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# POTENTIAL RISK OF EXPOSURE TO SELECTED XENOBIOTIC RESIDUES AND THEIR FATE IN THE FOOD CHAIN – PART I: CLASSIFICATION OF XENOBIOTICS

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**Abstract:** Consumers are exposed to a diversity of chemicals in all areas of life. Air, water, soil and food are all unavoidable components of the human environment. Each of those elements influences the quality of human life, and each of them may be contaminated. We are exposed to toxic or potentially toxic compounds in many ways in our daily lives and toxicology is clearly a subject of great importance for society. This becomes apparent when we look at the types of poisons and the ways in which we are exposed to them. Indeed, the categories cover virtually all the chemicals one might expect to encounter in the environment. After consideration of this, one might well ask "Are all chemicals toxic?" Phrase as an answer: "There are no safe chemicals, only their safe use". Xenobiotics are defined here as those compounds, both organic and inorganic, produced by human beings and introduced into the environment, as well as into the food chain at concentrations that cause undesirable effects. Xenobiotics in the food chain are monitored in two forms: by testing – the objective of which is to discover unsuitable foodstuffs in the consumer's network, and by monitoring – to obtain objective information about environmental components contamination and to harmlessness health of available foodstuffs.

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## INTRODUCTION

Xenobiotics are chemicals foreign to life, which are usually derived synthetically or from an abiotic process. The term "xenobiotic" is a combination of the Greek words "*xenos*", meaning strange or foreign, and "*bios*", meaning life. Thus, xenobiotic chemicals are pollutants in the biosphere, although not all pollutants are xenobiotic chemicals. The synthetic xenobiotic chemicals are often of enormous value to human society, and are usually the majority of the chemicals in such important groups of substances as petrochemicals, pesticides and plastics. Increasingly, humans are subjected to exposure to various xenobiotics. The situation is well summarized in the following quotation from Rachel Carson: "As crude a weapon as the cave man's club, the chemical barrage has been hurled against the fabric of life." [17, 18]

Since the very beginning of the chemicals industry there has been interest in producing more efficacious products. This has led to continuing research into the prediction of the likely properties of a chemical prior to its use. Concurrently, research has been in progress which will give a better understanding of the mode of action of chemicals. One of the most important properties of a chemical, in situations involving a biological effect or application, is how well it is absorbed or bioaccumulated. Bioaccumulation usually means the accumulation of chemical in an organism to a higher concentration than is present in an external source [4]. When a foreign organism or the macromolecule enters the body, our immune system may produce antibodies that

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interact with and destroy it. However, some xenobiotics do not trigger an antibody response. Instead, the body's numerous enzymes metabolize such foreign molecules and toxins to less reactive water-soluble metabolites that can be readily excreted [25].

Xenobiotics biotransformation is the process – actually the series of enzyme-catalysed processes – that alters physiochemical properties of xenobiotics from those that favour the absorption across biological membranes (namely lipophilicity) to those favouring elimination in urine or bile (namely hydrophilicity). Without xenobiotic biotransformation the numerous xenobiotics to which we are exposed (which include both man-made and natural chemicals such as industrial chemicals, pesticides, pollutants, pyrolysis products in cooked food, alkaloids, secondary plant metabolites and toxins produced by moulds, plants, etc.) would, if they are sufficiently lipophilic to be absorbed from the gastrointestinal tract and other sites of exposure – eventually accumulate to toxic levels [26]. The overall purpose of the two phases of metabolism of xenobiotics is to increase their water solubility (polarity) and thus excretion from the body. Very hydrophobic xenobiotics would persist in adipose tissue almost indefinitely if they were not converted to more polar forms. In certain cases, phase 1 metabolic reactions convert xenobiotics from inactive to biologically active compounds. In these instances, the original xenobiotics are referred to as "procarcinogens". In other cases, additional phase 1 reactions (further hydroxylation reactions) convert the active compounds to less active or inactive forms prior to conjugation. In a very few cases, conjugation may actually increase their biological activity. Xenobiotics can produce a variety of biological effects, toxicity, immunological reactions, cancer, etc. [18] Moreover, the outcome of xenobiotic biotransformation can be even an more toxic substance than the original one (for instance VCM – Vinyl Chloride Monomer).

