the level of physiological or anatomical changes. While EDs have been recognized to be of particular concern in the regulatory context, no experimental strategy to identify them has been generally agreed and validated.

Whether the substances defined as EDs are indeed a serious threat to the environment or public health is not clear. Many of the human diseases and disorders predicted to increase as a result of low environmental exposure to EDs have not yet done so. However, the ED hypothesis has drawn attention to the challenge of understanding the complexity of hormone action. Many studies are underway on breast cancer, endometriosis, testicular cancer, and other plausible end points but it must be remembered that these illnesses may have multiple causes and may be influenced by individual life styles and habits. Thus, the idea that endocrine disruption in humans is caused by environmental chemicals is still a hypothesis requiring more evidence before it can be considered proven.

8. EPIGENETICS

IUPAC definition

epigen/esis n., **-etic** adj.

Phenotypic change in an organism brought about by alteration in the expression of genetic information without any change in the genomic sequence itself.

Note: Common examples include changes in nucleotide base methylation and changes in histone acetylation. Changes of this type may become heritable.

Explanatory comment

Until recently it has been dogma that gene expression is dependent on the primary DNA sequence (the genetic code), with regulation of gene transcription being determined by transcription factors or repressors whose transcription is in turn regulated in a tissue-dependent series of feedback mechanisms. In this paradigm, inherited genetic diseases arise from alterations in the primary DNA sequences that give rise to one or more proteins with consequently altered amino acid sequences. Epigenetics acknowledges that numerous factors

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outside the DNA sequence can affect the pattern of gene expression, that these alter phenotype and may cause disease, and that they can be heritable.

In earlier usage, epigenetics referred to any change in phenotype that was not accounted for specifically by changes in DNA sequence. In cancer biology, for instance, carcinogens that induce specific DNA mutations are considered to have no threshold effect for induction of cancer, whereas other (i.e., epigenetic) exposures may have a level below which excess cancers are not produced. Thus, carcinogens are sometimes divided into genotoxic and epigenetic carcinogens. Mechanisms of epigenetic changes in cancer can include silencing of tumor-suppressor genes, activation of oncogenes, and defects in DNA repair. They can also include effects of substances with broad actions that modify the immune or endocrine systems. It has been practice to distinguish cancer initiators from promoters, and promoters have been considered to exemplify epigenetic mechanisms. These considerations would lead to a very broad definition of epigenetics. Today, the term is acquiring the more specific meaning of changes in DNA or histone structure, other than base sequence changes, that alter gene expression.

The human chromosome does not consist of elongated pieces of naked DNA awaiting the transcriptional machinery, but rather of DNA coiled and packed in a highly organized manner, notably by interactions with various proteins known as histones. The winding of DNA around successive multimeric histone structures gives rise to a ‘beads-on-a-string’ type of structure. Each individual histone / DNA ‘bead’ is referred to as a nucleosome and the ‘string’ is chromatin. To be transcribed, a given DNA sequence must partially unwind or dissociate from the histone core. A higher level of structure is determined by interactions among nucleosomes that may give rise to tighter or looser regions of chromatin packing along the chromosome, or expose regions between nucleosomal or chromatin domains to binding of repressor proteins that influence structure and transcription of extended regions of chromatin. In general, a more open chromatin structure, referred to as euchromatin, is active (more conducive to gene expression) while more condensed regions called heterochromatin are silent (suppressing expression). From steric arguments, it is logical that accessibility to the transcriptional machinery depends both on loosening of the heterochromatin structure and unwinding of DNA within the nucleosome. Chemical modifications of both DNA and histones influence the degree of compaction and winding/unwinding.

Various modifications of histones are documented, including acetylation, methylation, and phosphorylation. Ubiquitination also occurs and targets the histone protein for degradation by the proteasome. It has been proposed that the pattern of N-terminal modifications of

histones represents a new kind of genetic code that determines gene expression by changing histone conformation. The enzymology of histone acetylation is widely studied. The extent of acetylation depends on the balance of activity between histone acetylases and histone deacetylase. Acetylation of lysine residues in the N-termini of histones H3 and H4 masks positive charges that otherwise interact with acidic residues in H2 histones of adjacent nucleosomes. This leads to a more open chromatin structure and favours gene expression. The histone acetylase / deacetylase system is of great importance in toxicology and is visited again in the discussion of mutagenicity.

The major epigenetic chemical modification of DNA is methylation. Most important is the relative hyper- or hypomethylation of position 5 of cytosine in CpG dinucleotides that tend to cluster in promoter regions of genes. The degree of methylation of a stretch of DNA is generally inversely correlated with its degree of expression. Increased methyl-CpG content is associated with increased heterochromatin formation. That hypermethylation tends to occur in promoter regions suggests it may interfere with transcription factor binding, and there is evidence that some transcriptional repressors bind with increased affinity to methylated CpG sequences. However, it is not yet clear whether methylation of DNA is directly responsible for suppression of transcription or whether it acts in conjunction with histone tagging or through influencing nucleosomal structure. Furthermore, there is evidence that DNA methylation and histone deacetylation may be linked. A protein MECP2 has been identified that binds to methylated CpG sequences and recruits histone deacetylase, amplifying the transcriptional suppression.

DNA methylation participates in other aspects of epigenetic control. Imprinted genes are those in which one allele (either the maternal or paternal) is effectively silenced in early development, and this usually involves hypermethylation of the silent allele. In addition, certain DNA sequences called transposons are prone to self-cloning themselves into other regions of the chromosome, where they may either disable or hyperactivate a target gene. Methylation tends to suppress the mobility of transposons.

The dogma that only DNA that ultimately codes for a protein represents a gene has also been upset by the demonstration of numerous RNA-only genes. (Some purists who prefer to reserve the word ‘gene’ for a DNA sequence that is expressed as protein also prefer the term ‘transcriptional unit’ for any DNA sequence that is actively transcribed.) These are genes that encode RNA which is not translated, but affects the expression of other genes nevertheless. Numerous examples are known where the complementary strand is transcribed as an antisense RNA that blocks translation of its sense partner. Other genes code short hairpin RNAs that

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silence other genes through RNA interference. The involvement of untranslated RNA sequences in epigenetic phenomena is an active area of investigation.

Various definitions have indicated that the epigenetic phenomenon must persist for at least one generation, or be heritable. However, if heritability is taken as part of the definition of epigenetics in current usage, then it is necessary to demonstrate inheritance in the F3 generation. This is because an exposure that affected (injured) the F1 daughter in utero, for example, could also affect her eggs and thus have a direct but not heritable affect on the F2 generation. Thus, true demonstration of heritability, is not always easy to achieve. When it occurs, it is reminiscent of Lamarckianism, the once discredited view that characteristics acquired from environmental exposures during an individual's lifetime could be passed on to progeny.

How are these patterns of chemical modification passed on from cell to cell or generation to generation independent of the genetic code? The answers are complex and not well understood. An early example was the inheritance of the trait *callipyge* (Gr., beautiful buttocks) in sheep developing advantageous hindquarters. A decade of experimentation revealed the unusual inheritance pattern of this trait is due to a conserved protein-coding gene and RNA-only genes on the same chromosome, that are transcriptionally regulated epigenetically in response to a G-to-A base change in a region of 'junk DNA' 30,000 bases away from the nearest known gene. Loss of imprinting (e.g., of the insulin-like growth factor IGF2) has been associated with some sporadic cases of colon cancer and several rare genetic diseases. Identical twins discordant for development of Beckwith-Weidemann syndrome were found to differ with respect to imprinting on a region of chromosome 11, the affected twin having failed to imprint.

Epigenetic phenomena are beginning to take a prominent place in toxicology. Their involvement in nickel and arsenic toxicity are good examples. Although divalent nickel cations are at best weakly mutagenic, nickel compounds have been classified by IARC as human carcinogens, and there is mounting evidence that any such carcinogenic effects may be through heritable changes in DNA methylation and/or histone acetylation. In the nucleus, Ni^{2+} competes with Mg^{2+} and binds selectively to heterochromatin, probably through specific interactions with various histone sites. Resulting decondensation and damage to heterochromatin correlates with morphologic transformation. Damage includes heritable chromosome deletions and hypermethylation of a senescence gene on the X-chromosome. Silencing of the tumor suppressor gene p16 has also been demonstrated following exposure to various nickel compounds.

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A unique epigenetic mechanism is also suggested for toxicity of carcinogenic arsenic compounds. Arsenic in divalent and pentavalent states is detoxified through mechanisms that involve formation of methylated derivatives. The methyl donor is S-adenosyl methionine, the same donor involved in DNA methylation. Exposure to some arsenicals leads to depletion of S-adenosyl methionine, and consequently to global DNA hypomethylation. One idea is that hypomethylation of protooncogenes may lead to their overexpression and ultimately to cell transformation. This also illustrates the point that both DNA hypermethylation and hypomethylation may lead to cancer, depending on whether the targets are pro- or anti-carcinogenic genes.

The above examples reflect a recent focus on toxicological aspects of epigenetics relating to cancer. The idea that cancer can be explained by single or multiple mutational hits on genes that stimulate or inhibit proliferation is being reconsidered. Clearly, heritable effects that affect gene expression give rise to at least some cancers, independent of any alterations in the DNA sequence of the gene itself, and this adds a new layer of complexity to our attempts to understand malignant transformation. However, because these epigenetic phenomena involve dynamic chemical modifications to the genome that may switch on or off during the life of the cell, they present potentially more tractable targets for therapeutic intervention than changes in the primary DNA sequence itself.

Finally, it should be noted that the term epigenomics has come into prominent use. This refers to the study of the epigenetic factors in illness or susceptibility using a genomics approach (see Genomics, Proteomics, and Related Terms). While the term epigenetics refers to changes resulting from one or more individual genes, epigenomics refers to a more global analysis of epigenetic effects across the genome. Thus, it relies on techniques that give a genome-wide picture of epigenetic phenomena, for instance using high-throughput microarray technology. Currently these approaches are best developed for DNA methylation, and for histone modifications using DNA crosslinking followed by chromatin immunoprecipitation and microarray (ChIP-on-chip) analysis. Epigenetic control of stem cell differentiation and organ development may underlie a broad spectrum of disease. Initially studies are being concentrated on cancer and aging. There is now considerable hope that insight into major psychiatric illnesses like schizophrenia and bipolar disorder will be found through epigenomics research.

9. GENOMICS, PROTEOMICS, AND RELATED TERMS

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IUPAC definitions

genomics

1. Science of using DNA and RNA based technologies to demonstrate alterations in gene expression.

2. (in toxicology) Method providing information on the consequences for gene expression of interactions of the organism with environmental stress, xenobiotics, etc.

proteomics

Global analysis of gene expression using a variety of techniques to identify and characterize proteins.

> *Note*: It can be used to study changes caused by exposure to chemicals and to determine if changes in mRNA expression correlate with changes in protein expression: the analysis may also show changes in post-translational modification, which cannot be distinguished by mRNA analysis alone.

Explanatory comment

The publication of the first drafts of the complete human genome in the journals *Nature* and *Science* in 2001 [28, 29] heralded a new age in biology, and a proliferation of terminology – some good and some bad – that accompanied the new way of thinking. It is now believed that we can know the complete set of genes for a given organism – its genome. If that is so, presumably it should be possible to know its complete set of proteins, carbohydrates, metal-binding species, and even all the possible phenotypic traits expressed by the organism with the same certainty. Some of the methods and successes of genomics and proteomics will be discussed here. Problematic issues relating to other comprehensive "-omics" will be reviewed at the end of this section.

The human genome project, promulgated in the 1990s, finished ahead of schedule and revealed that the human species has fewer genes than expected. While prior estimates had ranged to upwards of 100,000, we now know that about 25,000 DNA sequences code for the proteins that contribute to our phenotype. This number does not correlate well with what we understand as biological complexity. We have fewer coding genes than the rice plant, and the minute worm, *Caenorhabditis elegans,* has more than the fly *Drosophila melanogaster*. However, we have come to realize that the total picture of an organism includes genes that code only for RNA that is not translated into protein, and a host of other epigenetic phenomena (see Section 8 - Epigenetics). It is clear that cataloguing the set of coding DNA genes for a given species is a long way from understanding the complete phenotype of that species, but

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nevertheless provides a wealth of information to approach the biology and pathology of the species. With the draft human genome behind us, other genomes that have been tackled early on include those of model organisms for biological research (*Drosophila*, *C. elegans*, *Arabidopsis thaliana*, *Danio rerio*, the mouse, and various yeasts) and those of agricultural significance, including rice and maize. These successes have evolved hand in hand with technologies for large scale sequencing and bioinformatic techniques for sorting, assimilating, and storing sequence data.

Activities referred to as genomics today include not only the automated pursuit of more (and more complete) genome sequences, but the use of surveying techniques for gene expression under various circumstances. These survey approaches commonly include microarray technology, where nucleic acid sequences, typically either primary DNA sequences or complementary cDNA sequences are immobilized on a support. In practice, several thousand sequences from an organism are now immobilized on a patch of a glass microscope slide. Total RNA being translated by a given cell or tissue preparation is tagged for detection, usually with fluorophores, and then hybridized against the target array and detected by scanning laser fluorometry. Quantitation of the fluorescent signal on each spot gives a snapshot of the expression of genes in the cell or tissue at the moment it was harvested. An exciting application of these genomic and proteomic approaches is the possibility of high through-put screening of substances for a mechanistic understanding of toxic effects that could bypass the need for animal studies. Additionally, the full range of toxic potential of a substance could be gained from a few animals, without sacrificing individual animals for specific endpoints.

Three considerations are worth mentioning in the interpretation of such experiments.

i) Description of the experiment. It was recognized early in the microarray era that replication of experiments between laboratories was necessary to validate results that are so prone to artifact, and this requires careful description of the experimental protocol. An international consortium of journal editors devised the “Minimum Information About a Microarray Experiment” (MIAME) protocol that should be provided in order to contribute the results of a microarray analysis to the literature. The protocol requires details of a) experimental design, b) array design, c) sample selection, d) hybridization protocol, e) image analysis, and f) normalization and controls for comparison. It is optimistic to think that this information will be sufficient to consolidate data among many laboratories, but it is a starting point.

ii) Statistical analysis. Because there are many levels of potential analytical variability, there is much discussion of how many replicates of a microarray experiments should be performed and how statistical validity should be determined. Costs still frequently limit the number of

replicates, at least in the academic research laboratory. Normalization of the signal intensity is a necessary precursor to meaningful analysis, and data processing through available routines such as Significance Analysis of Microarrays (SAM) and non-linear regression by the LOESS method is mandatory to minimize artifact.

iii) Data management. Because one microarray experiment can potentially generate meaningful data on the expression of thousands of genes, data management is a consideration. It is the rule, rather than the exception, that a well-considered microarray experiment provides a research laboratory with enough information to fuel months or years of ancillary confirmation and intriguing follow-up.

Another powerful aspect of genomics is phylogenetic analysis. This uses analysis of gene sequences to study and establish evolutionary relationships between and among organisms. Gene trees are built up, sometimes referred to as 'cladistics' (from Gr. *clados*, a branch, referring to descent from a single ancestor). The end product from cladistic analysis is a phylogenetic tree that represents the evolutionary proximity of organisms based on similarities in the sequences of one or more genes.

Proteomics is the next logical step after definition of the genome. One attempts to identify all the proteins expressed by the genome, and while in principle this might be achieved by *in silico* translation of all the genes, this is not useful because we still cannot predict cell- and tissue-specific controls on transcription, mechanisms of alternative splicing, or epigenetic controls on gene expression/modification. Therefore, we need to measure the protein complement expressed at a given time in a given cell or tissue to understand the manifestation of the genome in a given state of health, disease, or an environmental situation. The standard approach here is two-dimensional electrophoresis followed by mass spectrometry. After careful isolation of all proteins in a sample, in the presence of broad inhibitors of proteases, phosphatases, and other degradative enzymes as the desired result dictates, the solubilized protein sample is first separated by isoelectric focusing on an ampholine gradient, which separates proteins based upon their isoelectric points. This partially separated mixture is then subjected to electrophoresis in the second dimension on a polyacrylamide gel, based on differences in size and charge. Individual proteins are recognized non-quantitatively and non-specifically, by stains based on Coomassie blue dye or much more sensitive silver staining. This approach is currently able to resolve a few thousand proteins in a sample. Spots are then cut from the gel, eluted, and subjected to analysis by mass spectrometric techniques.

