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Introduction to the Discipline of Toxicology

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“In all things there is a poison, and there is nothing without a poison. It depends only upon the dose whether a poison is poison or not.”

Paracelsus (1493–1541)

1.1 Introduction

The discipline of toxicology is concerned with the health risks of human exposure to chemicals or radiation. According to Paracelsus’ paradigm toxicology is charged with describing the adverse effects of chemicals in a qualitative sense, and with evaluating them quantitatively by determining how much of a chemical is required to produce a given response. Taking these two together, we can describe the intrinsic properties of an agent (hazard identification) and we can estimate the amount of the chemical required to produce these properties (risk characterization).

Humans may be exposed to chemicals in the air, water, food, or on the skin. The external dose at which a chemical exerts its toxic effects is a measure of its potency, i.e. a highly potent chemical produces its effects at low doses. Ultimately, the response to the chemical depends upon duration and route of exposure, the toxicokinetics of the chemical, the dose–response relationship, and the susceptibility of the individual. To characterize the risk, the dose–response can be evaluated and the exposure at which the chemical will produce adverse effects identified. It is obvious from this that risk characterization comprises three elements:

- hazard identification, i.e. a description of the agent’s toxic potential
• evaluation of the dose response, including information on the concentration above which the agent induces toxic effects to identify the no observable adverse effect level (NOAEL)
• exposure assessment to understand the concentration of the agent in the relevant medium, time, and routes of human exposure.

It is necessary to establish toxicological profiles of each chemical, either pre-existing or newly developed, to ensure that it can be utilized safely either by the public or under specific conditions of use such as in the workplace. Toxicological evaluations may take different forms for new and existing chemicals. In the case of newly developed drugs, pesticides or new chemicals a stepwise procedure starting from structure–activity evaluation and simple in vitro and in vivo short-term tests and proceeding to life-time testing in experimental animals. Depending on the hazardous potential of the agent, studies can be extended to evaluate toxicokinetics and the toxic mode of action. For existing chemicals the available information is collected and a risk assessment based on exposure data, knowledge of the dose–response relationship, and the mode of action is performed.

The parameters that determine toxic potential and potency are discussed in the following chapters. Here they are briefly discussed to indicate their importance for the risk characterization process, which is presented in detail in Chapter 2.1.

1.2 The Risk Assessment Process

1.2.1 Hazard Identification

Chemicals induce local and/or systemic effects such as embryotoxicity, hepatotoxicity, neurotoxicity, etc. after absorption from the gastrointestinal tract, through the skin or via the lungs. Reactivity, solubility, and metabolism of the chemical, its metabolites, and their distribution within the organism determine the target organ of the critical effects.

Acids or bases can be directly acting agents which cause irritation or corrosion at the site of exposure such as skin, mucous membranes of the eye, the gastrointestinal tract or the respiratory system. However, most chemicals induce systemic effects such as embryotoxicity, hepatotoxicity, neurotoxicity, etc. after absorption from the gastrointestinal tract, through the skin or via the lungs. Depending on exposure concentration and time of exposure, acute or chronic effects may result. Acute intoxication usually occurs in response to large doses. Chronic effects are seen after repeated exposure, during which time the chemical reaches critical concentrations at the target organ, e.g. liver, kidney or central and peripheral nervous system. Histopathological and biochemical changes have been the major parameters used to detect organ toxicity. Increasing availability of sensitive methods in analytical chemistry and molecular-biological approaches including toxicokinetics and the various “omics” (Chapter 4.6) have significantly improved the understanding of the mechanisms by which cellular and subcellular functions are impaired and how the cells respond to toxic insults. This results in a better understanding of toxic mechanisms, species differences and the consequences of exposures at high and low concentrations over different times.

Exposure to some chemicals, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), can result in retention and long-lasting effects even after a single high exposure (Chapter 6.1). This is because TCDD is lipophilic and not well metabolized, which results in very slow elimination. The consequence is accumulation in adipose tissue. In humans the half-life of excretion is about 8 years. In laboratory animals and humans TCDD induces tumors in various organs. Since TCDD does not induce DNA damage or mutations, the carcinogenic effect is considered to have a threshold, i.e. there are doses below which no adverse effects will be observed.
Induction of sensitization and of allergic responses by sensitizing agents are also considered to require to reach a threshold dose, although at very low doses and the NOAELs of these effects are rarely known. When establishing acceptable exposure standards thresholds are not considered to be a property of the dose–response curves for genotoxic carcinogens because so far any genotoxic event is considered irreversible. (For more detailed discussion of this concept see Chapters 2.8 and 2.9.)