In a general sense, toxicology can be defined as "toxin science" or the "science of poisons". The main divisions of the study of toxicology involve the sources of toxin, physical, chemical and biological properties of toxins, toxic doses, and the changes that occur in the living organism and their effects, treatment of toxic diseases, isolation of toxins, analysis of toxins and regulation about toxins. Many toxic events have occurred in recent years due to the increased use of industrial, agricultural and household chemical substances, and with the use of nuclear energy. These harmful effects concern the whole biosphere as well as human beings, and not only food chain. The investigations of these chemical substances in biological systems and in the environment, and the methodology related to this investigation, are the subjects of the science of chemistry, while their metabolism, effects and changes at molecular levels are the subject of biochemistry. The investigations of the toxic effects that occur as the results of the use of these chemical substances, as well as the treatment and research on the safety, production and processing of their use, are related to the sciences of medicine, agriculture and food. Toxicology is a multidisciplinary science with close relationships with other sciences, such as pharmacology, immunology, biology, pathology, physiology, chemistry, biochemistry, food and public health branches, namely, hygiene and occupational health [1]. Toxicology, as it seeks to reveal the adverse effects of xenobiotics, may be subdivided into two phases: toxicity and toxicokinetics. Toxicity is the study of the toxic action of xenobiotics on the body, including the dose-response relationship, receptor interactions and mechanisms of toxicity or can be defined as "the capacity of the substance to cause adverse health effects (injury, hazard) on a living organism". Toxicokinetics is the study of the action of the body on xenobiotic, including absorption, distribution, metabolism, and excretion (ADME) [13]. The type of toxicity that occurs can have local effects, such as skin irritation, or general effects, such as in impaired coordination, behavioural changes, organ structure changes, or death. The toxicity of chemical substance is related to the amount, or dose taken into the organism. The amount of a chemical a person is exposed to is important in determining the extent of toxicity that will occur [1]. The $LD_{50}$ values determined from acute oral systemic toxicity tests are used to place chemical substances in various toxicity categories that determine the hazard or precautionary statements that are used on product labels. The $LD_{50}$ for a particular substance is essentially the amount that can be expected to cause death in half (i.e. 50%) of a group of a particular animal species, when entering the animal's body by a particular route. The Globally Harmonized System of Classification and Labelling of Chemicals (GHS) provides a basis for harmonization of rules and regulations on chemicals at national, regional and worldwide level, an important factor also for trade facilitation. Substances that can be allocated to one of five toxicity categories based on acute toxicity by oral, dermal or inhalation route according to the numeric cut-off criteria, are shown in Table 1.

The significance of the $LD_{50}$ has been examined by many scientists who have concluded that it is an imprecise value and not a biological constant. The numeric value of $LD_{50}$ has been widely used to classify and compare the toxic potential of chemicals, the importance placed on the $LD_{50}$ and how it is used in a safety evaluation has almost reached the level of abuse. Although determining the $LD_{50}$ under a set of experimental conditions can provide valuable information about the toxicity of compound, the numeric $LD_{50}$ *per se* is not equivalent to acute toxicity. It should always be remembered that lethality is only one of many reference points used to characterize acute toxicity [12].

$LD_{50}$ values for the same substance can vary from species to species (e.g., mice, rabbits, dogs to humans), breed to breed/strain, or even animal to animal. A host of other variables also affect $LD_{50}$ results, including gender, age, and diet, housing and environmental conditions, and health status. Given the difficulties of extrapolating $LD_{50}$ values from animals to humans, scientists themselves have

**Table 1.** Criteria for classification of substances as acutely toxic. [a]

| Exposure route | Oral (mg/kg bw) | Dermal (mg/kg bw) | Gases (ppmV) [b] | Vapours (mg/l) | Dusts and Mists (mg/l) |
|---|---|---|---|---|---|
| **Acute toxicity hazard categories** | **Acute toxicity estimate** (ATE) – acute toxicity values are expressed as approximate $LD_{50}$ (oral and dermal) or $LC_{50}$ (inhalation) values or as ATE which is derived using the $LD_{50}/LC_{50}$ where available. | | | | |
| Category 1 | ATE ≤ 5 | ATE ≤ 50 | ATE ≤ 100 | ATE ≤ 0.5 | ATE ≤ 0.05 |
| Category 2 | 5 < ATE ≤ 50 | 50 < ATE ≤ 200 | 100 < ATE ≤ 500 | 0.5 < ATE ≤ 2.0 | 0.05 < ATE ≤ 0.5 |
| Category 3 | 50 < ATE ≤ 300 | 200 < ATE ≤ 1000 | 500 < ATE ≤ 2500 | 2.0 < ATE ≤ 10.0 | 0.5 < ATE ≤ 1.0 |
| Category 4 | 300 < ATE ≤ 2000 | 1000 < ATE ≤ 2000 | 2500 < ATE ≤ 20 000 | 10.0 < ATE ≤ 20.0 | 1.0 < ATE ≤ 5.0 |
| Category 5 | Criteria for this category are intended to enable the identification of test substances which are of relatively low acute toxicity hazard but which, under certain circumstances, may present a danger to vulnerable populations. Testing animals in this category ranges is discouraged and should only be considered when there is a strong likelihood that results of such a test would have a direct relevance for protecting human health. [c] | | | | |

[a] Adapted from Regulation (EC) No. 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No. 1907/2006; and Globally Harmonized System of Classification and Labelling of Chemicals (GHS) – ST/SG/AC.10/30/Rev. 3, United Nations, New York and Geneva 2009. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:353:0001:1355:EN:PDF http://www.unece.org/trans/danger/publi/ghs/ghs_rev03/03files_e.html

[b] Gas concentrations are expressed in parts per million per volume (ppmV).