The genome is well defined. The proteome is approachable by current methods. After this, we must exercise linguistic restraint. There is a trend to classify other collections of

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molecules as if in a complete descriptive context, and thus emerge terms like kinome (the set of expressed kinases), glycome (the complement of synthesized carbohydrates), and metallome (sometimes meant to refer to the set of expressed metalloproteins). For example, surveys of the kinome depend on immunoblotting techniques and thus are governed by the availability, specificity, and affinity of antibodies. They are incomplete and non-quantitative; the practice is without guidance, and the nomenclature is imprecise. The origins of the term 'genome' are not helpful in constructing terms to describe these other sets of molecules. It is an amalgamation of gene (from Gr. *genea*, generation or race) and chromosome (again Gr. *chroma,* colour and *soma,* body, based on the staining properties observed by the early cell biologists). By loose analogy, various –ome and –omics words come into the language. IUPAC has also defined interactome, phenome, and transcriptome (see Glossary of Terms Used in Toxicology, 2nd edition [4]). While they may serve some scientific purpose, they can also be problematic. As an example, the term metallome has been used to describe the set of molecular metal-binding species in a given organism. But should this refer to metal-binding proteins (a subset of the proteome), or to all metal-binding species, even including metal salts that may form with available counterions? This is a problem of speciation, not of comprehensive description. The concept has even been further subdivided in coining terms such as zincome and selenome, to refer to the species that bind zinc or selenium. This practice seems to be both scientifically distorting and etymologically unfounded, and should surely be discouraged. Even the more accepted terms "metabolomics" and "metabonomics" are a case in point. Ambiguity in definition belies the fact that no complete set of metabolites can be defined in the same way that a genome can be sequenced, as these will depend on individual differences in enzyme activities, environmental exposures to xenobiotics, etc. However, it should be noted that some biologists consider metabolomics to be the study of the collection of metabolites themselves, and take metabonomics to describe the study of the metabolic changes that cells undergo in response to stressors. The term epigenomics has a special status, and is dealt with in section 8 on Epigenetics.

10. IMMUNOTOXICITY, IMMUNOSUPPRESSION, AND HYPERSENSITIVITY

IUPAC definitions

hypersensitivity

State in which an individual reacts with allergic effects following exposure to a certain substance (allergen) after having been exposed previously to the same substance.

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Note: Most common chemical-induced allergies are type I (IgE-mediated) or type IV (cell-mediated) hypersensitivity

immunosuppression

Reduction in the functional capacity of the immune response; may be due to:

1. Inhibition of the normal response of the immune system to an antigen.
2. Prevention, by chemical or biological means, of the production of an antibody to an antigen by inhibition of the processes of transcription, translation or formation of tertiary structure.

immunotoxicity

Ability of a physical, chemical, or biological agent to induce adverse effects in the immune system.

After [4]

Explanatory comment

There are many possible toxic effects on the immune system, and in a broad sense they may be classified as either immunosuppression or unwanted immune activation. In broadest terms, immunosuppression leads to a deficit in the immune response to microbial pathogens and transformed cells, whereas inappropriate activation is associated with allergy, hypersensitivity, and autoimmunity. The immune system plays a key part in protecting us from infectious disease by attacking and destroying infectious organisms and potentially harmful macromolecules. It also monitors our own cells and attacks and destroys many of those that become transformed to potentially cancerous progeny. This host resistance to infectious agents and neoplasms depends on the presence of immunocompetent cells that may be stimulated to proliferate in the presence of antigenic substances.

The immune system depends for its efficient function on the intrinsic cooperation of various cells, which is strongly dependent upon the biological actions of soluble mediating molecules such as immunoglobulins, hormones, growth factors, and cytokines acting through membrane receptors. Any substance that affects these interactions can cause agent-specific or species-specific damage that, in many cases, causes immunosuppression (resulting in e.g., decreased resistance to infectious agents and development of tumors). Immunosuppression may be either systemic, or occur at the local level (e.g., in lungs or skin). As noted above, an inappropriate increase in the immune response can also occur, leading to hypersensitivity, as exemplified by respiratory tract allergy or allergic contact dermatitis. Furthermore, some substances can cause the development of autoimmune diseases.

Diseases associated with abnormal immune function, including common infectious

diseases and asthma, are more prevalent at younger ages. Several factors may explain this increased susceptibility, including functional immaturity of the immune system and age-related differences in metabolism. Although not yet conclusively demonstrated, it is generally believed that the immature immune system is more susceptible to xenobiotics than the fully mature system, and that the effects of immunotoxicant exposure in the very young may be particularly persistent. This contrasts with effects observed following exposure in adults, which generally occur at higher doses and do not persist for long after exposure ends. Experimental animal studies show that harmful effects on the developing immune system may be qualitative (e.g., affecting the developing immune system without affecting the adult immune system) or quantitative (e.g., affecting the developing immune system at lower doses than in adults). Immune maturation may simply be delayed by xenobiotic exposure and may recover to normal adult levels over time. However, if exposure interferes with a critical step in the maturation process, lifelong defects in immune function may follow. These defects may be expressed as immunosuppression or as dysregulation of the immune system, resulting in decreased resistance to infection or development of a functional phenotype that is associated with allergy and asthma. Experimental evidence indicates that development can be hindered or delayed to such an extent that certain effector mechanisms either are absent or do not function properly for essentially the lifetime of the individual as has been reported following exposure to diethylstilbestrol (DES) or tetrachlorodibenzo-*p*-dioxin (TCDD). In humans, the clinical effects of immunotoxicant exposure during development may be expressed immediately or later in life, presenting as either increased infectious or neoplastic diseases, or as increased incidences (or severity) of allergic or autoimmune disease.

The complexity of the immune system results in there being many potential target sites and pathological consequences of toxicity. The strategies devised by immunotoxicologists working in safety assessment have all applied a tiered panel of assays in order to identify immunosuppressive and immunostimulatory agents in laboratory animals. These testing stategies include measurement of one or more of the following: altered lymphoid organ weights and histology; changes in the cells of lymphoid tissue, peripheral blood leukocytes, and (or) bone marrow; impairment of cell function at effector or regulator level; and altered susceptibility to challenge with infectious agents or tumor cells. With respect to clinical evaluation and research on human responses for immunoactivation, the lymphocyte transformation (proliferation) test performed on peripheral blood lymphocytes, cytokine profiling of blood or serum, and analysis of lymphocyte subpopulations by flow cytometry are of greatest current interest.

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Assessment of immunotoxicity

Many factors must be considered in evaluating the potential of an environmental agent or drug to influence adversely the immune system of experimental animals and humans. As for assessment of any type of toxicity, these of course include selection of appropriate animal models and exposure variables, an understanding of the biological relevance of the end-points being measured, use of validated measures, and quality assurance. The experimental conditions should take into account the potential route and level of human exposure and any available information on toxicodynamics and toxicokinetics. The doses and sample sizes should be selected so as to generate clear dose-response curves, needed for the determination of no-observed-adverse-effect (NOAEL) or no-observed-effect levels (NOEL). Testing must be continually refined to allow better prediction of conditions that may lead to disease. In addition, techniques should be developed that will help to identify mechanisms of action; these may include methods for in vitro examination of local immune responses (such as in the skin, lung, and intestines), and techniques of molecular biology and the study of genetically modified animals. These obvious criteria require special attention in assessing immunotoxicity, where appropriate measurements and endpoints are not always clearly agreed upon.

Unfortunately, detection of immune changes after exposure to potentially immunotoxic substances is much more difficult in humans than in experimental animals. Testing possibilities are limited, levels of exposure to the agent (i.e., dose) are often difficult to establish, and the immune status of populations is extremely heterogeneous. Age, race, sex, pregnancy, acute stress and the ability to cope with stress, coexistent disease and infections, nutritional status, tobacco smoke, and medications may all contribute to this heterogeneity. This heterogeneity renders less effective some of the clinical tests mentioned above.

Since most of our knowledge regarding human toxicity of environmental chemicals comes from epidemiological studies, epidemiological study design for such investigations must be appropriate if we are to reach correct conclusions. The commonest design used in studies of immunotoxicity is the cross-sectional study, in which exposure status and disease status are measured at one time or over a short period. The immune function of 'exposed' subjects is then compared with that of a comparable group of 'unexposed' individuals. Because many of the immune changes seen in humans after exposure to a chemical may be sporadic and subtle, recently exposed populations must be studied and sensitive tests must be used for assessing the immune system. Conclusions about immunotoxic effects should be based on changes not in a single parameter but in the immune profile of an individual or population.

Examples of immunotoxicants

Examples of environmental and industrial chemicals reported to be immunotoxicants in humans are asbestos, benzene, and halogenated aromatic hydrocarbons, including TCDD (often referred to just as dioxin) and polychlorinated biphenyls (PCBs), that give rise to immunosuppression. TCDD and PCBs are widespread environmental pollutants which are resistant to biodegradation and have lipophilic properties facilitating bioaccumulation in, e.g., fish, and thus in humans who eat the fish. Other economically important chemicals known to be powerful hypersensitizers are the isocyanates. In studies of the toxicity of metallic elements, a dose-response relationship is sometimes seen showing stimulation of some immune functions at low doses while high doses cause immunosuppression. Nickel ions and chromium ions cause hypersensitivity reactions (see below) and dermatitis in humans. Beryllium is another metal with hypersensitizing and immunotoxic properties.

Hypersensitivity

When exposed to an antigen, the body may produce antibodies specific to that antigen. These antibodies may provide immunity against later exposures to the antigen. Under some physiological conditions, or in people with defective immune systems, an excessive immune reaction may occur that can cause cell and tissue damage. Histamines released from mast cells following such damage can cause dilation of small blood vessels, tissue inflammation, and constriction of the bronchi of the lungs. The result may be anaphylaxis, an immediate, sometimes fatal, hypersensitivity reaction to some substances including macromolecules occurring in the diet, notably to some found in peanuts. In humans, the clinical signs and symptoms of anaphylaxis include reaction of the skin with itching, erythema, and urticaria; reaction of the upper respiratory tract with edema of the larynx; reaction of the lower respiratory tract with dyspnea, wheezing, and cough; reaction of the the gastrointestinal tract with abdominal cramps, nausea, vomiting, and diarrhea; and reaction of the cardiovascular system with hypotension and shock. Individuals undergoing anaphylactic reactions may develop any one, a combination, or all of the signs and symptoms. Anaphylaxis may be fatal within minutes, or fatality may occur days or weeks after the reaction. Death is a result of the damage suffered as a result of the decrease in blood pressure following extreme dilation of the blood vessels.

Serum sickness is a similar but milder hypersensitivity to serum proteins or drugs that occurs several weeks after injection of foreign material. Delayed reaction hypersensitivity

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occurs when lymphocytes react to certain antigens. The lymphocytes slowly infiltrate an area, such as skin exposed to poison ivy toxin, and cause local inflammation reactions which may be followed (or accompanied by) tissue damage. Anaphylaxis, serum sickness, and delayed sensitivity may occur in otherwise normal, individuals as well as those inclined to allergies. Individuals with allergic, or atopic, hypersensitivity form antibodies that react with antigens to cause local tissue damage and such symptoms as hives, hay fever, and asthma. Antihistamines are drugs that prevent histamine from acting on blood vessels, bronchioles, and other organs. Acute reactions, such as anaphylaxis, are treated by giving epinephrine and other sympathomimetic drugs to support the blood circulation.. Steroids such as cortisone are also given to suppress inflammation and depress the immune system. In some cases, hypersensitized individuals receive injections of gradually increasing quantities of the antigenic material to which they are sensitive, in order to build tolerance and avoid or lessen their hypersensitivity to that particular substance.

Briefly, hypersensitivity has been classified into four types by Gell and Coombs [30] as shown in the table below.

Gell and Coombs Classification of Types of Hypersensitivity

Type	Alternative name	Related health problems	Mediators
1	Allergy	Atopy Anaphylaxis Asthma	IgE
2	Cytotoxic, antibody dependent	Erythroblastosis fetalis Goodpasture's syndrome Autoimmune hemolytic anemia	IgM IgG Complement
3	Immune complex disease	Serum sickness Arthus reaction Systemic lupus erythematosus (SLE)	IgG Complement
4	Cell-mediated	Delayed hypersensitivity Contact dermatitis Hypersensitivity in tuberculosis Chronic transplant rejection	Lymphocytes

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Autoimmunity

Autoimmunity is a condition in which the immune system fails to differentiate self-antigens from foreign antigens and begins to attack self-tissues. In effect, the body generates an immune response against itself. An autoimmune reaction results in inflammation and in tissue damage. Some of the more common autoimmune disorders are rheumatoid arthritis, systemic lupus erythematosus, and vasculitis. It is also possible that some glomerulonephritides, Addison's disease, mixed connective tissue disease, polymyositis, Sjögren's syndrome, and progressive systemic sclerosis, have autoimmune components. It is likely that some cases of infertility may be explained by autoimmunity. Some common multifactorial diseases have an autoimmune component, and Type I diabetes is an important example. Common antigens in generalized autoimmunity are the nuclear antigen recognized by the anti-nuclear antibody that is important in the diagnosis of systemic lupus erythematosus, and the anti-mitochondrial antibody elevated in primary biliary cirrhosis. Some autoimmune diseases are poorly understood but may, in certain circumstances, be a consequence of exposure to a toxic substance or substances. For example, hemolytic anemia has been linked to an autoimmune response affecting the red cells following exposure to the pesticide dieldrin.

Skin sensitivity

Allergic contact dermatitis (ACD) is a major cause of minor discomfort to people. It is also the most tested form of immunotoxicity and is largely a preventable disease. Prevention of the effects can be achieved by correct identification of skin sensitizers, characterization of their potency, understanding human skin exposure, and application of good risk assessment and management strategies.

Skin sensitization is caused by substances that behave as electrophiles and can react with skin proteins, altering them so that the immune system recognizes them as ‘foreign’ [31]. This leads to proliferation of lymphocytes which recognize the substance and (or) the altered protein which it produces. Subsequent contact with the substance can then cause an enhanced effect on the skin, producing redness, swelling, itching etc. Substances which do this are called skin sensitizers. People who are sufficiently exposed may develop the sensitized state called contact allergy; once they have acquired that state, further exposure can lead to the development of allergic contact dermatitis.

For assessing the risk of developing ACD from exposure to a given substance, it is important to know the relative potency of an identified chemical allergen, to understand how people are exposed to it, and to integrate this information into the risk assessment. Having done

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this, one can formulate a plan for management and reduction of the risk. For consumers, this may mean voluntary or regulatory imposition of concentration limits in products, often with suitable labeling. Control of occupational exposure may involve both an equivalent of the consumer limit process and a greater emphasis on controlling skin exposure, if necessary by the use of personal protective equipment.

Multiple chemical sensitivity (MCS)

Multiple chemical sensitivity (MCS) is essentially an adverse reaction of susceptible persons to a large number of chemicals. When exposed to the relevant chemicals, people with MCS react with symptoms such as nausea, headache, dizziness, fatigue, impaired memory, rash, and respiratory difficulty. Many household and industrial chemicals, including cleaning products, tobacco smoke, perfumes, inks, and pesticides, have been mentioned as triggers for MCS. It can be argued that immunotoxicity provides a mechanism for some of the observed symptoms but there is no conclusive evidence of this.

Most toxicologists and physicians do not regard MCS as a legitimate medical syndrome, arguing that the depression that frequently accompanies it is an indication that the symptoms are psychosomatic. Further, descriptions of the syndrome are largely anecdotal and difficult to verify scientifically. It is also true the imprecisely defined syndrome is easily misused as a diagnosis, leading to a large number of worker's compensation cases involving MCS. Nevertheless, many putative sufferers do seem to improve when they eliminate contact with the chemicals suspected of triggering their condition; in extreme cases, this seems to require confinement to specially treated living quarters.

11. MUTAGENICITY

IUPAC definition

mutagenicity

Ability of a physical, chemical, or biological agent to induce (or generate) heritable changes (mutations) in the genotype in a cell as a consequence of alterations or loss of genes or chromosomes (or parts thereof).

Explanatory comment

Our heritable information consists of deoxyribonucleic acid (DNA) base sequences, or genes, that code for proteins and are arranged end-to-end with much intervening non-coding DNA.

The linear arrangement of genes and non-coding DNA constitutes a chromosome, and in humans the genetic information of the nucleus is packaged into 23 pairs of chromosomes plus the X and Y sex chromosomes. In addition, mitochondrial DNA sequences encode information that is processed within the mitochondria, and may also be subject to mutation. The nuclear chromosomes are coated with various proteins including histones and an additional amount of nonhistone proteins that help package them into complex tertiary structures. This is discussed further in Section 8 - Epigenetics. When an alteration in the readable sequence of a gene occurs, changes in the transcript of the gene will occur and an abnormal, generally malfunctioning result, or the protein may even be absent. Such changes, called mutations, drive evolution, but beneficial mutations are negligible in number compared to mutations that harm the organism. In addition to changes in sequence, whole segments of genes or even sections of chromosomes may be deleted or rearranged, and these changes are also referred to as mutations, although the term clastogenic is used to refer to the subset of mutations that result from chromosomal breaks and rearrangements. In contrast, changes in gene expression that do not result from changes in sequence are called epigenetic. Mutagens that lead to cancer are called carcinogens (see Section 5 - Carcinogenicity); those that cause birth defects or malformations in the offspring are referred to as teratogens (see Section 15 - Reproductive Toxicology).

Mutations can arise spontaneously from errors in DNA replication or DNA repair. However, many are caused by agents that increase the frequency of mutations by interacting with, or chemically modifying DNA, and these agents are referred to as mutagens. Many mutagens are chemical substances, but ionizing and ultraviolet radiation can also alter DNA structure physically (e.g., by inducing thymine dimer formation that interferes with transcription), and is also considered a mutagen. Some chemical mutagens intercalate with DNA, thus interfering with the fidelity of transcription. These are generally planar hydrophobic structures such as acridine orange or ethidium bromide that fit in the major or minor groove of the DNA helix (intercalation). The anticancer drug cisplatin is an inorganic compound that intercalates with DNA. Alkylating agents, on the other hand, form covalent adducts with DNA bases that result in faulty transcription. Commonly, a site of attack of monoalkylation is the 7-N of guanine. Dialkylating agents can cause DNA strand crosslinking through this site. Alkylating agents include many anti-cancer agents such as nitrogen mustards (cyclophosphamide, chlorambucil), alkyl sulfonates (busulfan), and nitrosoureas. Base substitution in RNA or DNA with analogues such as halogenated pyrimidines, and base deamination with nitrous acid, are further examples of actions of mutagens. Some substances

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require activation within the cell, e.g., by cytochrome P450, before they become mutagenic. An example here is the activation of benzopyrene to its epoxide by CYP1A1.

Several different types of mutations are well documented. A change in one base pair may result in a new triplet codon that codes for a different amino acid in the final protein, giving rise to a dysfunctional protein product. This is a missense mutation. A nonsense mutation occurs when the single base pair change results in a stop codon and a truncated product. One or more base pairs may be inserted into (insertion mutation) or deleted from (deletion mutation) the DNA sequence, resulting again in a different or absent protein product. If the insertion or deletion is not a multiple of three base pairs, the reading frame of codons will be changed and this is called a frameshift mutation. Duplication occurs when a DNA sequence is erroneously copied more than once during chromosomal replication. Duplications of genes such as *myc* and *Flt3* are often associated with a poor prognosis in cancer patients. If multiple copies of a gene result, this may result in the overexpression of the encoded protein. Expansion refers to a sequence of DNA that is replicated multiple times within a gene. For example, the sequence GAA in the first intron of the *FRDA* gene encoding frataxin has been found to be repeated up to 1700 times in patients with Friedreich's ataxia.

Heritability refers to the passing on of a DNA sequence from a cell to its daughters, and, if they give rise to gametes, to the progeny of these gametes. Thus, a mutation that becomes embedded in the germ line is passed on from one generation to the next, in either an autosomal or X-linked manner in humans, depending on whether or not the mutation is carried on one of the sex chromosomes. If a mutagen causes an alteration in DNA sequence of a non-germ line cell, division of that cell in an organism may result in dysfunctional or neoplastic tissue. It is now well recognized that mutations in mitochondrial genes may also be inherited.

Testing for the mutagenic potential of a substance is often carried out with the Ames test. Here, several strains of *Salmonella typhimurium* are used. These strains have been rendered deficient in histidine synthesis through both deletion and frameshift mutations in the genes involved in histidine biosynthesis. After exposure to a substance, the occurrence of *S. typhimurium* colonies growing on histidine-deficient medium is an indication of the ability of the substance to have produced reversing or counteracting mutations of one or more of the different types. Rat liver microsomes may be included to allow for the possible requirement for bioactivation before the substance becomes mutagenic. This test is very sensitive, but it is also recognized as not necessarily predictive for eukaryotes. Further, the sensitivity of the Ames test is improved by using *Salmonella* strains deficient in DNA repair mechanisms, which at the same time potentially exaggerates the mutagenicity of test substances. Tests with whole

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animals, such as the Legator test, have been mentioned briefly under the heading "Acute and Chronic" in Part I of this explanatory dictionary [1]. They may be more realistic, in that host factors may also modify the mutagenic potential of a substance, but end points are less well-defined and the high through-put feature of a screening test is lost.

12. NANOPARTICLES AND ULTRAFINE PARTICLES

IUPAC definitions

nanoparticle

Microscopic particle whose size is measured in nanometers, often restricted to so-called nano-sized particles (NSPs; <100 nm in aerodynamic diameter), also called *ultrafine particles*.

ultrafine particle

Particle in air of *aerodynamic diameter* < 0.1 μm.

Note: The fraction of the air particulates referred to as ultrafines is often referred to as the $PM_{0.1}$ fraction.

Explanatory comment

The term, 'nanotechnology' was introduced in 1974 by Norio Taniguchi [32] to describe new technology for designing semiconductors on an atom-by-atom or molecule-by-molecule, basis. In essence, nanotechnology deals with formation and usage of small structures that have useful properties and can be manipulated at the atomic or molecular level.

The term 'nanoparticles' originated in relation to nanotechnology to describe the synthetic particles that make it possible. Nanoparticles are increasingly used in body lotion, facial creams and sun screen protection; in food stuffs; and in a great variety of other products used by humans. Previously, the term 'ultrafine particles' was applied to particles in this size range of < 100 nm in aerodynamic diameter, referring to naturally formed particles found in the air. It is difficult to see any toxicological value in distinguishing between nanoparticles and ultrafine particles on the basis that one group is synthetic and the other natural. This situation mirrors the distinction commonly made between natural and synthetic chemicals, a distinction that has limited toxicological significance.

Ultrafine particles (and nanoparticles) can be absorbed in the lungs, mostly from the alveoli. The uptake in an organism of particles via the lungs reflects the balance between deposition and clearance. Deposition results from the impaction of large particles and the sedimentation and diffusion of small particles. Total deposition is a measure of all the particles

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deposited. For particles of aerodynamic diameter of about 3 µm, deposition is found to be approximately 50%. Larger particles are deposited in the upper respiratory tract, while smaller particles deposit in the lower airways and alveoli. Clearance of larger particles from the bronchial tree is by mucociliary transport into the pharynx. This typically takes about 24 hours from the time of deposition to clearance, but depends on status of the mucociliary clearance. Transport of smaller particles by macrophages from the alveoli may take much longer.

A large percentage of ultrafine (and nano-) particles are exhaled, i.e., are breathed out again, although a significant number of inhaled particles are deposited in the respiratory tract. In addition, there is evidence of uptake of these very small particles into the skin and the olfactory system, as well as through the gut wall following ingestion. Uptake through the skin may require special attention because of the large surface area available for absorption. Uptake through the olfactory mucosa may be followed by transport through nerves of the olfactory system into the brain. There are medical uses of nanoparticles that may entail injection of nanoparticles.

Chemistry of nanoparticles

The term 'nanoparticles', as mentioned above, has been applied particularly to man-made ultrafine particles containing, e.g., titanium oxide, zinc oxide, silica, and carbon. Sometimes these are called 'engineered nanoparticles', implying nanoparticles manufactured to have definite properties or a definite composition. The surface properties of nanoparticles are likely to be important in determining their toxicity. New and improved techniques will be required to characterize them and consequently their toxicologically significant properties.

There has been particular concern about nanotubes that consist of graphene sheets which form single-walled (SWNT) or multi-walled (MWNT) nanotubes. The single-walled carbon nanotubes (SWCNT) have unique properties, are stiffer than diamonds, and are estimated to be 10 times stronger than steel. In the preparation of carbon nanotubes (CNTs) metals, amorphous carbon and other compounds are used. Nanotubes may include catalytically active metals like nickel and iron. Both nickel and iron catalyse oxidative reactions. Ultrafine particles (and nanoparticles) can adsorb lipopolysaccharides and cause pro-inflammatory effects. Contamination of gold nanoparticles with lipopolysaccharides can interfere with the immune system.

Risk assessment of nanoparticles

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Respirable nanoparticles may be more toxic than larger particles of equivalent mass and of the same composition. Thus, characterising the exposure to such particles involves more than simply analysing the content of molecules or elements. It is unclear to what extent macrophages can phagocytose different kinds of ultrafine or nanoparticles of different types, sizes, and shapes. Nanoparticles have been reported to contribute to development of coronary heart disease, but again the harm of specific nanoparticles has not been well characterized. Asbestos fibres represent naturally occurring ultrafine particles of well-established toxicity and carcinogenicity. Their length and diameter are crucial to their toxic effects, and similar defined dimensions may determine the toxicity of nanoparticles. It may be that the surface area of nanoparticles is the best measure of dose, but the lesson learned from the extensive characterization of the health effects of asbestos fibres is that the three-dimensional shape of nanoparticles will likely affect their toxicity.

Whether nanoparticles can generally cause cancer remains to be established. Some metal compounds that are used for making nanoparticles, e.g., titanium oxide, have been classified as potential human carcinogens, and nanoparticles incorporating such metallic elements will require further careful assessment from the regulatory point of use.

Further considerations

The study of the toxic effects caused to living systems, particularly human beings, by exposure to nanoparticles brings together chemistry, bioengineering and toxicology to predict harmful effects which may occur as exposures of humans and their environment increase. The aim of nanotoxicology is to define the steps required to avoid such effects. There is an immediate problem in defining appropriate units of dose and exposure. Mass or substance measurement is not appropriate since it does not correlate in any simple way to the number of particles or their surface area, both of which are likely to be important factors in biological interactions. Surface shape is also important in any interaction of particulates with living cells and tissues. Such interaction involves surface chemistry and electric charge, and so these become further considerations in nanotoxicology. After interaction with the body surface, wherever this may occur, further reactions will be affected by the structure of the nanoparticles, including such physical characteristics as crystallinity and porosity. Put another way, after the initial interaction, distribution of nanoparticles in the body of an organism will be a function of both the surface characteristics of the particles and the surface characteristics of different parts of the body. In addition, a critical size may exist beyond which the movement of nanoparticles is restricted. Thus, dose-effect and dose-response studies may require to be

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stratified according to defined particle characteristics including mass, number, size, shape, surface area, crystallinity, and, if known, mechanism of action.

The above factors have not yet been well characterized. There will surely be characteristics of nanoparticles that will target them to specific organs, tissues and cells; these remain to be elucidated. The presence of components such as metals present in nanotubes may determine their health effects. An increased risk of cardiopulmonary diseases associated with nanoparticle exposure requires special attention, if it is not to become a serious problem. This raises questions about the ethical justification of experiments that have been carried out with volunteers to study the effects of ultrafine particles and that have demonstrated harmful effects, even if apparently minor.

Conclusions

Although there may be common risks associated with all exposures to nanoparticles, each type of nanoparticle must be treated individually for the assessment of health risks. Traditional toxicological testing is, in general, inappropriate when carrying out risk assessments for nanoparticles. The general properties of nanoparticles as a group require the design and validation of a set of special toxicity tests for adequate risk assessment. Nanoparticles designed for drug delivery or as food components should be given particular attention in this regard.

13. PERSISTENCE

IUPAC definition

persistence

Attribute of a substance that describes the length of time that the substance remains in a particular environment before it is physically removed or chemically or biologically transformed.

Explanatory comment

This section will concentrate on substances which have the potential to be either water or fat soluble or may become so as a result of chemical reactions in the environment or of biotransformation by living organisms. Persistence is also associated with insoluble particulates (usually dusts, e.g. silica, or fibres, e.g. asbestos) which may have adverse effects following deposition in the lung and inadequate removal by lung clearance mechanisms. Such

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particulates may also cause harm following reactions with the gut and, if small enough (see Nanoparticles), with other organs after absorption into the body. In nonmammalian organisms, they may interact with gills and body or cell surfaces to produce adverse effects. They may be phagocytosed with consequences for both prey and predators. They may trap nutrients, restricting their bioavailability. They may act as catalysts for reactions which may be biologically harmful or beneficial. To consider these possibilities properly requires a book and is not possible further here.

Persistence of potentially water-soluble or fat-soluble substances reflects the ability of such substances to remain in an environment unchanged, or changed to another potentially toxic form, for a long period of time, sometimes for many years. The longer a substance persists, the greater the potential for exposure of humans and other organisms, and for bioaccumulation to toxic concentrations. The persistence of a substance is usually measured or estimated as a half-life in air, water, soil, and (or) sediment. The persistence of a substance in air is important because, once in the atmosphere, it may travel global distances from its original point of release. Persistence in air is a property of volatile persistent organic pollutants (POPs). POPs have been defined as organic chemicals that are stable in the environment, are liable to long-range transport, may bioaccumulate in human and animal tissue, and may have significant impacts on both human health and the environment. Such chemicals are resistant to hydrolysis and to breakdown by the action of ultraviolet light and oxygen.

It is important to distinguish between persistence in a single medium and overall environmental persistence. Persistence in an individual medium is controlled by transport of the substance to other media, as well as by transformation to other chemical species. Together, these factors determine the residence time in the medium. Persistence in the environment as a whole is a distinct concept. The environment behaves as a web of interconnected media, and any substance released to the environment will become distributed in these media in accordance with its intrinsic reactivity and physicochemical properties.

Multimedia mass balance models are the most convenient means for determining the overall environmental persistence from information on sources and loadings, chemical properties and transformation processes, and inter-media partitioning [33]. It is important to note that these models reflect an equilibrium or steady-state situation within a defined geographic space (which is why these models are sometimes referred to as box models). On the global scale, an equilibrium situation will never be reached and a steady-state situation is not likely.

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Multimedia mass balance models may be subdivided into three categories on the basis of the level of complexity addressed:

Level I - Predicts the equilibrium distribution of a fixed quantity of conserved chemical, in a closed environment at equilibrium, with no degrading reactions, no advective processes, and no inter-media transport processes (e.g., no wet deposition, or sedimentation).

Level II - Predicts the result of continuous discharge of a substance at a constant rate into the total environmental system until steady-state and equilibrium conditions are reached at which the input and output rates are equal. Loss of the substance is assumed to occur by degradation reactions and by advection. Inter-media transport processes (e.g., wet deposition, or sedimentation) are not quantified.

Level III - Predicts the result of a chemical being continuously discharged at constant rates independently into each environmental medium such as air, water, and soil, until it achieves a steady state in which input and output rates are equal. Loss is assumed to be by degradation reactions and advection. Unlike the Level II model, occurrence of equilibria amongst media is not assumed and, in general, each medium is at a different fugacity. A mass balance applies not only to the system as a whole, but also to each compartment. Rates of inter-media transport are calculated using socalled D values introduced by Donald Mackay [33] to derive expressions for transport between media (e.g., evaporation) or for transformation (e.g., biodegradation). D values are expressed in mol / h Pa and contain information on mass transfer coefficients, areas, deposition and resuspension rates, diffusion rates, and soil runoff rates. When a substance present in a phase is subject to a number of transport and transformation processes, each has its own D value and the relative importance of each process is made clear by the magnitude of the D value (see [33]). Note that, unlike the assumptions in Level II models, inputs to each medium in Level III models are assumed to occur independently. In Level II only the total input into the whole system is considered.

An estimate of overall environmental persistence may be obtained using a Level III Mackay fugacity model [33] for a standardized set of environmental conditions in which net advection and sediment burial are ignored. The purpose of such a model is to provide the user with a measure of overall environmental persistence based on chemical reactivity only. This may be useful in making decisions for the purpose of pollution prevention because ultimately, on a global scale, there is no advective removal. If advective loss is included, the residence time is reduced and may give a misleading impression of a short persistence. Advective losses merely relocate the chemical; they do not destroy it.

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It is important to note the difference between residence time and half-life in each environmental medium when interpreting the overall persistence. The half-life is a measure of how fast a chemical is eliminated from each environmental medium by chemical transformation or degradation. The residence time in each medium includes the half-life but it also takes into account transport into and out of that medium. The residence time may, therefore, be substantially different from the media-specific half-life for some chemicals.

Bioaccessibility, bioavailability, bioaccumulation, bioconcentration, and biomagnification

These terms are discussed in other Sections of this document but must be referred to here because of their central importance in relation to the consequences of persistence of POPs and similar substances. POPs, if they are bioaccessible, have the physicochemical properties, notably lipid solubility and octanol / water partition coefficient (see 'Absorption' in Part 1 of this explanatory dictionary[1]), required to be readily bioavailable. The same properties facilitate uptake by living organisms (bioaccumulation and bioconcentration) and, consequently, biomagnification through food webs, to potentially toxic levels in predators.

Toxicity

Toxicity is a function of the concentration of the chemical and the duration of exposure. Because persistent and bioaccumulative chemicals are long-lasting substances that can build up in the food chain to high levels, they have a higher potential to express toxicity and to be harmful to humans and the ecosystem.

Media

There are four major environmental compartments in which a chemical may be found: air, water, soil, and sediment. Air is also referred to as the atmosphere or atmospheric compartment. The atmospheric compartment includes the area closest to the Earth's surface (the troposphere) and beyond (the stratosphere). Water refers to surface water, and includes oceans, rivers, lakes, and ponds. This definition does not include aquifers found below the Earth's surface (groundwater).