[c] Council Regulation (EC) No. 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), App. 3; Available from: http://eur-lex.europa.eu/JOHtml.do?uri=OJ%3AL%3A2008%3A142%3ASOM%3AEN%3AHTML

spoken out against the test, especially the classical form of the test [30]. It has been suggested that the modern approach to the toxicological testing should be based on three R's – reduction, refinement and replacement – to optimize the balance between the needs of society and the welfare of animals [24, 29]. The conventional acute oral toxicity test (formerly OECD Test Guideline 401) as the most heavily criticised test in terms of animal welfare was the driving force behind the development of alternative tests for acute systemic toxicity testing. At the present time, alternative tests are proving to be equally useful, such as the acute toxic class method, fixed dose procedure, up-and-down procedure, etc. (for a more detailed description of these alternative tests see references) [5, 20, 21, 22]. Before actually conducting tests on animals, first of all possibilities offered by alternative approaches should be checked and considered. The following strategies for avoiding unnecessary testing on animals are:

• *In vitro* methods – a test performed *in vitro* is performed in a controlled environment, such as a test tube or Petri dish, and does not use a living organism. A test performed *in vivo* is one using a living organism, e.g. a vertebrate animal. Results obtained from suitable *in vitro* methods may indicate the presence of a certain dangerous property, or may be important in relation to understanding the mode of action of the substance. In this context, "suitable" means sufficiently well developed according to internationally agreed test development criteria (e.g. the European Centre for the Validation of Alternative Methods – ECVAM; Centre for the Evaluation of Alternative Toxicological Methods – NICEATM). Data generated from *in vitro* test methods (validated and pre-validated) can provide the information for the hazard endpoint and is sufficient for the purpose of classification and labelling and/or risk assessment. Advanced *in vitro* technologies may provide valuable information on the mode of action of the substances and can be part of a read-across and category justification.

• Grouping of substances and read-across – animal tests on a substance can be avoided if there is enough evidence on similar substances which the registrant can show should be "read across" to their own substance. Substances which have physicochemical, toxicological and ecotoxicological properties are likely to be similar, or follow a regular pattern as a result of structural similarity, may be considered as a "group", or 'category' of substances. Applying the group concept means that the physicochemical properties, human health effects and environmental effects or environmental fate may be predicted from data for one substance within the group by interpolation to other substances in the group (read-across approach). This avoids the need to test every substance in the group for every hazard endpoint. Preferably, a category should include all similar substances.

• Non-testing methods (*In silico* approach) – animal tests can be avoided if the hazardous properties of a substance can be predicted using computer models. The Quantitative Structure-Activity Relationship (QSAR) approach seeks to predict the intrinsic properties of chemicals by using various databases and theoretical models, instead of conducting tests. Based on knowledge of chemical structure, QSAR quantitatively relates the characteristics of the chemical to a measure of a particular activity. QSAR should be distinguished from SAR, which makes qualitative conclusions about the presence or absence of a property of a substance, based on a structural feature of that substance.

• Weight of evidence approach – animal tests can be avoided if there is a weight of evidence which points to the likely properties of a substance. This approach, which also involves expert judgment, may be applied if there is

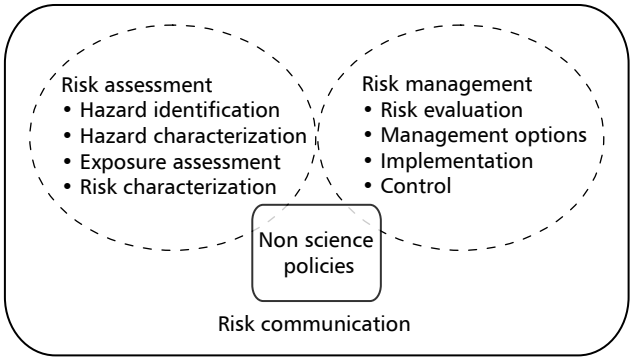
sufficient information from several independent sources leading to the conclusion that a substance has (or has not) a particular dangerous property, while the information from each single source alone is regarded insufficient to support this assertion [9].

The European Union is committed to promoting the development and validation of alternative techniques which can provide the same level of information as current animal tests, but which use fewer animals, cause less suffering, or avoid the use of animals completely. Most of these methods are developed within the framework of the Organization for Economic Co-operation and Development (OECD) programme for testing guidelines, and should be performed in conformity with the principles of Good Laboratory Practice (GLP), in order to ensure as wide as possible 'mutual acceptance of data' in assessment of chemicals agreement internationally accepted. The OECD is an intergovernmental organisation with representatives from 30 industrialised countries in North America, Europe and the Pacific, as well as the European Union, and provides information on internationally approved testing methods used by government, industry and independent laboratories to assess the safety of chemical products. The OECD also issues guidance documents on the validation and international acceptance of new or updated test methods for hazard assessment.

Modern toxicology goes beyond the study of the adverse effects of exogenous agents to the study of molecular histology, using toxicants as tools. Historically, toxicology formed the basis of therapeutics and experimental medicine. Toxicology in the previous centuries (1900 to the present) continues to develop and expand by assimilating knowledge and techniques from most branches of biology, chemistry, mathematics, and physic. A recent addition to field of toxicology (1975 to the present) is the application of discipline to safety evaluation and risk assessment [3].