Soil refers to the topmost layer (the first few inches) of the Earth's surface that is not covered by water. Soil includes all the material near the surface that differs from the underlying rock material. Sediment is the particulate matter found under surface water.

Usually soil is found to be aerobic (oxygenated) and sediment is anaerobic (free of oxygen). However, soil can become anaerobic and sediment may be aerated following dredging. This can have a profound effect on persistence of substances.

Half-life

Half-life is the length of time it takes for the concentration of a substance to be reduced by one-half relative to its initial level, assuming first-order decay kinetics. It is a useful approximation to consider "complete removal" as taking approximately six half lives, i.e., $1 / 2^6 = 1 / 64$ of the amount of substance remains. This may be quite misleading for highly toxic substances. Laboratory-based half-lives for any given substance are generally related only to reactivity in a given defined medium under one or a few sets of defined conditions. Laboratory studies may also fail to replicate the microbial potential for biodegradation of an environment at risk.

Percentage in each medium

Once a substance is released into the environment, it may move from one environmental compartment to another. For example, volatile substances deposited on land will move into the air. Some substances such as dichlorodiphenyltrichloroethane (DDT) or dichlorodiphenyltrichloroethane (DDE) enter the air as vapor in hot climates and then condense in the cold air of the Arctic and Antarctic, or in the snow at the top of high mountains. Water soluble substances will end up in rivers and the sea. The expected distribution of a substance in air, water, soil, and sediment is expressed as the estimated percentage in each medium relative to the total amount in the environment. The amount in each medium at steady state may be calculated using the Mackay fugacity models available freely from the website of the Canadian Environmental Modeling Centre [34].

14. PHARMACOGENETICS AND TOXICOGENETICS

IUPAC definitions

pharmacogenetics

Study of the influence of hereditary factors on the effects of drugs on individual organisms.

After [4]

toxicogenetics

Study of the influence of hereditary factors on the effects of potentially toxic substances on individual organisms.

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Explanatory comment

The term pharmacogenetics acknowledges the role of genetic factors in pharmacology, especially in drug metabolism. In the 1950s it was recognized that certain individuals (about 1 in 3500 Caucasians) had an impaired ability to hydrolyze the muscle relaxant succinylcholine, leading to prolonged muscle paralysis after anesthesia. This was shown to be due to a genetic variant of the phase I enzyme, butyrylcholinesterase, and thus was pharmacogenetics born. Other examples soon followed. One of the best-known examples of genetic diversity of drug metabolism is variation in isoforms of the phase II enzyme *N*-acetyltransferase that affects the rate at which certain drugs such as hydralazine, isoniazid, and procainamide are acetylated. Based on differences in half lives of these drugs, the population is divided into "slow acetylators" and "fast acetylators". Another example is provided by thiopurine methyltransferase, which metabolizes 6-mercaptopurine and azathioprine, drugs used in treating leukemia. In thiopurine methyltransferase deficiency, metabolism by alternative pathways produces a metabolite that is toxic to the bone marrow and potentially leads to bone marrow suppression. In most affected people, this deficiency results from one of three variant alleles. One in 300 people have two variant alleles and need only 6-10% of the standard dose of the drug. They are at risk of developing severe bone marrow suppression.

The cytochrome P450 enzymes are an extremely important group in phase I metabolism and their genetics have been widely studied. One of the best examples is CYP2D6. Many drugs as diverse as codeine, metoprolol, and dextromethorphan are metabolized by CYP2D6. Five-to-10% of Caucasians show decreased metabolism of the antihypertensive debrisoquin, with increased ratios of parent drug to 4-hydroxydebrisoquin in the urine as a consequence of genetic variation in the CYP2D6 gene that leads to a less active or inactive cytochrome. They have an exaggerated response to debrisoquin resulting in hypotension. Bearing in mind the large number of drugs metabolized by CYP2D6, such patients are classed as "poor metabolizers". On the other hand, some people have multiple copies of the CYP2D6 gene. They have an inadequate response to standard doses of drugs metabolized by CYP2D6, and are termed "extensive metabolizers". Relatively infrequent in Northern Europe, the occurrence of multiple copies of the CYP2D6 gene is as high as 29% in East African populations.

Allelic variants are now known that affect virtually all classes of drugs. These not only affect metabolizing enzymes, but drug targets, and transporters that determine absorption, distribution, and excretion of drugs. Single nucleotide polymorphisms (SNPs) are defined as

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allelic variants with a frequency of greater than 1 %. There are approximately ten million SNPs in the human genome. Association studies linking genotype with drug-response phenotype are now being considered on a broad scale. Drug efficacy is, of course, affected by metabolism, and it is hoped that by understanding individual differences in metabolic profiles, therapy can be targeted more effectively to the individual. Adverse and idiosyncratic drug reactions, too, often result from genetic variation in drug metabolism.

Extending the discussion beyond drugs, the toxicity of any substance often depends on its disposition and metabolism, and hence on genetic variations in the responsible carriers, transporters, and enzymes. In the past, occupational exposure to benzidine occurred, and slow acetylators were at greater risk of developing benzidine-related cancer of the bladder. Polymorphisms in CYP1A1 (aromatic hydrocarbon hydroxylase) are associated with variable risk of cancer caused by polyaromatic hydrocarbons. The glutathione-S-transferase allele GSTM1 has been associated with lung cancer, e.g., in exposure to cigarette smoke, and skin cancer caused by arsenic in drinking water. NAT1 and NAT2 alleles of *N*-acetyltransferase are involved in metabolizing aromatic and heterocyclic aromatic amines, and have been associated with lung cancer in smokers and also colorectal cancer.

The terms pharmacogenomics and toxicogenomics refer to the study of the genome as it relates to pharmacology and toxicology. That is, they refer to the application of the science of genomics to understanding differences in toxicity and drug metabolism. It is noted, however, that in common usage a clear distinction between, for example toxicogenetics and toxicogenomics is not always made. Apart from understanding individual susceptibilities, the techniques of toxicogenomics promise new mechanistic insights into mechanisms of toxicity. Using microarrays to study gene expression at the RNA level can reveal early changes that precede clinical or gross histopathological changes. This in turn could lead to development of early biomarkers of toxic injury. With more prolonged exposure, adaptive changes may be expected in clusters of genes that represent signatures characteristic of certain pathways of toxicity. An interesting newer application of toxicogenomics is to assessing validity of extrapolation from test organisms to man. The genomic sequences of humans and, for example, mice are compared and conserved regions identified. These are used to construct microarrays of orthologous* genes for the two species. Measurement of gene expression against these microarrays in both species following exposure to a toxic substance allows assessment of conserved toxicological endpoints at a molecular level.

Finally, it will be realized that there are ethical issues in determining individual differences in susceptibility to toxic substances. For example, if an individual had an increased

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susceptibility to a toxic effect from an exposure likely to occur in a particular work place, the employer might decide to limit employment, independently of the worker's acceptance of the risk.

**Note*: 'Orthologous' is a term applied to genes (in different genomes) that are derived from a common ancestral sequence (homologous) by phylogenetic descent. It may also be applied to proteins or other gene products encoded by such genes. A closely related term is 'paralogous' that is applied to genes or nucleotide sequences within a single genome that arose from a single ancestral sequence by duplication and subsequently evolved independently. Again, this term may be applied to proteins or other gene products encoded by such a gene, or by the same gene in different individuals

15. REPRODUCTIVE TOXICITY

IUPAC definitions

reproductive toxicant

Substance or preparation that produces non-heritable *adverse effects* on male and female reproductive function or capacity and on resultant progeny.

reproductive toxicity

Ability of a physical, chemical, or biological agent to induce adverse effects in the reproductive system.

reproductive toxicology

Study of the nonheritable *adverse effect*s of substances on male and female reproductive function or capacity and on resultant progeny.

Explanatory comment (See also Section 7, Endocrine Modification, and Section 18, Teratogenicity)

For purposes of classifying toxicities, the known induction of genetically based heritable effects in the offspring is considered under the heading of 'Mutagenicity' (see Section 11 above) since it is generally considered appropriate to include such effects under the separate hazard class of germ-cell mutagenicity. This leaves the classification of reproductive toxicity to cover two groups of effects as in the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) [35] and as proposed as working definitions in IPCS/EHC Document N° 225, Principles for Evaluating Health Risks to Reproduction Associated with

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Exposure to Chemicals [36]. The groups of effects covered are:

1. Adverse effects on sexual function and fertility;

2. Adverse effects on development of the offspring.

Adverse effects on sexual function and fertility may include, but not be limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems. Adverse effects on development, taken in the widest sense, includes any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, up to the time of sexual maturation. However, classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women and for men and women of reproductive capacity. Thus, for regulatory classification, developmental toxicity means adverse effects induced during pregnancy, or as a result of parental exposure. The major manifestations of developmental toxicity include (a) death of the developing organism, (b) structural abnormality, (c) altered growth, and (d) functional deficiency.

Some reproductive toxic effects cannot be clearly assigned to either impairment of sexual function and fertility or to developmental toxicity. Nevertheless, substances with these effects would still be classified as reproductive toxicants. Adverse effects on or via lactation are also included in reproductive toxicity, but for regulatory classification, such effects are treated separately. This is because it is desirable to be able to classify chemicals specifically for an adverse effect on lactation so that a specific hazard warning about this effect can be provided for lactating mothers.

Thus, from the above considerations, reproductive toxicants are classified into categories as follows:

CATEGORY 1: Known or presumed human reproductive toxicant.

This Category includes substances which are known to have produced an adverse effect on sexual function and fertility or on development in humans or for which there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. For regulatory purposes, a substance can be further distinguished on the basis of whether the evidence for classification is primarily from human data (Category

1A) or from animal data (Category 1B).

CATEGORY 1A: Known human reproductive toxicant.

Placing a substance in this category is based largely on evidence from humans.

CATEGORY 1B: Presumed human reproductive toxicant.

The placing of the substance in this category is based largely on evidence from experimental animals. Data from animal studies should provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or, if occurring together with other toxic effects, the adverse effect on reproduction is considered not to be a secondary non-specific consequence of these other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

CATEGORY 2: Suspected human reproductive toxicant

This Category includes substances for which there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility or on development, in the absence of other toxic effects, or, if occurring together with other toxic effects, the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects, and where the evidence is not sufficiently convincing to place the substance in Category 1. For instance, deficiencies in the study may make the quality of evidence less convincing, and, in view of this, Category 2 could be the more appropriate classification.

Effects on or through lactation

Effects on or through lactation are a separate toxicity classification. Substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, may be classified to indicate this. Classification can be assigned on the basis of:

a) absorption, metabolism, distribution and excretion studies that would indicate the likelihood the substance would be present in potentially toxic levels in breast milk; and (or)

b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and (or)

c) human evidence indicating a hazard to babies during the lactation period.

Interpreting the available data on reproductive toxicity

Classification as a reproductive or developmental toxicant should be used for chemicals which have an intrinsic, specific property to produce an adverse effect on reproduction or development, and not if such an effect is produced solely as a non-specific secondary consequence of other toxic effects. In assessing toxic effects on developing offspring, it is important to consider the possible influence of maternal toxicity.

For human evidence to justify Category 1A classification there must be reliable evidence of an adverse effect on reproduction in humans. Evidence used for classification should be from well conducted epidemiological studies which include the use of appropriate controls, balanced assessment, and due consideration of bias or confounding factors. Less rigorous data from studies in humans should be supplemented with adequate data from studies in experimental animals and classification in Category 1B should be considered.

In some reproductive toxicity studies on experimental animals the only effects recorded may be considered to be of low or minimal toxicological significance and classification may not be justified. These include, e.g., small changes in semen parameters or in the incidence of spontaneous defects in the fetus, small changes in the proportions of common fetal variants such as are observed in skeletal examinations or in fetal weights, and small differences in postnatal developmental assessments.

Data from animal studies should provide clear evidence of specific reproductive toxicity in the absence of other, systemic, toxic effects. However, if developmental toxicity occurs together with other toxic effects in the dam, the potential influence of the generalised adverse effects should be assessed. The preferred approach is to consider adverse effects in the embryo or fetus first, and then to evaluate maternal toxicity, along with any other factors, which are likely to influence these effects. Developmental effects that are observed at maternally toxic doses should not be automatically discounted. Discounting developmental effects that are observed at maternally toxic doses can only be done on a case-by-case basis once a causal relationship is established or disproved.

If appropriate information is available it is important to try to determine whether developmental toxicity is due to a specific maternally mediated mechanism or to a non-specific secondary mechanism, like maternal stress and the disruption of homeostasis. Generally, the presence of maternal toxicity should not be used to negate findings of embryo or fetal effects, unless it can be clearly demonstrated that the effects are secondary non-specific effects. This is

especially the case when the effects in the offspring are significant, e.g., irreversible effects such as structural malformations. In some situations it is reasonable to assume that reproductive toxicity is due to a secondary consequence of maternal toxicity and discount the effects, e.g., if the chemical is so toxic that dams fail to thrive, they are incapable of nursing pups, or they are prostrate or dying.

Fertility

Fertility includes aspects of spermatogenesis and oogenesis. Fecundity, fertility and "sperm quality" are distinct parameters that are not equivalent and are frequently confused. Sperm count and sperm quality do not necessarily predict whether conception will take place for a given couple. A 'fertile couple' has conceived at least one child. Fecundity is the ability of a couple to conceive a child and is often evaluated by the time necessary to achieve pregnancy (Time to Pregnancy (TTP)).

Infertility may be thought of as a negative outcome. A couple that is infertile is unable to have children within a specific time frame. Therefore, the epidemiological measurement of reduced fertility or fecundity is indirect and is accomplished by comparing birth rates or time intervals between births or pregnancies. This may be done by several methods, including the standardized birth ratio (SBR; also referred to as the standardized fertility ratio) and the length of time to pregnancy or to birth (TTP). In these evaluations, the couple's joint ability to produce children is estimated. The SBR compares the number of births observed with those expected based on the person-years of observation, preferably stratified by factors such as time period, age, race, marital status, parity, and contraceptive use. The SBR is analogous to the standardized mortality ratio, a measure frequently used in studies of occupational cohorts, and has similar limitations in interpretation. The SBR is obviously less sensitive in identifying an effect than semen analyses.

"Time to pregnancy" is also a useful tool and has clearly demonstrated a difference in fecundity among smokers and non-smokers. Analysis of the time between recognized pregnancies or live births is a more recent approach to indirect measurement of fertility. Because the time between births increases with increasing parity, comparisons within birth order (parity) are more appropriate.

A specific effect that may reduce fertility is damage to sperm chromatin. This is assessed by the sperm chromatin structure assay (SCSA), first described by Evenson et al. (1980). SCSA is a flow cytometric technique which identifies the spermatozoa with abnormal chromatin packaging, defned by susceptibility to acid denaturation *in situ*. Acridine orange

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staining is used, after a low pH challenge, to distinguish between denatured (red fluorescence = single stranded) and native (green fluorescence = double stranded) DNA regions in sperm chromatin, the former being a result of DNA breaks and (or) of changes in protamine quantity and composition and (or) of an insufficient level of disulfide groups. The level of DNA breaks is conveniently expressed by the DNA fragmentation index (DFI), which is the ratio of red to total (red plus green) fluorescence intensities in the flow cytometric analysis. In two independent studies, DFI levels > 30 ± 12 were incompatible with fertility *in vivo* of otherwise normal sperm.

In reproductive toxicology, it is important to be aware of factors such as the genotype of the fetus and possible interactions between genes and the environment. Most knowledge is based on animal data from laboratory animals. The differences in reproductive toxicity between species are exemplified by the consequences of exposure to the drug, thalidomide. Humans and monkeys are very sensitive to the effects of thalidomide while mice and rats are not. Thus, tests on mice and rats indicated that thalidomide had low toxicity and failed to identify its toxic effects on the fetus. The ability to cause gross structural (anatomical) malformations in the developing embryo or fetus exemplified by the effects of thalidomide is called teratogenicity. Teratogenicity is discussed below (Section 18).

By the time metabolic pathways and excretion are fully developed in the newborn, the probability of adverse effects may decrease for some toxicants and increase for others. The kidney does not have full function before delivery and is not fully functional during the immediate postnatal period. This leads to limited excretion of potential toxicants *via* the urine. Metabolism and excretion of some potentially toxic substances like caffeine, for example, is very limited in the fetus. An example of the consequences of deficient metabolism in the fetus is the gray baby syndrome seen in newborn infants. In 1959 this syndrome was reported in association with the use of the antibiotic chloramphenicol. Infants developed abdominal distension, vomiting, cyanosis, cardiovascular collapse, irregular respiration and subsequent death shortly after therapy with chloramphenicol was started. Pharmacokinetic studies in the neonate showed accumulation of chloramphenicol in plasma due to poor drug metabolism. In other cases when bioactivation of xenobiotics can take place to form active metabolites, toxicity may also be increased.