Risk Analysis aims to ensure that the available food for consumers is safe. It also provides consumers with information so they can make informed choices when purchasing food. Three main components can be distinguished in the Risk Analysis process: Risk Assessment (scientific advice and information analysis), Risk Management (regulation and control), and Risk Communication. Risk Analysis is a detailed examination including risk assessment, risk evaluation, and risk management alternatives, performed to understand the nature of unwanted, negative consequences to human life, health, property, or the environment. It is an analytical process to provide information regarding undesirable events; a process of quantification of the probabilities and expected consequences for identified risks. Risk assessment is the process of evaluating the toxic properties of chemicals and the conditions of human exposure to ascertain the likelihood that humans will be adversely affected, and to characterize the nature of the effects which may be experienced. The risk assessment process can be divided into 4 steps: hazard identification, hazard characterization, exposure assessment and risk characterization. In hazard identification, a determination is made of whether the chemical of concern, be it an industrial chemical, environmental pollutant, etc., can be linked to an adverse effect. Dose-response assessment establishes the relationship between the magnitude of exposure and the occurrence of adverse effect. The major activities of toxicologist are concentrated in these two steps. In exposure assessment, human exposure to the substance of concern is identified through characterization of the exposed population, routes of exposure, and magnitude of the exposure under various conditions. All the information derived in these three steps of the risk assessment process is used in the risk characterization step. In this fourth and final stage of the risk assessment process, a determination is made of the likelihood that humans may experience the identified adverse effect under actual or plausible hypothetical conditions of exposure. Based on risk characterization, the need for and the degree of risk management will be determined. A number of options are available to the risk manager, including education and communication of risk, exposure monitoring and controls, limitations in the use of the chemicals of concern, or a total ban of the chemical. Risk management decisions are influenced by economic, political and social concerns and are considered separately from the risk assessment process (Fig. 1).

**Figure 1.** Risk analysis as a concept.




## HISTORY

The origins of toxicology appear to be deeply rooted in the history of human civilization. Our ancestors in their quest for food must have attempted to eat a variety of foods of both plant and animal origin, and soon recognized that there were harmful as well as beneficial consequences associated with the consumption of such material. The rise of agricultural knowledge has been traced back to ancient times, when humankind progressed from nomadic hunting/gathering tribes to more settled societies supported by domesticated animal herds and cultivated crops. In terms of archaeological findings, primitive agriculture may have developed as early as 9000–7000 BC in the Near East [11]. The first attempt of identification and classification, and

the introduction of the first antidotes, took place during Greek and Roman times. Dioscorides categorized poisons by their origin: animal, vegetables and mineral. This categorization remained the standard classification for the next 1500 years [31]. In the 3rd century BC, Aristotle commented that "coal fumes (carbon monoxide) lead to a heavy head and death" [14]. The recognition, classification, and use of poison in Ancient Greece and Rome where accompanied by an intensive search for universal antidotes. In fact, many of the physicians of this period devoted significant parts of their careers to this endeavour [32]. Paracelsus' study on the dose-response relationship is usually considered the beginning of the scientific approach to toxicology. He was the first to emphasize the chemical nature of toxic agents [23]. Paracelsus, the best known Renaissance toxicologist, stressed the need for proper observation and experimentation regarding the true response to chemicals. He underscored the need to differentiate between the therapeutic and toxic properties of chemicals when he stated in his *Third Defence*, "What is there that is not poison?" All things are poison and nothing (is) without poison. Solely, the dose determines that the thing is not a poison." [7] The development of toxicology as a modern science, as a distinct branch deity, began during the 18th and 19th centuries. Attention focused on the detection of poisons and the study of toxic effects of drugs and chemicals in animals [19]. The poison mystique – mythological and magical – was gradually replaced by an increasingly rational, scientific, and experimental approach to the study of these agents. Much of the lore of poisons that had survived for almost 2,000 years was finally debunked and discarded [15]. Table 2 shows important early figures and events in the history of toxicology.

## CLASSIFICATION

History has taught us how to classify all substances in two classes: Those that are safe and the others that are harmful. Traditionally, the term food was used for those materials that where beneficial and essential for the functioning of the human body. Substances that were distinctly harmful to the body were classified as poisons. This

**Table 2.** Historic overview of important events in the evolution of toxicology from antiquity to 1900s. [a]

| Date | Person | Importance |
|---|---|---|
| c.a. 4500 B.C. | Gula | First deity associated with poisons |
| c.a. 850 B.C. | Homer | Wrote how Ulysses anointed arrows with the venom of serpents |
| 384–322 B.C. | Aristotle | Described the preparation and use of arrow poisons |
| c.a. 470–389 B.C. | Socrates | Executed by the poison Hemlock |
| c.a. 132–36 B.C. | King Mithridates VI | Fanatical fear of poisons; developed first mithradatum, one of first universal antidotes |
| 81 B.C. | Sulla | Issued *Lex Cornelia*, the first anti-poising law |
| 69–30 B.C. | Cleopatra | Committed suicide from deliberate cobra envenomation |
| 40–80 A.D. | Dioscorides | Wrote *Materia Medica*, which classified poison by animal, vegetable and mineral |
| 9th Century | Ibn Wahshiya | Farm Arab toxicologist; wrote toxicology treatise *Book of Poisons*, combining contemporary science, magic and astrology |
| 1135–1204 | Moses Maimonides | Wrote *Treatise on Poisons and Their Antidotes* |
| 1250–1315 | Petrus Abbonus | Wrote De Venenis the major work on poisoning |
| 1493–1541 | Paracelsus | Introduced dose-response concept to toxicology–Paracelsus' study on the dose-response relationship is usually considered the beginning of scientific approach to toxicology. He was the first to emphasize the chemical nature of toxic agents. |
| 1611–1678 | William Piso | First to study emetic properties of ipecacuanha (plant *Psychotria ipecacuanha*) |
| 1633–1714 | Bernardino Ramazzani | Father of occupational medicine; wrote *De Morbis Artificum Diatriba* |
| 1714–1788 | Percival Pott | First description of occupational cancer, relating to chimney sweeps |
| 1730–1805 | Felice Fontana | First scientific study of venomous snakes |
| 1820 | Edward Jukes | Self-experimented with orogastric lavage apparatus known as Juke's syringe |
| 1787–1853 | Bonaventure Orfila | Father of modern toxicology; wrote *Traite des Poisons*; first to isolate the arsenic of humans organs |
| 1797–1882 | Robert Christison | Wrote *Treatise on Poisons*, one of the most influential texts in the early 19th century |
| 1848 | O.H. Ostill | Wrote first book on symptoms and treatment of poisoning |
| 1847–1915 | Max Gutzeit | Developed a method to quantify small amounts of arsenic |
| 1850–1929 | Luis Lewin | Studied many toxins, including methanol, chloroform, snake venom, carbon monoxide, lead, opiates and hallucinogenic plants |
| 1869–1970 | Alice Hamilton | Conducted landmark investigations associating worksite chemical hazards with diseases; led reform movement to improve worker safety |