It is very important to be aware of differences between individuals and between species. Studies combining molecular biology with classical epidemiological approaches have demonstrated the existence of allelic variants for developmentally important genes that may enhance the susceptibility of the embryo. For example, the association between heavy maternal

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cigarette smoking (>10 cigarettes / day) and cleft lip and (or) palate in the offspring is marginally significant until an allelic variant for tumor growth factor (TGF-) is considered. The combination of smoking and the uncommon variant for the gene raises the odds ratio to a highly significant level. With regard to species variation, endocrine disruption and subsequent reproductive toxicity have been identified as an effect of environmental chemicals, such as DDT and PCBs, that can cause feminizing effects in the population (see Section 7 - Endocrine Modification).

Historically, reproductive toxicity in relation to development has concentrated on the effects of exposure of females. However, it is now clear that exposure of males to certain agents can adversely affect their offspring and cause infertility and cancer. The hazards associated with exposure to ionizing radiation have been recognised for nearly a century and there is evidence for harmful effects of paternal exposure from X-ray studies in mice resulting in heritable tumors. In humans, smoking fathers appear to give rise to tumors in the F1 generation, i.e., the first filial generation produced by two parents. Cyclophosphamide, 1,3-butadiene, and urethane have been tested in rodents and, after exposure of males only, each compound produced an adverse effect in F1 male offspring.

In order to protect the public from the harmful effects of reproductive toxicants, labeling must include appropriate warnings. Directives have already been published by the EU covering about about 70 CMR (carcinogenic, mutagenic and reprotox) substances. Recent changes to the cosmetics laws in some countries have banned the use of CMR substances in cosmetics. There is continuing revision of CMR classification because of the international movement, promoted by the United Nations, towards a Globally Harmonized System of Classification and Labelling of Chemicals (GHS) [35].

16. RISK ASSESSMENT, RISK MANAGEMENT, AND SAFETY

IUPAC definitions

risk assessment

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

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

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risk assessment management process

Global term for the whole process from hazard identification to risk management.

risk management

Decision-making process involving considerations of political, social, economic, and engineering factors with relevant risk assessments relating to a potential hazard so as to develop, analyze, and compare regulatory options and to select the optimal regulatory response for safety from that hazard.

Note: Essentially risk management is the combination of three steps: risk evaluation; emission and exposure control; risk monitoring.

safety

Reciprocal of risk: practical certainty that injury will not result from a hazard under defined conditions.

Note 1: Safety of a drug or other substance in the context of human health: the extent to which a substance may be used in the amount necessary for the intended therapeutic purpose with a minimum risk of adverse health effects.

Note 2: Safety (toxicological): The high probability that injury will not result from exposure to a substance under defined conditions of quantity and manner of use, ideally controlled to minimize exposure.

Explanatory comment

Risk assessment, risk management and risk communication, combined as a process for controlling situations where an organism, system or (sub) population could be exposed to a hazard, has been defined as 'risk analysis' by IPCS and OECD [37], following the precedent of the Joint Expert Committee on Food Additives (JECFA) of the World Health Organization (WHO). This use of the term 'analysis' must be deprecated as the process is actually one of integration and not analysis. By any conventional usage, 'analysis' refers to 'the resolution or breaking up of anything complex into its various simple elements', as defined in the current Oxford English Dictionary [38]. There is no precedent for the perverse usage in the OECD / JECFA definition which tends to confuse rather than to clarify thought.

The concept of risk has already been discussed in part 1 of this explanatory dictionary [1]. Here the concern is with the provision of safety, i.e., the reduction of risk to an acceptable level. 'Acceptability' of risk varies from person to person and is , therefore, subjective, but risk assessment, as defined, should be objective. Thereafter, risk management should aim to reduce risk to levels acceptable to those at risk. This raises issues of risk communication which are

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outwith the remit of this explanatory dictionary since they involve consideration of public relations and the related psychological issues which vary as much as the groups at risk. Each group at risk has a different perception of risk, often dependent upon deeply rooted fundamental ideas, which are not open to debate, and must be accommodated for effective communication of risk and how it can be minimized.

Since all substances are toxic at a high enough dose, there is none for which one can guarantee absolute chemical safety, i.e., no risk of harm. Thus, the aim of chemical safety management is to reduce the risk associated with defined chemical processes to an acceptable minimum. This requires consideration of intrinsic hazard and the probability of exposure at a level that may be high enough to cause harm under defined conditions; in other words, risk assessment. Risk assessment is followed by management of the risk situation to minimize risk and therefore to optimize safety.

Risk to be considered for risk management purposes should include known quantifiable risks, known, but not quantifiable risks (which may be identifiable by testing), and unknown risks (which are identifiable only once the chemical is in use, i.e., through risk monitoring). Risk monitoring is an essential part of risk management. An example of risk monitoring is pharmacovigilance, another is observational environmental monitoring.

Risk assessment

There are four steps in risk assessment:

1. Hazard identification. Identify agents responsible for potential harmful effects, collect information on their physicochemical and harmful properties, on the group(s) of people or other living organisms liable to exposure, and on the exposure circumstances (e.g., environmental conditions);

2. Risk characterization. Identify potential effects of possible exposures and quantify dose-effect and dose-response relationships;

3. Exposure assessment. Quantify exposure, by measurement or biological monitoring if it is already occurring or by predictive modeling if it is not;

4. Risk estimation. Quantify risk in the exposed group, making clear any assumptions involved. Ideally, this should give a statistically based measure of probability of harm to the defined group under the defined circumstances. Often this is not possible and a *risk quotient* is calculated which is related to probability of harm but only as a rough approximation.

Risk management

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Following risk assessment, risk management is the process of deciding how to reduce to a practicable minimum the risk assessed. Again, various stages have been defined for this process:

1. Risk evaluation. Comparison of risks resulting from different actions and their probable consequences. This includes comparison of costs and benefits. These comparisons are used to decide what might be an acceptable risk level, bearing in mind the any irreducible level of risk that may already exist;

2. Exposure control. Actions required to keep exposure below the level associated with unacceptable risk. Normally this will be an exposure level which causes no risk but for mutagens and carcinogens a risk of 1 in a million is generally regarded as acceptable.

3. Risk monitoring. Observation, assessment, measurement to determine how effective the actions taken to control exposure have been. This may include biomonitoring;

4. Risk management evaluation and improved exposure control. There is always an element of risk associated with failure of control for which accident (emergency) planning should be in place. If previous actions have not been as effective as expected, further actions must be taken to reduce exposure. Then stage 3 is repeated and so on until exposure control, and thus safety, is acceptable. Risk management is concerned with the consequences of risk evaluation. If risks are evaluated as broadly acceptable, no further management action may be needed. However, management may be required to reduce tolerable risks to a level at which they are broadly acceptable. Even if it is not possible to do this, risks should always be reduced as far as is reasonably practicable.

Risk management is a combination of prevention (or minimization) of exposure to a potentially harmful amount or concentration of a chemical and prevention (or minimization) of any consequences (ill health and or pollution). These objectives are often achieved by one or more of the following approaches:

a) Substitution (favored in the European Risk Evaluation and Authorization of CHemicals (REACH) Regulation). Substitution of a substance by a less hazardous alternative or use of an alternative, less risky process, would seem to be an obvious way to reduce risk, but such a change may reduce one risk at the cost of increasing another. For example, chlorofluorocarbons, which attack the ozone layer but are of low toxicity to humans, were replaced as a refrigerant by ammonia that is highly toxic to humans and has injured people following accidental releases. DDT, which is of low toxicity to humans but poisons birds of prey after biomagnification, was replaced as an insecticide by organophosphates which are highly toxic to humans. Now, DDT is being used again, with more care than before, since it is

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the most effective insecticide against *Anopheles* mosquitoes which were spreading malaria killing millions of people. Fire retardants with a potential for human toxicity may be substituted by less effective fire retardants because of fears of human toxicity and this may result in more fires, with accompanying mortality. These are a few examples of problems in substituting one substance by another that is apparently less hazardous. Perhaps the biggest problem in looking for a substitute for a given substance is that substances for which toxicity data are not fully available will always appear to be safer than those that have been thoroughly investigated. Often assurance that there is "no evidence of toxicity" simply means that available data are minimal and approximate to "no evidence" at all on which to make an informed judgment. It should be remembered that 'absence of evidence' is not 'evidence of absence';

b) Change of process. Changing the process may prevent or reduce exposure and (or) minimize emissions. Change may involve improving process containment or improving ventilation, and (or) secondary containment, e.g., collecting a substance before it is vented or discharged, perhaps by means of suitable filters, followed by reclamation and (or) recycling. In some circumstances, dilution may permit hazardous waste material to be safely discharged to an appropriate receiving environment where it can be degraded without harm;

c) Use of personal protective equipment. This is the least favored approach to reducing risk. Ideally, any exposure occurring during the use of a substance should be lower than that which can harm humans or their environment. Any process which requires wearing protective equipment, clothing, gloves or footwear is intrinsically of high risk. Care must always be taken to ensure that the equipment, clothing, etc., are appropriate and functioning properly.

As a general principle substitution is preferred to change of process, which is preferred to use of protective equipment. Measurements of concentrations of substances in the immediate environment, where the chemical is used, and health and ecological monitoring are necessary to ensure that controls are adequate. Biomonitoring (see above) may also be used, but invasive biomonitoring is generally not welcomed by people, and may itself cause undue stress on workers.

Risk management must include measures to ensure safety (and pollution) awareness amongst those at risk, especially members of the general public. Such awareness is dependent on public perceptions, as already mentioned. There are prejudices to be overcome, e.g., that manmade chemicals are more toxic than those that occur naturally, or that one cannot have too much of a substance which is essential to health such as a vitamin, or even water. Perhaps the greatest prejudice is that hazardous substances should be avoided on the basis of the

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'precautionary principle'. Since every substance is hazardous at some exposure level, this prejudice is, in essence, suicidal. Thus, risk management demands public education to ensure that every person at risk understands the rationale of regulatory requirements and thus implements them in an appropriate manner.

17. SPECIATION: CHEMICAL AND BIOLOGICAL

IUPAC definitions

speciation (in chemistry)

Distribution of an element amongst defined chemical species in a system.

speciation (in biology)

1. Systematic classification of of groups of organisms of common ancestry that are able to reproduce only among themselves, and that are usually geographically distinct.
2. Segregation of living organisms into groups that are able to reproduce only among themselves, and that are usually geographically distinct.

species (in biology)

In biological systematics, group of organisms of common ancestry that are able to reproduce only among themselves, and that are usually geographically distinct.

species (in chemistry)

Specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure.

Explanatory comment

Speciation has two distinct meanings, one in chemistry [39] and one in biology. Chemists speak of distinct species of an element referring to different chemical forms, whereas biologists use the term as derived from species as genetically distinct but closely related organisms. IUPAC has provided a definition of chemical species, and also recognizes the meaning of species in biology.

A. Speciation in chemistry

The terms "species" and "speciation" have become widely used in the chemical and toxicological literature, and it is now well established that the occurrence of an element in different compounds and forms is crucial to understanding the environmental and occupational toxicity of that element. A number of definitions of chemical speciation can be found in the

literature. In the past, the term "speciation" has been used to refer to "reaction specificity" (rarely); in geochemistry and environmental chemistry, to changes taking place during natural cycles of an element (species transformation); to the analytical activity of measuring the distribution of an element among species in a sample (speciation analysis); and to the distribution itself of an element among different species in a sample (species distribution). After a series of International Symposia on Speciation of Elements in Toxicology and in Environmental and Biological Sciences, the organizers formulated the definition "Speciation is the occurrence of an element in separate, identifiable forms (i.e., chemical, physical or morphological state)". The aim was to include determinants of reactivity, and produced a definition that goes well beyond speciation in a chemical sense and would include different phases of a pure substance, and even different-sized particles of a single compound. This selection of definitions illustrates the circularity in defining a species in terms of an entity, form, or compound and indicates the lack of prior consensus in use of the term speciation. To attempt to harmonize the field and offer at least partial solutions to the ambiguities present in some of the earlier definitions, IUPAC formulated and adopted the definitions cited above.

Fundamental to these concepts is the meaning of the term (chemical) "species". A chemical species is a specific form of an element that can be defined as distinct on several levels. Thus, at the lowest level two distinct species may differ only in their isotopic composition. In terms of human health and risk assessment, though, some structural aspects of speciation are more important than others. For instance, of much greater importance in toxicology than isotopic composition are differences in electronic or oxidation state. Also critical for understanding toxicity are differences in the molecular structures and complexes in which the element participates. Macromolecular species are excluded from the definition unless a macromolecular ligand is specifically defined. For example, a metal ion bound to two isoforms of a protein with defined amino acid sequences could be considered two species, but an ion bound to a polyelectrolyte such as humic acid or heparin would not be defined in terms of multiple species representing individual molecules in the heterogeneous and polydisperse population. In this case, it is advisable to refer to a fraction. "Fractionation" can be defined as the process of classification of an element according to physical (e.g. size, solubility) or chemical (e.g. bonding, reactivity) properties. In this context, "speciation" is defined as the distribution of an element among defined chemical "species" in a system, and "speciation analysis" is the analytical activity of identifying and (or) measuring the quantities of one or more individual chemical species in a sample.

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Some examples of the importance to toxicology of speciation at the level of oxidation state, inorganic and organic complex formation, and organometallic compounds are illustrative. The greatly differing toxicities of Cr^{III} (non-toxic at usual exposures in the cation $[Cr(H_2O)_6OH]^{2+}$) and Cr^{VI} in the chromate anion $(Cr^{VI}O_4)^{3-}$ (a carcinogen) result because chromate anion $(Cr^{VI}O_4)^{3-}$ is taken up more readily by cells through anion transporters and subsequently Cr^{VI} releases electrons during its intracellular reduction to Cr^{III}. On the other hand, As^{III} in arsenite is more toxic than As^{V} in arsenate, in part due to its greater ability to bind thiols in the lower oxidation state. Oxidation of Hg^0 vapour to Hg^{2+} by intracellular enzymes (e.g., in erythrocytes, neurons) causes the mercuric ion to become trapped in cells. Fe^{II} and Fe^{III} differ in their solubilities and have distinct transporter systems to get in and out of cells. Good examples of the importance of inorganic complexation are the various nickel compounds, which differ greatly in their carcinogenicity. For example, Ni_3S_2 is a potent carcinogen in rats but amorphous NiS is not. Nickel salts are immune sensitizers, and the species of nickel can affect the physical form and thus the route of effective bioavailability. For example, nickel tetracarbonyl is a gas that is readily inhaled and absorbed from the lungs. Complexation of metals with organic ligands can affect their availability for cell uptake and for excretion from the body. Organometallic compounds in general are lipophilic and one consequence of this is an ability to penetrate the blood-brain barrier. Thus, whereas mercuric salts are peripheral neurotoxicants, alkylmercurials are potent central nervous system toxicants, as are many other alkylmetallics such as those of lead and tin.

Strictly speaking, whenever an element is present in different states according to isotopic composition, electronic or oxidation state, and (or) complex or molecular structure, it must be regarded as occurring in different species. In practice, however, usage will depend on the relevance of the species differences for our understanding of the system under study. One would not generally describe a living organism or define an organic reaction mechanism by carbon speciation. Nevertheless, a pair of stereoisomers are certainly distinct species, with different biochemical properties (e.g., only *S*-aminoacids and *R*-sugars are used by living organisms), and if each formed a chelation complex with a metal ion, these would be referred to as distinct species of the metal. Further, while the definition of species is general, in practice it is used mainly in the context of metallic and metalloid elements. Usage of speciation terminology also depends on our ability to distinguish the various species analytically. This practical analytical consideration governs whether different species should be grouped together or measured separately. Separate measurement implies minimum lifetimes and thermodynamic

stabilities to allow detection, the values of which will change with developments in instrumentation.

B. Speciation in biology

In the taxonomic subdivision of organisms, species is the lowest level of distinction. It is an uncapitalized subdivision of *genus*, and refers to organisms that are so closely related genetically that they successfully breed amongst themselves and generally not with other species; thus they carry on the genetic lineage. As the definition underscores, this close genetic similarity arises from common ancestry. Geographic isolation allows for the development of distinct species that subsequently become unable to interbreed with others. Indeed, the variety of unique species Charles Darwin encountered on his voyage to the geographically isolated Galapagos Islands was fundamental to the development of evolutionary theory.

Speciation, then, refers to the processes by which new species arise through evolution. While these now include deliberate laboratory manipulations and interventions such as animal husbandry and genetic modification of crops, natural speciation is worthy of discussion. There are generally considered to be four mechanisms of natural speciation, defined according to the degree and nature of geographic separation of the population undergoing speciation (the "speciating population"): these are allopatric, peripatric, parapatric, and sympatric.