[a] Adapted from Thompson CJ, 1931 and Pachter HM, 1961.

concept involving the division of chemicals into two categories has persisted to the present day. Loomis (1978), however, suggested that such classification, in a strictly scientific sense, is not warranted, primarily because a strict line of demarcation classifying and separating the beneficial and harmful chemicals cannot be drawn, and because the degree of harmfulness of any compound is essentially related to the amount consumed [6].

Toxic agents are classified in a variety of ways, depending on the interests and needs of the classifier. For example, toxic agents can be discussed in terms of their target organs (liver, kidney, hematopoietic system, etc.), use (pesticides, solvent, food additives, etc.), source (animal and plant toxins) and effects (cancer, mutation, liver injury, etc.) The term *toxin* generally refers to toxic substances that are produced by biological systems, such as plants, animals, fungi or bacteria. The term *toxicant* is used in speaking of toxic substances that are produced by or a by-product of anthropogenic activities. Some toxicants can be produced by both natural and anthropogenic activities. For example, polyaromatic hydrocarbons are produced by the combustion of organic matter which may occur both through natural processes (e.g. forest fires) and through anthropogenic activities (combustion of coal for energy production; cigarette smoking). Generally, such toxic substances are referred to as toxicants, rather than toxin, because although they are naturally produced, they are not produce by biological systems.

Toxic agents may also be classified in term of their physical state (gas, dust, liquid), their chemical stability or reactivity (explosive, flammable, oxidizer), general chemical structure (aromatic, amine, halogenated hydrocarbon, etc.) or poisoning potential (extremely toxic, very toxic, slightly toxic, etc.) Classification of toxic agents on the basis of their biochemical mechanisms of action (e.g. alkylating agent, sulfhydryl inhibitor, methemoglobin producer) is usually more informative than classification by general terms such as irritants or corrosives [8].

Toxic substances can be classified in several ways, according to the areas of interest of the individuals who deal with the subject of toxicology.

**Classification according to the methods of isolation from natural sources.** Historically, where toxins classified according to their way of isolation from natural source of plant, animal or mineral origin. The animal sources used in these classifications included the toxins produced in the specialized organs of snakes, spiders, marine animals, etc. Current classification based on this approach include marine organisms because fish poisons such as ciguatoxin, saxitoxin and tetrodoxin are produced by marine organisms in the diet of fish, and these toxins may concentrate in the process of preparing food or protein sources. Examples of plant sources from food toxins are caffeine, yellow rice, gossypol poisoning (cotton seed) and certain fungi, while trace metals can be given as examples of toxins isolated from mineral sources.

**Classification according to physical state.** Toxic substances can be classified according to their physical states; examples of this method of classification are toxins in a gaseous state (e.g. hydrogen sulphide and sulphur dioxide; toxins as vapour – e.g. benzene and hexane; toxins in aerosol form – e.g. insecticides and herbicides; and toxins in dust form – e.g. aflatoxins and asbestos powder).

**Classification according to their use, labels and chemical structures.** Toxins can also be classified according to their use and labels, such as explosive, pesticides, solvents, food additives, plasticizers, etc. They can be classified according their chemical structures, such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), organometallic compounds, ametallic compounds, etc. The chemical structure and the biological activity of toxins are related to each other because specific functional groups can show specific toxic effects. In addition, the isomerism in the chemical structure (optical activity and structural isomerism) can affect the biological activity of toxins.

**Classification according to pathophysiological effects.** Toxins can be classified according to their impact on physiological effects. In this method of classification, the tissue or target organs affected by the toxin (hepatotoxins, bone marrow toxins, kidney toxins), the pathophysiological changes that occurred (central nervous system depressors, teratogenesis, carcinogenesis, mutagenesis) and biochemical effect mechanism (toxins producing methemoglobinemia) are taken into consideration [1].

The EU distinguished between three types of categories of chemicals. These sets of categories are useful for statistical analysis of the uses and functions of chemical substances in the European Union [10]. The two last categories are present in Table 3:

1. The 4 main categories give an indication of the level of the emission factor.
2. The 16 industrial categories cover all areas of society where chemicals are used.
3. The 55 use categories cover the many specific functions substances may have.

But from the other point of view, Table 4 shows xenobiotics in food classified into the following groups:

- Chemical substances that are unintentionally introduced into food of animal and vegetable origin where their residues remain.
- Contaminating substances that are unintentionally introduces into food during its industrial processing and distribution.
- Contaminating substances found randomly in natural products.
- Substances that are added to food intentionally during its production, processing and distribution.
- Substances created in food by its decomposition, or by interaction of its components during production, processing and distribution [27, 28].

**Table 3.** List and description of categories used by EU for new and existing chemicals.

**Industrial category**

The 16 industrial categories listed below represent industrial use areas for chemicals. Some substances are used in more than one industrial category.

1. Agricultural industry (e.g. plant protection products, fertilisers)
2. Chemical industry: basic chemicals (e.g. solvents, pH-regulating agents – acids, alkalis)
3. Chemical industry: chemicals used in synthesis (e.g. intermediates – including monomers, process regulators)
4. Electrical/electronic engineering industry (e.g. electrolytes, semiconductors; not: galvanic, electroplating agents)
5. Personal/domestic (e.g. consumer products such as detergents – including additives, cosmetics, agricultural pesticides for domestic use)
6. Public domain (e.g. professional products used in public areas as non-agricultural pesticides, cleaning agents, products used in offices such as correction fluids, printing inks)
7. Leather processing industry (e.g. dyestuffs, tanning auxiliaries)
8. Metal extraction industry, refining and processing industry (e.g. heat transferring agents)
9. Mineral oil and fuel industry (e.g. gasoline, motor oil, gear oil, hydraulic fluid, colouring agents, fuel additives, anti-knock agents, waste oil detoxification agents)
10. Photographic industry (e.g. anti-fogging agents, sensitizers)
11. Polymers industry (e.g. stabilisers, softeners, anti-static agents, dyestuffs)
12. Pulp, paper and board industry (e.g. dyestuffs, toners)
13. Textile processing industry (e.g. dyestuffs, flame retardants)
14. Paints, lacquers and varnishes industry (e.g. solvents, viscosity adjusters, dyestuffs, pigments.
15. Engineering industry: civil and mechanical – new substances (e.g. agents used in construction work, agents used in automobile, aircraft and ship building) Other – existing substances. Substances not described elsewhere.

16/999 Other – new substances. Substances not described elsewhere.

**Function category/Use category**

The 55 use categories listed below represent various functional uses of substances. Some of them are subdivided into sub-categories where appropriate. For clarity, exclusions are indicated in some cases.

1. Absorbents and adsorbents (filter material/media, molecular sieves, silica gel, etc.)
2. Adhesives, binding agents (dispersion-based adhesives, hot melt, resins for polymer-based hardening adhesives, solvent based adhesives)
3. Aerosol propellants
4. Anti-condensation agents (anti-dim agents, condensation removers, etc.)
5. Anti-freezing agents (anti-freeze liquids, de-icing agents, etc.)
6. Anti-set-off and anti-adhesive agents (spraying powder and anti-set-off additives for printing, oils and waxes for laths and shuttering, casting slip, etc.)
7. Anti-static agents (anti-static additives, substances for surface treatment against static electricity)
8. Bleaching agents Not: cosmetics, photographic bleaches, optical brighteners.
9. Cleaning/washing agents and additives Sub-categories: detergents, soaps, dry cleaning solvents, optical brighteners in detergents.
10. Colouring agents Sub-categories: dyestuffs, pigments (including toners), colour forming agents, fluorescent brighteners (but see below re detergents). Not: cosmetics; food colours; photo-chemicals, optical brighteners used exclusively in detergents, reprographic agents.
11. Complexing agents
12. Conductive agents Sub-categories: electrolytes, electrode materials.
13. Construction materials additives (wall construction materials, road surface materials, ceramic, metal, plastic and wooden construction materials)
14. Corrosion inhibitors (corrosion inhibiting additives, rust preventives)
15. Cosmetics
16. Dust binding agents
17. Electroplating agents
18. Explosives Sub-categories: blasting agents, detonators, incendiaries.
19. Fertilisers
20. Fillers
21. Fixing agents
22. Flame retardants and fire preventing agents
23. Flotation agents (flotation oil, flotation depressants)
24. Flux agents for casting
25. Foaming agents Sub-categories: chemical or physical blowing agents, frothers.
26. Food/feedstuff additives
27. Fuels Sub-categories: gasoline, kerosine, gas oil, fuel oil, petroleum gas, non-mineral oil.
28. Fuel additives Sub-categories: anti-fouling agents, anti-knock agents, deposit modifiers, fuel oxidisers.
29. Heat transferring agents Sub-categories: cooling agents, heating agents.
30. Hydraulic fluids and additives
31. Impregnation agents Not: flame retardants, conserving agents, biocides.
32. Insulating agents
33. Intermediates Sub-categories: monomers, pre-polymers.
34. Laboratory chemicals
35. Lubricants and additives (oils, fats, waxes, friction reducing additives)
36. Odour agents Not: food additives, cosmetics.
37. Oxidising agents
38. Pesticides Not: nutrients, fertilisers.
39. Pesticides, non-agricultural (Biocides) Sub-categories: disinfectants, preservative products, pest control products, specialist biocides. Not: plant protection products, veterinary products.
40. pH-regulating agents (acid, alkalis, buffers)
41. Pharmaceuticals Sub-categories: veterinary medicines.
42. Photochemicals Sub-categories: desensitisers, developers, fixing agents, photosensitive agents, sensitizers, anti-fogging agents, light stabilisers, intensifiers.
43. Process regulators Sub-categories: accelerators, activators, catalysts, inhibitors, siccatives, antisiccatives, cross-linking agents, initiators, photo-initiators etc.
44. Reducing agents
45. Reprographic agents Sub-categories: toner for photocopying machines, toner additives.
46. Semiconductors Sub-categories: semiconductors; photovoltaic agents.
47. Softeners Sub-categories: coalescing agents, bates (leather technology), devulcanising agents, emollients, swelling agents, water softeners, plasticisers.
48. Solvents
49. Stabilisers Sub-categories: antioxidants, heat stabilisers, light stabilisers, scavengers, charge stabilisers.
50. Surface-active agents
51. Tanning agents
52. Viscosity adjusters Sub-categories: pour point depressants, thickeners, thixotropic agents, turbulence suppressors, viscosity index improvers.
53. Vulcanising agents
54. Welding and soldering agents