(i) Allopatric speciation occurs when a large population has been isolated by geographic barriers. If the barriers break down, allopatric species are found to have evolved so as to be unable to interbreed with other populations of what was originally the same species. They then constitute a new species.

(ii) Parapatric speciation refers to the evolution of two divergent species when geographic isolation is incomplete, there is some contact, but genetic separation still occurs because the heterozygote is not favoured by the prevailing environmental conditions.

(iii) Peripatric speciation occurs when small populations are isolated for various idiosyncratic reasons and stop breeding with the main population. They evolve in niches.

(iv) Sympatric speciation allows for species divergence without geographic separation. For example, groups of insects may start to feed on two different plant species growing in the same location, and eventually the different feeding groups become genetically distinct. A subtlety here is when different groups or races coexist but exploit different niches for behavioural reasons. This has been referred to as heteropatric speciation.

Two other terms should be mentioned. Cladagenesis refers to the branching of a new species when the founding one continues to exist. A clade is a group of organisms descending

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from a common ancestor. Anagenesis is the evolution of one species into another without divergence of the phylogenetic tree and with loss of the founder species.

While some see evolution as a gradual process, Stephen Jay Gould and others have proposed a theory of "punctuated equilibrium" that proposes most species are stable throughout most of their geological history, and evolve in "bursts". Catastrophes and other stochastic events could account for these bursts, of which there is evidence in the paleontological record. However, isolation of small groups from the large gene pool is viewed as a primary event in allowing rapid selection of favourable characteristics. The debate between "punctuationalism" and "gradualism" remains one of the more interesting discussions in the field of biological speciation.

18. TERATOGENICITY

IUPAC definition

teratogenicity

Ability to cause the production of nonheritable structural malformations or defects in offspring. After [4]

Explanatory comment

Teratogenicity may be regarded as a special form of embryotoxicity or developmental toxicity. Unfortunately, the OECD Test Guidelines [40] state that "Developmental toxicology was formerly often referred to as teratology". This statement is misleading since the term 'developmental toxicology" covers toxicity to the embryo and fetus in the widest sense. The distinctive definition of 'teratology' is required in order to concentrate attention on a special type of toxicity which requires a mechanism which affects the embryo and interferes with organogenesis producing congenital malformations, i.e., irreversible functional or morphological defects present at birth. Malformations caused by exposures after birth are to be regarded separately as postnatal developmental disturbances. Typical teratogenic agents and the effects that they cause in humans are:

Alkylating agents	degeneration of the embryonic disc and intrauterine death
Androgenic hormones	adrenal hyperplasia, virilisation of the female genitalia
Chlorobiphenyls	dark-brown staining of the skin, exophthalmos
Diethylstilbestrol	virilization of the female fetus
Lithium	cardiovascular defects

Retinoic acid	multiple CNS and cardiac malformations, thymic aplasia
Thalidomide	limb malformations, phocomelia, ear defects, etc.
Valproic acid	neural tube defects, heart defects

Prevalence

Although there are many figures quoted for the prevalence of major congenital malformations, these figures are greatly dependent on the population studied, the point at which the data is collected after birth, and the classification of congenital defect (e.g., minor, major, cosmetic). Most sources use the figure of 3 % prevalence rate for major congenital malformations recognized at birth, and another 3 % rate for major congenital malformations unrecognized during the neonatal period. This combined 6 % rate does not include mental and physical growth retardation, or minor congenital malformations such as hydrocele, angiomas, hernias, and nevi which have not been regarded as serious enough for medical treatment. Of course, not all malformations can be attributed to drug use during gestation. The cause of approximately 40 % of malformations is unknown. About 12 % – 25 % of these congenital malformations are purely genetic defects. Down's syndrome is the most common of this group. Another 20 % are due to interactions between hereditary factors and environmental factors, the latter of which are largely unknown. Environmental factors alone, such as maternal disease or infection, chemicals and drugs, account for between 5 % and 9% of the malformations. This category includes infections such as rubella (German measles), cytomegalovirus, and *Toxoplasma gondii* (a protozoan) as definitive teratogens. It also includes maternal diseases such as diabetes and seizure disorders. Rubella infection is the most well-known of the viral infectious teratogens. Maternal rubella can result in a group of defects, including heart disease, cataracts, and deafness, known as fetal rubella syndrome. Diabetes is the most common chronic disease causing teratogenesis. Congenital malformations due to strictly environmental factors are estimated to occur in 0.1 % – 0.2 % of all live births. However, it is possible that accidental high exposures may lead to a much higher local incidence. Only a small portion of congenital malformations are due to drugs acting as teratogens.

Teratogenicity

Changes in embryonic tissues can be produced by chemicals at concentrations far below those causing target organ toxicity in adults. Further, teratogenic effects may occur in embryonic organs different from the target organs showing toxicity in adults. Possible effects in the offspring at birth or in the postnatal development period include embryo lethality (death); mild-

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to-severe dysmorphogenesis in one or more organ systems, resulting in structural malformations or physiological and biochemical dysfunction; and psychological, behavioral, and cognitive deficits. Substances causing such effects are called teratogens. Teratogens are a major concern to the public, to industry and to regulatory agencies because of the low levels required to cause damage. Unfortunately many Material Safety Data Sheets (see Section 19) do not include much information on this increasingly recognized form of toxicity.

Timing is of the utmost importance in teratogenic studies, because exposure must occur during the period of organogenesis. Organogenesis occurs for different organs at different times after the implantation of the fertilized ovum. In other words, there are different windows of sensitivity for the different organs. In humans, organogenesis occurs in the first 8 weeks of pregnancy. Frequently, the period of chemical susceptibility is only a few days, with exposures before or after this time showing no effect upon a particular developing target organ, but perhaps causing adverse effects at another site. When using surrogate animal models to test teratogenicity, timing of administration is critical because of the shorter gestation time (e.g., 21 days in mouse and rat and 32 days in rabbit, as compared to 36 weeks in the human) and a corresponding reduction in the periods of organogenesis, often of only a few hours duration for certain organs. Because the sensitive stages of differentiation and development of embryos to the teratogenic effects of chemicals are often short in duration, the incidence of observable teratogenic effects of a given chemical may be rare in the potentially exposed population, as the combination of effective exposure and sensitive embryonic developmental stage may be a rare occurrence. This is a challenge both for the laboratory scientist and the epidemiologist trying to identify teratogens in order to regulate them.

Mechanisms of teratogenicity are not well understood and are probably of many differerent kinds, ranging from inactivation of key enzymes to small changes in hormonal balance at key stages in development. Oxidative stress and reactive oxygen species have been implicated in some teratogenic processes. Most recently, attention has focussed on the developing knowledge of epigenetics (see Section 8) and inhibitions of histone acetylase or DNA methylation have been suggested as possible causes of teratogenic change.

Teratogenicity testing is routinely conducted in two species, a rodent (e.g., mouse or rat) and a nonrodent (usually the rabbit), and involves the daily administration of a range of three appropriate dose levels to different groups of timed-pregnant animals throughout the established period of organogenesis (e.g., days 6 to 15 for mice and rats or days 6 to18 for rabbits). The animals are killed 24 hours prior to the calculated day of parturition (day 20 for mice and rats, day 31 for rabbits) and undergo a complete necropsy. The number of live and

dead fetuses is determined, and the uterine muscle is examined for re-absorptive sites (small scars) indicative of early embryonic deaths. Each fetus is weighed, the sex is determined and each is examined for external abnormalities (e.g., missing digits, limbs, tail; or eye, ear, or cranial anomalies) prior to dissection to detect internal malformations (e.g., heart, lungs, intestinal tract, gonads). Whole fetuses are fixed in special fluids for histological examination of neural structures and for the detection of skeletal anomalies.

While the brain and peripheral nervous tissue begin to develop early in organogenesis, there is also considerable postnatal development that can be affected by prenatal exposure to potentially toxic agents, causing overt but subtle behavioral and cognitive deficits that cannot be detected by morphological examination of the brain. The possibility of such effects has resulted in modifications of the testing protocols, with some treated animals being allowed to give birth and to rear their young for 6 weeks after parturition (with or without continuous treatment of the dams). At 6 weeks of age, the young animals are examined by a battery of testing protocols designed to assess behavioral and cognitive development.

Test protocols

There are two distinct sets of test protocols for reproductive toxicity including teratogenicity, one to be found in the OECD set of test guidelines, applied to industrial chemicals, and one established by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). When interpreting data, it is important to know which of these has been used to obtain the data.

19. TOXICITY CLASSIFICATION, LABELING, AND MATERIAL SAFETY DATA SHEETS

IUPAC definitions

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).

safety data sheet

Single page giving toxicological and other safety advice, usually associated with a particular preparation, substance, process, use pattern, or exposure scenario.

Explanatory comment

Introduction - Toxicity classification and labelling

Most countries have legislation requiring that all chemicals should have a clear label to indicate their identity, hazardous properties, and safety precautions. The label should draw attention to the inherent danger to persons handling or using the chemical. Symbols and pictograms have been established for various hazard categories. These symbols or pictograms are an integral part of the label and give an immediate idea of the types of hazard that the substance or the preparation may cause.

As already mentioned above, there is a move to establish a global harmonization system (GHS) for toxicity classification and labeling. At the World Summit on Sustainable Development (Johannesburg, South Africa) in August-September 2002, governments adopted the Johannesburg Plan of Implementation which "encourages countries to implement the new globally harmonized system for the classification and labelling of chemicals as soon as possible with a view to having the system fully operational by 2008." (para. 23c) [41]. Several countries have already implemented this system fully.

It should be noted that classification and labeling for supply of chemicals may be different from that for transport of chemicals. Classification and labeling for transport is dealt with by United Nations Organizations while that for supply is regulated at European Union level for the component nations and at national level otherwise.

Risk and safety phrases

There is an increasing international use of standard risk and safety phrases on labels [42]. In the course of time, some phrases have been removed as improved phrases are introduced. Thus, there are some gaps in the numbering.

It should be emphasized that the so-called "risk phrases" are really "hazard phrases" since they give no indication of the probability of harm. Accurate determination of the probability of harm associated with defined circumstances is the key to risk assessment (see Section 16), and is dependent on the probability of exposure and resultant harm. If there is no exposure to a hazard, there is no risk. Examples of the so-called risk phrases (**R**) relating to toxicity are:

R 20 Harmful by inhalation.

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R 21 Harmful in contact with skin.
R 22 Harmful if swallowed.
R 23 Toxic by inhalation.
R 24 Toxic in contact with skin.
R 25 Toxic if swallowed.
R 26 Very toxic by inhalation.
R 27 Very toxic in contact with skin.
R 28 Very toxic if swallowed.

These may be used in combinations, e.g., R 20 / 21/ 22. 'Harmful' is the lowest level of toxicity classification. It implies that a large or continuing exposure is required in order to produce any harmful effect. 'Toxic' or 'very toxic' implies that a small exposure can cause harm fairly quickly. Thus, substances labeled 'Toxic' or "Very Toxic" must be handled with great care. Substances labeled "Harmful" must also be handled with care but there is less risk of harmul effects folowing a small exposure.

The standard safety phrases (**S**) give advice on the precautions necessary in handling chemicals. In effect, these phrases describe how to reduce the risk in handling the substances to which they apply. Such precautions do not, of course, reduce the intrinsic hazards of the relevant substances. For example:

S 1 Keep locked up.
S 2 Keep out of the reach of children.
S 3 Keep in a cool place.
S7 Keep container tightly closed
S9 Keep in a well-ventilated place
S14 Keep away from *(incompatible materials to be indicated by the manufacturer)*
S49 Keep only in the original container

As with the risk phrases, these are often used in combinations.

Labeling requirements

In general, a label must clearly show:

1. The trade name
2. The name and the address, including telephone number, of the manufacturer, the importer or the distributor
3. The chemical name of the substance (in the case of a preparation, the chemical names of the hazardous components)
4. Danger symbols
5. Risk phrases (R-phrases)

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6. Safety phrases (S-phrases)
7. The quantity of the contents of the package or container

Labels should be in the national, official language(s) of the country where the substance is to be used.

A label should show the chemical names of substances present that are the most serious hazards. In some cases, the list of names may be quite long; for example all cancer causing substances in a preparation must be identified and the corresponding R- and S-phrases must be given on the label.

If the preparation contains one or more of the substances requiring the following R-phrases, both the name of the corresponding substance(s) and the R-phrase should be mentioned in the label: R39, R40, R42, R43, R42 / 43, R45, R46, R47, R48, R49, R60, R61, R62, R63, R64. As a general rule a maximum of four R-phrases and four S- phrases should suffice to describe the risks and to formulate the most appropriate safety advice.

Symbols showing the most serious hazards should be chosen where more than one danger symbol has to be assigned. As a general rule a maximum of two danger symbols are used.

The explanation of the letter symbols appearing in the attached lists are given below. Each letter symbol refers to a danger symbol or pictogram (Fig. 7):

Letter symbol		Explanation
E	Explosive	This symbol with the word 'explosive' denotes a substance which may explode under the effect of a flame or if subjected to shocks or friction.
O	Oxidizing	The symbol with the word 'oxidizing' refers to a substance which releases excessive heat when it reacts with other substances, particularly with flammable substances.
F	Highly flammable	This symbol with the words 'highly flammable' denotes a substance which may become hot and finally catch fire in contact with air at ambient temperature, or is a solid and may readily catch fire after brief contact with the source of ignition, and which continues to burn or to be consumed by chemical reaction after removal of the source of ignition. If it is gas it may burn in air at normal pressure. If it is a liquid it

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		would catch fire with slight warming and exposure to a flame. In contact with water or damp air the substance may release highly flammable gases in dangerous quantities.
F+	Extremely flammable	The same flammable symbol as above with words ‘extremely flammable’ denotes e.g. a liquid which would boil at body temperature and would catch fire if vapors are exposed to a flame.
T	Toxic	The symbol with skull and crossed bones with the word ‘toxic’denotes a highly hazardous substance.
T+	Very toxic	The same symbol as above with the words 'very toxic' is used to label a substance, which, if inhaled or ingested or, if it penetrates the skin, may involve extremely serious immediate or long-term health risks and even death.
C	Corrosive	The symbol with the word 'corrosive' will be found on a label of a substance which may destroy living tissues on contact with them. Severe burns may result from splashes of such substance.
Xn	Harmful (less than T)	The symbol with word 'harmful' denotes substances which may cause health hazards less than toxic. It could refer to other types of risks, e.g., to allergic reactions.
Xi	Irritant (less than C)	The same symbol as above with the word 'irritant'.

When more than one danger symbol is used:

- obligation to apply symbol T or T+ will make symbols C, Xn and Xi optional;
- obligation to apply symbol C will make symbols Xn and Xi optional;
- obligation to apply symbol E will make symbols F and O optional.

If a preparation is classified both harmful (Xn) and irritant (Xi), it will be labeled Xn, and the irritant properties should be pointed out with appropriate R-phrases. The total amount of the

substance in the preparation must be considered in choosing the danger symbols, R- and S-phrases.

Generally, no account needs to be taken of substances if they are present in the following amounts, unless another lower limit has been specifically given:

less than 0.1% by weight for substances classified as very toxic T+, or toxic T

less than 1% for substances classified as harmful Xn, corrosive C, irritant Xi

Any pictorial symbol indicating danger is drawn in black and the background color should be orange.

The dimensions of the label are specified as follows:

Capacity of the package	Minimum dimensions (mm)
Not exceeding 3 liters	52 x 74
More than 3 liters but not exceeding 50 liters	74 x 105
More than 50 liters but not exceeding 500 liters	105 x 148
More than 500 liters	148 x 210

Note: Each danger symbol must cover at least 1/10 of the surface area of the label. The minimum size of the danger symbol shall not be less than 10mm x 10mm.

The Material Safety Data Sheet (MSDS)

Increasingly, there is also a requirement that manufacturers and suppliers provide a material safety data sheet, now often referred to only as a safety data sheet (SDS), with all the relevant hazard and safety information available. The term MSDS is slightly misleading since each MSDS may run to several A4 pages. The MSDS backs up the information that must be provided on the label attached to any container of a substance and is required by law in North America and the European Union. Similar laws are gradually being implemented worldwide as a result of the development of international agreement on a globally harmonized system (GHS) of chemicals management. MSDS must be kept on file, available to both workers and management, in the workplace at all times. However, as will be seen below, the usefulness of the MSDS is limited and its limitations must be appreciated if it is to be interpreted properly to guide safety precautions.

A standardized format is used in the preparation of an MSDS [43]. Unfortunately the information provided on some MSDSs is not validated or checked and must be considered critically before any use is made of it. However, International Chemical Safety Cards, IPCS validated versions of MSDSs, are available on the International Programme on Chemical Safety (IPCS) Chemical Safety Information from Intergovernmental Organizations)(INCHEM) website [44] with similar information for pure substances. In addition, there are also Concise International Chemical Assessment Documents (CICADS), which are short booklets produced by IPCS to summarize the key information required by industrial managements to protect their

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workforces. These documents also may be downloaded from the INCHEM website. CICADS contain copies of the relevant International Chemical Safety Cards.