55/999 Others – Substances whose technical functions are not described elsewhere.

**Table 4.** General classifications of xenobiotics in foods. [a]

**Substances added intentionally** (additive)

- Substances to improve appearance – food colorants, glazing agents (e.g. waxes, to give polished appearance), etc.
- Substances to extend storage stability – preservatives, antioxidants, etc.
- Substances to improve and modify consistence – emulsifiers, stabilizers, thickeners, etc.
- Substances to improve and modify flavour, aroma and biological value – sweeteners, aromas, essences, vitamins
- Adjuvants – enzymes

**Substances present unintentionally**
(contaminants and secondary products)

- Residues of agrochemicals: fungicides, herbicides, insecticides, etc.
- Residues of animal production: estrogens, antibiotics, tranquilizers, etc.
- Residues of adjuvants
- Others residues of: abstergents, disinfectants, chemical intermediates – metabolites, etc.
- Chemical contaminants present after microbiological processes: solvents, glazing agents, coagulators, neutralizing agents, acids and alkalis, enzymes, catalysts, bacterial and mould toxins, etc.
- Environment pollution: residues of toxic chemical elements, carcinogenic hydrocarbons, etc.
- Pollution from articles in daily use: softeners, stabilizers, traces of toxic elements, etc.
- Secondary products: come from chemical, physical and biological processes

[a] Adapted from Rosival L, Engst R, Szokolay A, 1978.

## SUMMARY

**Environmental pollutants.** The main sources of pollution are industrial processes and the deliberate release into the environment of substances such as pesticides. Pesticides are deliberately sprayed onto crops or agricultural land with the potential for exposure either via the crop itself or through contamination of drinking water or air. With pesticides, the major problem is persistence in the environment and an increase in concentration during passage through the food chain. The most visible pollutant, but perhaps not the most significant, is smoke from power stations and factories. Environment pollutants maybe released into the air, river, or sea water, or dumped on to the land. Vehicle exhaust fumes with several known toxic constituents constitute a major source of pollution.

**Natural toxins.** Many plants and animals produce toxic substances for both defensive and offensive purposes. Natural toxins of animal, plant, mould and bacterial origin comprise a wide variety of chemical types, cause a variety of toxic effects and are a significant cause of human poisonings. The concept currently expounded by some individuals that "natural is safe" is, in many cases, very far from the truth, and some of the most toxic substances known to man are of natural origin. Natural toxins may feature in poisoning via contamination of food, by accidental ingestion of poisonous plants or animals, and by stinging and biting.

**Household poisons.** These may include some of the substances in other categories such as pesticides, drugs and solvents. Exposure to these types of compounds is usually acute rather than chronic. Many household substances used for cleaning are irritants and some are corrosive [15].

**Food contaminants.** pollutants derived from burning fossil fuels, radionuclides from fallouts, or emission of industrial processing (toxic trace elements, radionuclides, polycyclic aromatic carbons, dioxins); components of packaging material and of other frequently used products (monomers, polymer stabilizer, plasticizer, polychlorinated biphenyls, cleansing/washing agents and disinfectants); toxic metabolites from microorganism (enterotoxins, mycotoxins); residue of plant-protective agents; residue from livestock and poultry husbandry (veterinary medicaments and feed additives). Toxic food contaminants might also be formed within the food itself or within the human digestive tract by reactions of some food ingredients and additives (e.g. nitrosamines).

There are more than 200 mycotoxins produced under certain conditions by about 120 fungi or moulds. Most mycotoxins data are on the genera *Aspergillus* spp. and the aflatoxins they produce during growth. These are the most common and highly toxic fungal toxins, e.g. aflatoxin $B_1$, the most powerful carcinogen known. In the course of food monitoring between 1995–2002, more than 40 foods were tested for the presence of aflatoxins, deoxynivalenol, fumosis, patulin, ochratoxin A and zearalenone. Individual mycotoxins were detected in 21% of the samples; pistachios were especially conspicuous.