EXAMPLE LIST OF THE COMPONENTS OF A MATERIAL SAFETY DATA SHEET, WITH ANNOTATIONS

Note: This list is based on United States regulations and other countries, such as those of the European Union, have similar but slightly different requirements. Guidance on the preparation of Safety Data Sheets for the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) can be found at the following webpage http://www.unece.org/trans/danger/publi/ghs/ghs_rev02/02files_e.html .

SECTION I - IDENTITY INFORMATION

Identity of the product: Identification of the substance by manufacturer, common name and synonyms, product code(s), chemical formula, chemical class classification (e.g., alcohol, etc.), shipping name (for national and international recognition).

EMERGENCY TELEPHONE NUMBER: Must be included, but it does not need to be toll free.

TELEPHONE NUMBER FOR INFORMATION: May be the same as above for small companies.

NAME OF THE MANUFACTURER OR IMPORTER: Be sure that this name is exactly the same as the name of the manufacturer listed on the product label. Small manufacturers sometimes send out MSDS from the manufacturer of the raw materials they mixed to make the product or that they repackaged. This should not be the case.

ADDRESS OF THE MANUFACTURER: Be sure this address is complete: street or box, town, state, and postcode (zip).

DATE PREPARED: MSDSs prepared more than three years ago are acceptable in the U.S., but an attempt should be made to get an updated version. Three year old MSDSs are invalid in Canada.

SIGNATURE OF THE PREPARER (OPTIONAL).

SECTION II - HAZARDOUS INGREDIENTS

List of ingredients with common names and synonyms, Chemical Abstracts Service (CAS) numbers, concentrations, legal exposure limits, American Conference of Government Industrial Hygienists (ACGIH) threshold limit values (TLVs), and source of information. Toxic chemicals comprising more than 1 % of the product by weight must be listed. Cancer-causing chemicals that comprise 0.1 % of the product must also be listed. If exposure to amounts even smaller than the required 1.0 or 0.1 % is known to be hazardous, the manufacturer also must list these ingredients. In practice, however, such hazardous ingredients

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often go unlisted. For example, trace amounts of extremely toxic dioxins and PCBs in many pigments usually are not reported.

TRADE SECRET EXEMPTIONS: Information on the identity of hazardous ingredients can be withheld by the manufacturer it they are trade secrets. The MSDS should state by whose authority (usually the competent health department) the product's identity can be withheld. Trade secret products should be avoided whenever possible since it is very difficult and time-consuming for medical personnel to get this data if there is an accident or illness. Even then, the medical person must withhold from the victim the name of the chemical that caused his (her) problem.

SECTION III - PHYSICAL DATA

Physical state and appearance; odor and odor threshold (level at which it would be detected in the nasal passages); the specific gravity; the vapor pressure (an index of the volatility at a specified temperature, usually at 20^0C); the vapor density - lighter or heavier than air; the evaporation rate relative to a reference chemical; boiling point; freezing point; the pH, if applicable (acidic, neutral or basic in nature - identifying corrosiveness); and lastly, the oil/water partition coefficient (log K_{ow}).

Note: Without knowing anything about the biological properties of the chemical, the physical properties permit the reader to make educated guesses concerning whether or not the agent will pose a health hazard by inhalation or by dermal contact, where the highest concentrations might be in a room following spillage (e.g., near the floor if it is much heavier than air), etc.

SECTION IV - FIRE AND EXPLOSION HAZARD

Mobility of the vapor; the flash point, flammable limits, auto-ignition temperature; explosion data - upper and lower explosive (flammable) limits (UEL, LEL) in air; percent by volume in air as well as mechanism(s) of initiating an explosion. In addition, extinguishing media are given as well as the potentially hazardous combustion products.

Note: This information is essential to fire fighting and rescue personnel.

SECTION V - REACTIVITY DATA

Stability, incompatibility (materials to avoid), conditions to avoid, hazardous decomposition products, hazardous polymerization etc.

Note: It may be necessary to obtain MSDSs for decomposition products if they are likely to be formed during storage or use of the primary material.

SECTION VI - HEALTH HAZARD DATA

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Primary route of exposure (dermal, oral, ocular, inhalation), over-exposure effects (irritation, sensitization) for acute and chronic effects, special toxicity (mutagenicity) teratogenicity, carcinogenicity, reproductive/fertility. Emergency and first aid procedures

Note 1: Effects may differ widely depending on the route of exposure. Often the respiratory route is the most harmful but certain substances such as phenol penetrate skin rapidly. In the workplace, oral exposure is often neglected but carelessness can make it a significant source of harm.

Note 2: Sensitization leading to dermatitis or respiratory problems may result from low levels of exposure that do not themselves cause obvious immediate toxicity. Sensitization effects to humans may not be identified in animal studies and should always be considered possible and guarded against.

Note 3: Mutagenicity, teratogenicity, carcinogenicity, and reproductive toxicity require extreme caution in handling substances that have such properties. If possible, such substances should not be used and suitable substitutes identified. This requires careful risk assessment, as poorly characterized substitutes may appear safer because of ignorance of their potentially harmful properties. Further, different risks will require some comparative evaluation. Thus, a low potency carcinogen that is an effective fire retardant may be preferable to a noncarcinogenic alternative that is less effective in preventing fires.

SECTION VII - PRECAUTIONS FOR SAFE HANDLING AND USE

STEPS TO BE TAKEN IF MATERIAL IS RELEASED OR SPILLED:

The MSDS should list preferred methods for spill control (e.g. chemical sorbents, Fuller's earth, etc.) and protective equipment (respirators, gloves, emergency ventilation, etc.) needed to keep workers safe during clean up of large spills or accidents.

Note: Read carefully before use of any substance and be sure you can dispose of it safely and without harming your local environment.

WASTE DISPOSAL METHOD: Unless the material can be rendered completely innocuous, the MSDSs can only tell users to dispose of the material in accordance with the existing regulations. Disposal has become an extraordinarily complex problem and cannot be addressed in a few lines on an MSDS. Substances which pose severe environmental threats or whose release (spills) must be reported should be identified here.

SECTION VIII - CONTROL MEASURES

RESPIRATORY PROTECTION (SPECIFIC TYPE): If needed during normal use, a good MSDS explains precisely what type of respirator is proper. Even the type of cartridge type for air purifying respirators should be specified.

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VENTILATION: If needed during normal use, a good MSDS specifies the type of ventilation system that provides proper protection. This includes recommendations about the use of general (mechanical) ventilation, local exhaust (which captures the contaminants at their source), or any special ventilation system that might be needed.

PROTECTIVE GLOVES: Good MSDSs list the specific type of glove material needed (rubber, nitrile, etc.) and other glove attributes such as length and thickness. Workers should know that some solvents penetrate gloves without changing the glove's appearance. Often such solvents are perceived only as perspiration. Good MSDSs indicate which gloves will resist penetration by the product. When in doubt, contact the technical department of your glove supplier.

EYE PROTECTION: Good MSDSs list precisely what type of goggles or glasses are needed by their ANSI Z87.1 standard classification. The MSDS at least should indicate whether vented or unvented chemicals splash goggles, impact goggles, or other specific types are needed.

OTHER PROTECTIVE CLOTHING OR EQUIPMENT: Aprons, boots, face shields, or eye wash stations should be listed here if needed.

WORK/HYGIENIC PRACTICES: Practices such as proper daily clean up methods and equipment after normal use should be detailed here.

Note: Make sure that you are prepared for any emergency before you use a chemical. Have all the appropriate equipment and any neutralizing materials readily available.

SECTION IX - SPECIAL PRECAUTIONS

Handling, shipping and storage precautions, special warnings; regulatory information - national and (or) international; appropriate telephone numbers to contact expertise in cases of difficulties with a product.

Note: This information should be checked carefully, especially if your use of the chemical is out of the ordinary.

SECTION X - REGULATORY INFORMATION

DOT class number, departmental response numbers, government numbers, e.g. USA-TSCA, USA-RCRA, CERCLA status.

Note: These regulatory values are useful for tracking information in official databases.

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ANNEX 1: ABBREVIATIONS, ACRONYMS AND INITIALISMS

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
CPS&Q	Consumer Products Safety and Quality Unit (formerly ECB)
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic Acid
EEA	European Environmental Agency
EC	Effective Concentration

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ECHA	European Chemicals Agency
ECB	European Chemicals Bureau (now CPS&Q Unit)
ED x	Effective Dose for a biological effect in *x* % of the individuals in the test population
GFR	Glomerular Filtration Rate
GHS	Globally Harmonized System of Classification and Labeling of Chemicals
HPV	High Production Volume
HQ	Hazard Quotient
IAEA	International Atomic Energy Authority
ICH	International Conference on Harmonization (of Technical Requirements for Registration of Pharmaceuticals for Human Use)
IUPAC	International Union of Pure and Applied Chemistry
LC_{50}	Median Concentration Lethal to 50 % of a test population
LD_{50}	Median Dose Lethal to 50 % of a test population
LED x	Lowest Effective Dose for a biological effect in *x* % of the individuals in the test population
LOAEL	Lowest Observed Adverse Effect Level
MFO	Mixed Function Oxidase
NADPH	Nicotinamide Adenine Dinucleotide Phosphate (reduced)
NAG	*N*-acetyl-D-glycosaminidase
NAS	National Academy of Science
NOAEL	No-Observed-Adverse-Effect-Level
PBPK	Physiologically-Based Pharmacokinetic Modeling
PBPD	Physiologically-Based Pharmacodynamic Modeling
PBTK	Physiologically-Based Toxicokinetic Modeling
PEC	Predicted Environmental Concentration
PEL	Permissible Exposure Limit
PIPs	Persistent Inorganic Pollutants
PK	Pharmacokinetic
PNEC	Predicted No Effect Concentration
POPs	Persistent Organic Pollutants
QSAR	Quantitative Structure-Activity Relationship

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QSMR	Quantitative Structure-Metabolism Relationship
REACH	Registration, Evaluation and Authorisation of Chemicals
RfC	Reference Concentration
RfD	Reference Dose
ROS	Reactive Oxygen Species
SAR	Structure-Activity Relationship, Specific (Standard) Absorption Rate
SD	Standard Deviation
SMR	Structure-metabolism relationship
SE	Standard Error
TEF	Toxicity Equivalency Factor
TEQ	Toxicity equivalent
UDP	Uridine Diphosphate
USEPA	United States Environment Protection Agency
USFDA	United States Food and Drug Agency

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Fig. 1 - The tricarboxylic acid (TCA) cycle.

Fig. 2 - Glycolysis refers to the conversion of glucose into pyruvate with the generation of a net 2 ATP. The point where a 6-carbon structure is cleaved into two 3-carbon structures is indicated. The fate of pyruvate depends on the metabolic preference of the cell. In an aerobic environment where O_2 supports oxidative phosphorylation, pyruvate generates acetyl CoA that is diverted into the TCA cycle. In alcoholic fermentation, decarboxylation of pyruvate forms acetaldehyde that is converted to ethanol. Under anaerobic conditions, generation of ATP can continue for a time with the buildup of lactate.

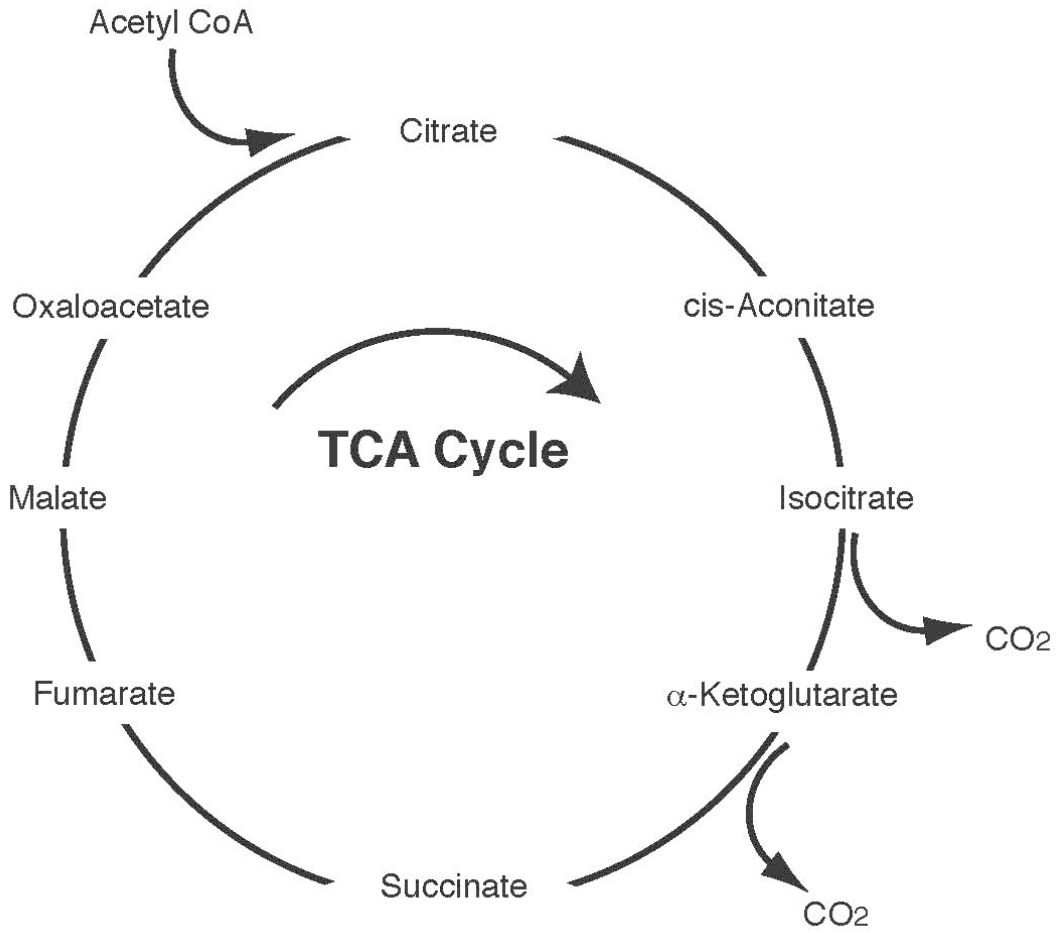
Fig. 3 – Schematic representation of the relationships between an extracellular substance, S, and the uptake of the substance by living cells.

Fig. 4 - A simple food web.

Fig. 5 - Soil food web. A food web showing trophic levels and relations between soil, organic matter, microorganisms, plants, insects etc., birds and mammals.

Fig 6 - Stages of carcinogenicity following genetic change (mutation).