**Food Additives.** This category of xenobiotics is directly ingested. However, food additives are usually of low biological activity. Many different additives are now added to food to alter the flavour or colour, prevent spoilage, or in the some other way change the nature of the foodstuffs. There are also many potentially toxic substances which may be regarded as contaminants occurring naturally in the food, resulting from cooking or from other contamination. Veterinary drugs and their breakdown products may also be found in foodstuffs. Most of these substances, both natural and artificial, may be present in food in very small amounts, but for the majority little is known of their long-term toxicity. In many cases they are ingested daily for perhaps a lifetime and the number of people exposed is very large. Although reliable data are still scarce, there certainly seems to be evidence that at least some additives may be associated with adverse effects.

**Plant protection agents (PPA).** Include all compounds used in agricultural food production to protect cultivated plants from plant- and insect-caused diseases, parasites or weeds, or from detrimental microorganism. The most important groups of PPA are: herbicides to protect the plant from weeds; fungicides to suppress the growth of undesired

fungi or moulds (inorganic fungicides – Bordeaux mixture, copper chloride oxide, lime and colloidal sulphur; organometallic compounds – dithiocarbamates of zinc and manganese and organic (metal-free) compound – lindane, linuron, vinclozin, ziram, etc.) and insecticides to protect the plants from damage caused by insects (organophosphate compounds, carbamates and pyrethroids have been used for many years). In addition to these main groups, there are acaricides to control mites, nematocides to control worms or nematodes, molluscicides to protect the plants from snails and slugs, rodenticides to control rodents (mice or rats) and plant growth regulators.

**Polychlorinated biphenyls (PCBs).** A complex mixtures of substances which were on the market from 1950. There were widely used, e.g. as transformer oil, hydraulic fluid, heat exchange medium, dielectric fluid in condensers, plasticizers, and additive for printing ink. The PCBs also came into contact with food, because of their persistence and solubility in fat, they accumulate, as in the case of DDT. Therefore, their discovery they were increasingly identified in fatty foods since. This, and the fact that PCBs can produce highly toxic dioxins in the combustion process, led to the banning of the production and application of PCBs in 1989.

**Harmful substances from thermal process.** Burning of organic materials, such as wood (wood smoke and its semi-dry distillation product, the wood smoke vapour phase), coal or fuel oil, results in pyrolytic reactions, which yield a great number of polycyclic aromatic hydrocarbons (PAHs – about 250 have been identified) with more than three linearly or angularly fused benzene rings, that are carcinogenic to varying extents. The quantity and diversity of the compounds generated is affected by the condition of the burning process. Benzo[a]pyrene (Bap) usually serves as indicator compound. Contamination of food with polycyclic compounds can be caused by fall-out from the atmosphere (as often occurs with fruit and leafy vegetables in industrial districts), by direct drying of cereals with combustion gases, by smoking or roasting of food (barbecuing or charcoal broiling; smoking of sausages, ham or fish; roasting of coffee). PAHs accumulate in high-fat tissues. Others are furan (possibly a carcinogenic substance, occurs in heated food, especially in roasted coffee), acrylamide (polyacrylamite, produced from monomeric acrylamide 2-propenamide, has been used for decades in various industrial processes, e.g. as a flocculant in the treatment of drinking water.

**Nitrate and nitrosamines.** The plants with the highest nitrate concentration 1,000–4,000 mg/kg fresh weight (chinese cabbage, endivie, corn salad, lettuce, fennel, kohlrabi, beetroot, radish, rocket and spinach) can store a lot of nitrate, their nitrate content depending, among other things, on the N supplied on fertilization. Apart of the properties of the soil, even light plays a role because some plants store more nitrate when there is a lack of light. Food of animal origin and drinking water are further sources of nitrate [2]. When nitrite reacts with secondary amines, nitrosamines are formed, and many are known to be powerful carcinogens.

**Metals.** Metals are probably some of the oldest toxicants known to humans. Health effect, such as colic, were reported following exposure to lead, arsenic and mercury over 2,000 years ago. Metals can be widely distributed in the environment by geological, meteorological, biological, environmental, and anthropogenic activities. For most individuals, the greatest cause of metal exposure is due to the metal content in food, with a smaller additional component coming from air (for example, consumer products such as deodorants – zirconium, vitamin and mineral supplements – selenium, hair dyes – silver, lead and cosmetics – lead, antimony, and cooper.

Exposure to metals and metal-containing compounds is common to many industrial, non-industrial, and environmental situations. Absorption of metals can have effects on the body, not all of them adverse. It must be remembered that some metals are essential for the normal function of the body – Co, Cu, Fe, Mn, Ma, Se, Zn [16].

**Industrial chemicals.** May contribute to environmental pollution and they may be a direct hazard in the workplace where they are used, formulated or manufactured. There is a huge range of chemical types and many different industries may involve the use or manufacture of hazardous chemicals. In the broadest sense, industrial exposure might include exposure to the solvents used in photocopiers and typist's correction fluid. Although in general exposure is controlled by law, often by the setting of control limits, realistic levels may still prove to be hazardous in the long-term, and acute exposure due to accidents will always occur. The long development time of diseases such as cancer often makes it difficult to determine the cause until a sufficient number of the workforce have presented with the disease for the association with a toxic compound to be made.

## CONCLUSION

It is evident that no single classification is applicable to the entire spectrum of toxic agents and that a combination of classification systems, or a classification based on other factors, may be needed to provide the best rating system for the special purpose. Nevertheless, the classification system that takes into consideration both the chemical and biological properties of an agent and the exposure characteristics are most likely to be used for legislative or control purposes and toxicology in general.

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