Fig. 7 - GHS pictograms used on labels to warn of properties hazardous to health



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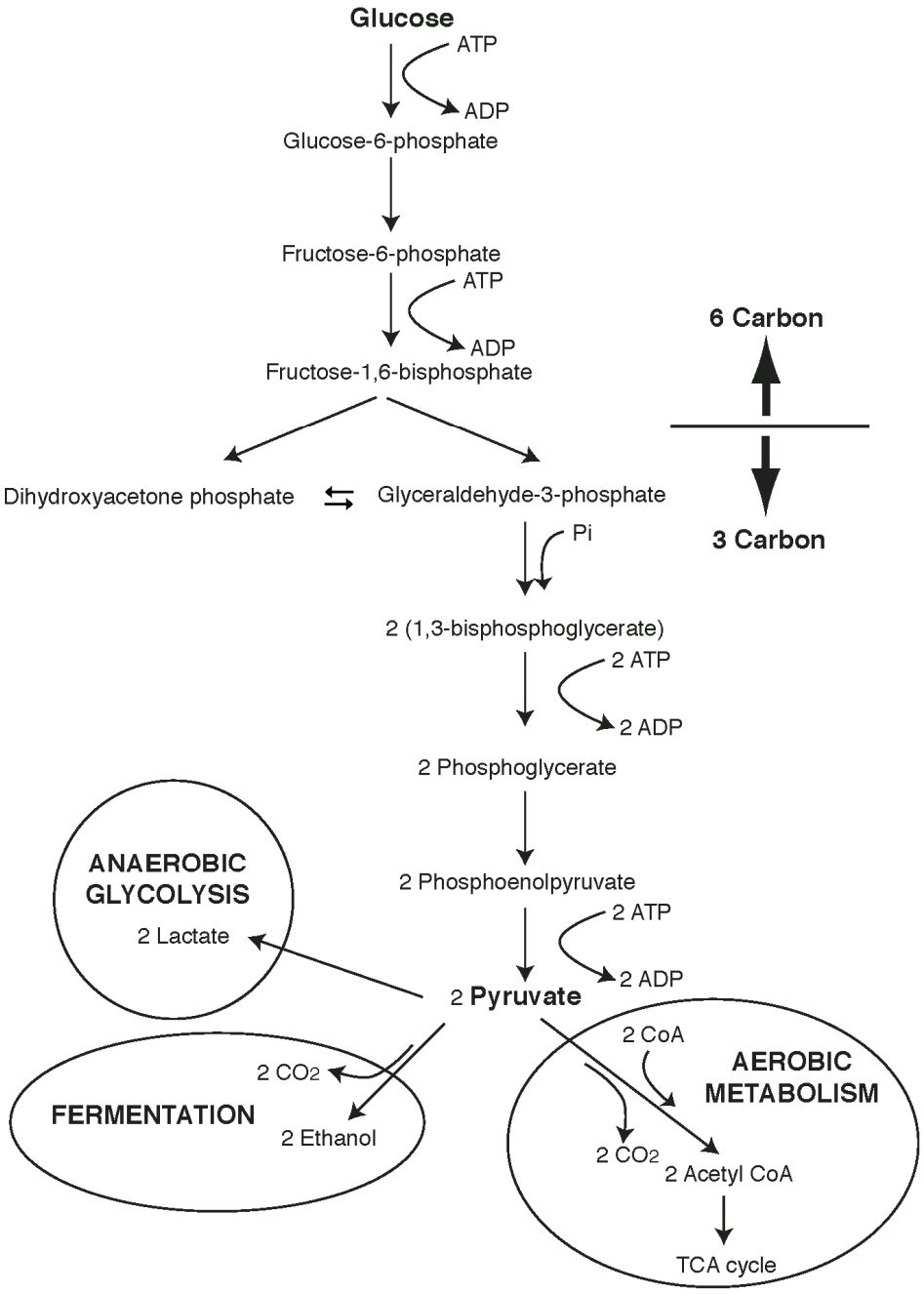

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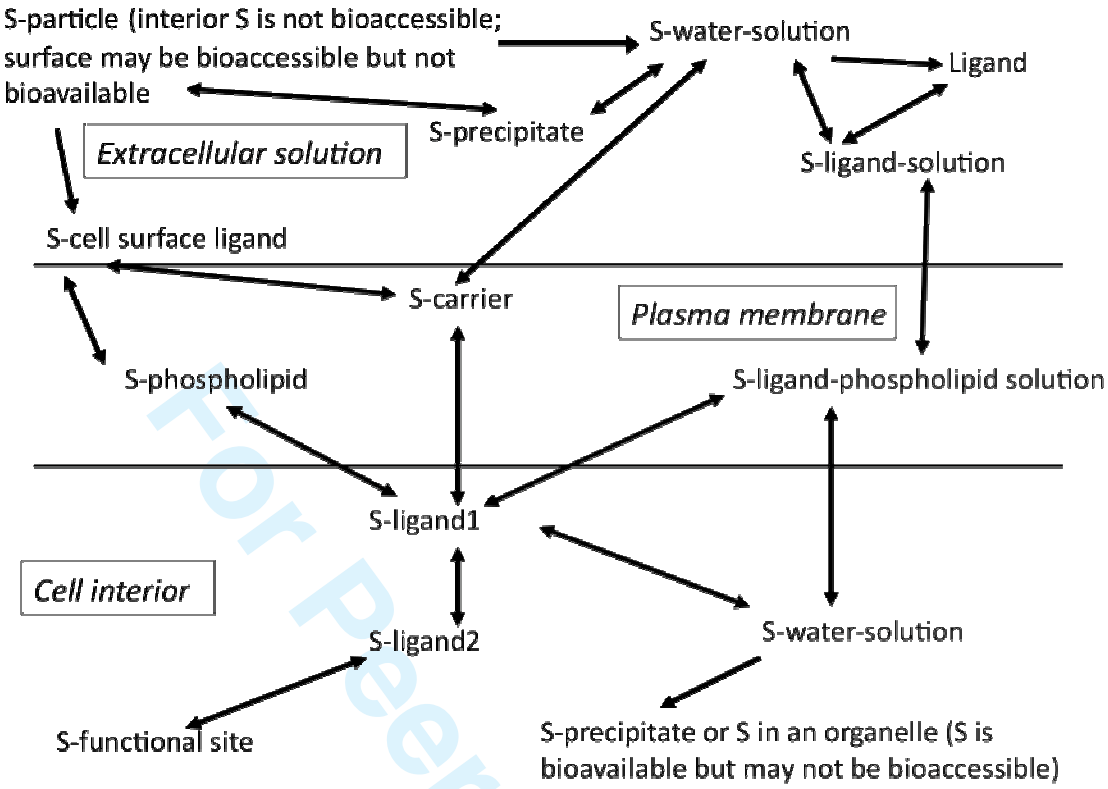


Fig. 3 - Schematic representation of the relationships, in terms of bioaccessibility and bioavailability, between an extracellular substance, S, and the uptake of the substance by a living cell.

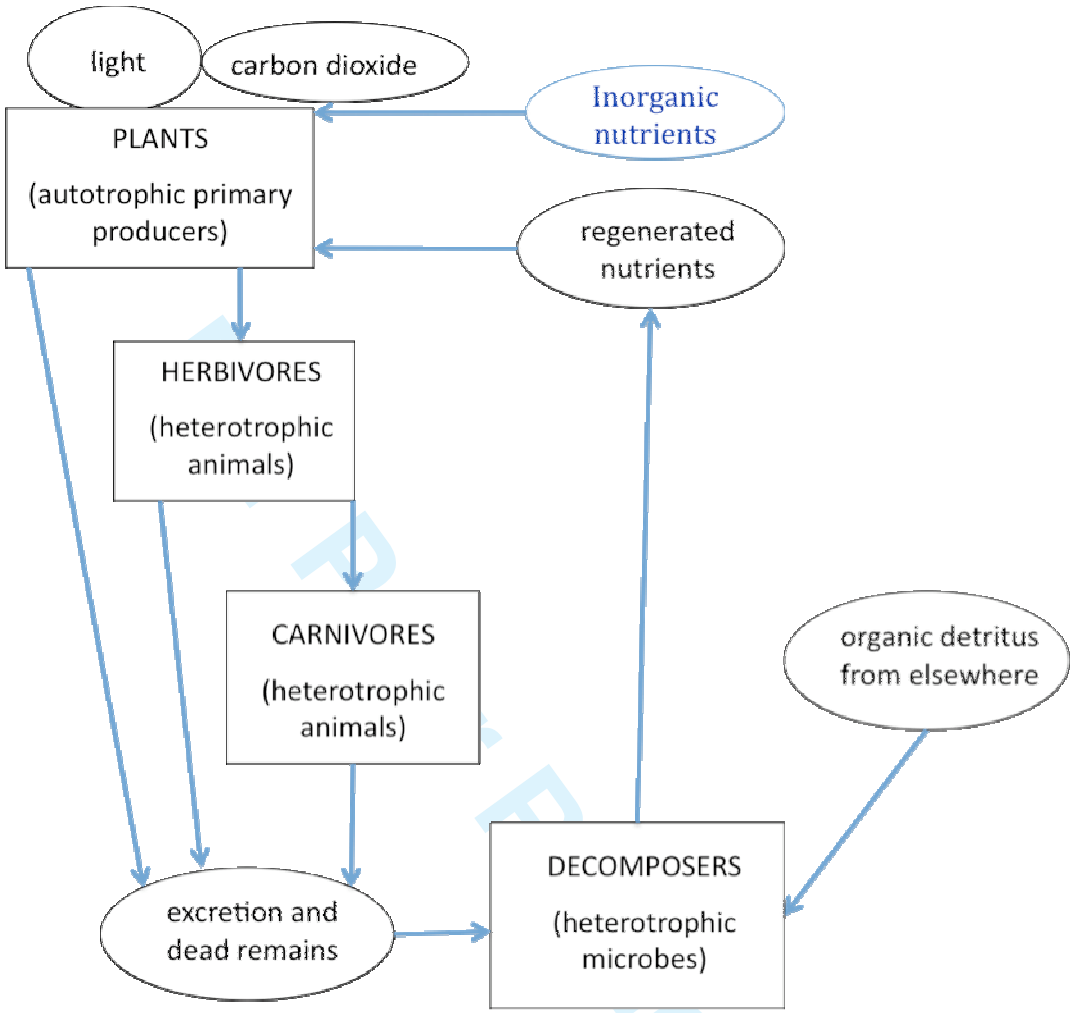
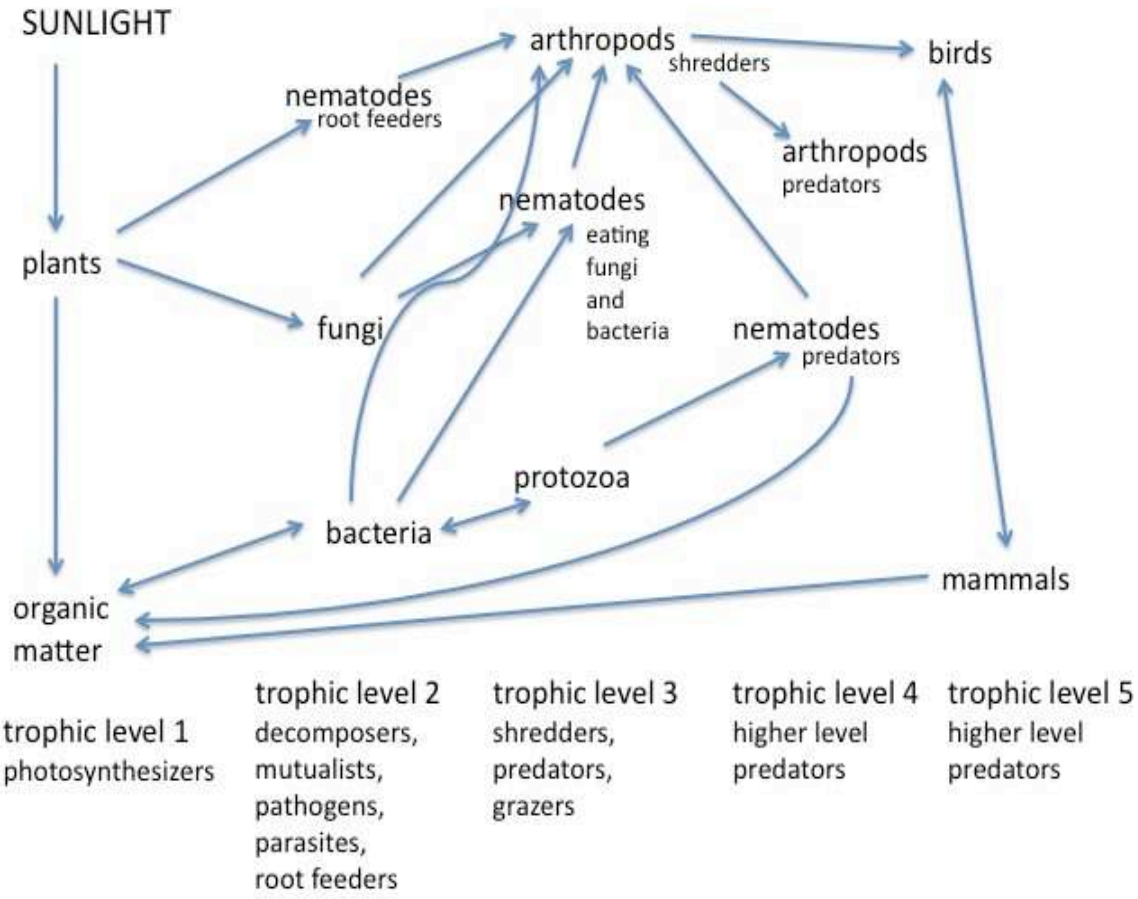


Fig. 4 - A simple food chain.



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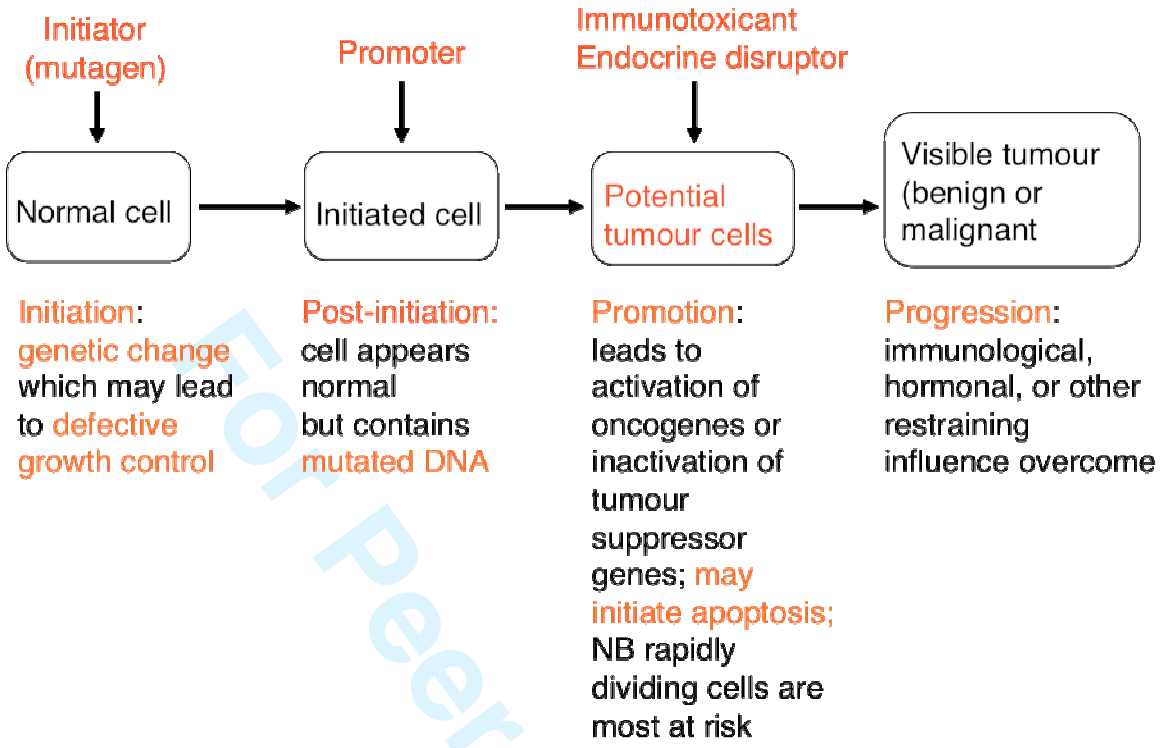

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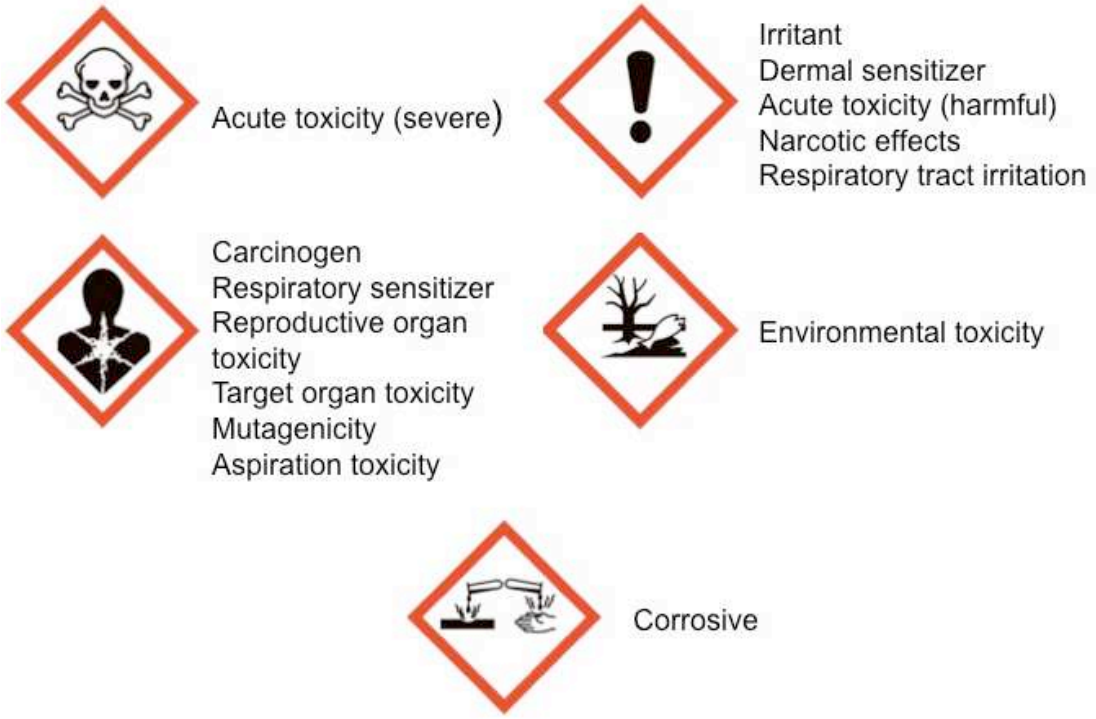

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