1.2.2 Dose Response and Toxic Potency

The Paracelsian admonition teaches us that the occurrence and intensity of toxic effects are dose dependent. This paradigm addresses the concept of threshold effects, which implies knowledge of the dose–response relationship. Animal or human exposure is usually defined as the dose, e.g. mg of the chemical/kg body weight/day. This daily dose may result from oral, inhalation or dermal exposure or as a sum thereof. The external dose leads to a specific internal dose, which depends on the amount absorbed via the different routes and the distribution to the critical target (tissue, organ). Absorption rates via the different routes can vary significantly, although oral and inhalation exposure usually lead to the highest internal dose. For example, about 50% of cadmium in inhaled air, e.g. in tobacco smoke, is absorbed in the lung, whereas cadmium absorption from the gastrointestinal tract is about 10%. Ultimately, it is the dose which reaches the cellular target over a given time period that results in the toxicological response. No toxic effects will be seen if the dose is below the NOAEL, whereas effects increase with increasing exposure. The dose–response curve may be expressed using a variety of mathematical formulas. Using the semilogarithmic form of the dose–response relationship the curve is sigmoidal in shape and varies in slope from chemical to chemical. Thus, if the curve is shallow a doubling of the dose results in a small increase in effects, whereas effects increase several-fold when the slope is steep (see Figure 1.1). The log of the dose is plotted on the abscissa (X axis) and increases toward the right. The location of the curve on the abscissa is a measure of the potency of the chemical.

![Dose–response curve showing log dose on the X axis and % response (effect) on the Y axis. The figure illustrates the location of regulatory values such as the NOEL (NOAEL), occupational exposure levels (OELs), and environmental standards such as acceptable daily intake (ADI). Note that a doubling of dose in the lower or upper part of the S-shaped curve results in small increases in effects, whereas it is much more prominent in the steep part.](image-url)
1.2.3 Exposure Assessment

Since toxic effects are dose dependent, knowledge of the extent and duration of exposure is an integral part of the risk assessment process. Exposure defines the amount of a chemical to which a population or individuals are exposed via inhalation, oral, and dermal routes. Animal or human exposure is commonly defined by mg of the chemical/kg body weight per day.

Toxicologists are concerned with exposure to any chemical, by any route, which may lead to adverse health effects. Exposure defines the amount of a chemical to which a population or individuals are exposed via inhalation, oral, and dermal routes. Animal or human exposure is commonly defined by mg of the chemical/kg body weight per day. According to the general principle of toxicology the consequences of human or environmental exposures depend on the amount and duration to which these individuals or populations are exposed. Thus, exposure assessment or prediction of exposure is an ultimate requirement for risk assessment and to decide whether regulations are needed. Since occupational exposure is regular and repetitive it can easily be measured in the air of the workplace by use of personal monitoring equipment or by biomonitoring.

Exposure of the general population is more difficult to assess. It usually is a combination of the presence of the compounds in indoor/outdoor air, drinking water or food, or use of products that contain the chemical (aggregate exposure). Moreover, frequency, duration, and site of exposure, and the concentration and weight of the substance in the products need to be considered. Children represent a special case of exposure. They may be exposed to chemicals that are released from articles such as toys during mouthing, via skin contact or from ingestion of contaminated dust or soil. Exposure can be modeled based on data such as information on frequency of mouthing, migration rates of the specific compound from the toy during mouthing, and absorption rates from the oral cavity and gastrointestinal tract.

The rate of absorption through the skin will also determine the internal exposure (body burden) of the chemical. Use of these parameters to assess exposure is plagued by many uncertainties, which often lead to overestimation of the actual exposure.

External exposure does not necessarily correlate with internal exposure. Therefore, risk assessment of internal exposure either requires knowledge of the dose response of internal exposure versus adverse effects or information to what extent external and internal doses correlate. Exposure assessment is even more complicated when mixtures of chemicals are the source of exposure (Chapter 2.3).

Ultimately, it is the dose that reaches the cellular target over a given time period that results in the toxicological response. Thus, the toxic potency of a chemical is the product of the interrelated external, internal, and target doses that result from the multiple pathways and routes of exposure to a single chemical (aggregate exposure). In the case of existing chemicals an appropriately designed program to measure the chemical in different media will provide the necessary information.

The measurement of external dose is either done on collected samples, i.e. food, or by direct measurement, i.e. in the ambient air. When collected samples are used representative sampling and appropriate storage conditions as well as accurate and reproducible measurement techniques are essential. This also applies to biomonitoring programs.
For new chemicals such data are not available and cannot be provided, so modeling of exposure is the only option.

In the EU Technical Guidance Document on Risk Assessment Part II the following core principles for human exposure assessment for new and existing chemicals and biocides are listed:

- Exposure assessments should be based upon sound scientific methodologies. The basis for conclusions and assumptions should be made clear and be supportable, and any arguments developed in a transparent manner.
- The exposure assessment should describe the exposure scenarios of key populations undertaking defined activities. Such scenarios that are representative of the exposure of a particular (sub)population should, where possible, be described using both reasonable worst-case and typical exposures. The reasonable worst-case prediction should also consider upper estimates of the extreme use and reasonably foreseeable other uses. However, the exposure estimate should not be grossly exaggerated as a result of using maximum values that are correlated with each other. Exposure as a result of accidents or from abuse shall not be addressed.
- Actual exposure measurements, provided they are reliable and representative for the scenario under scrutiny, are preferred to estimates of exposure derived from either analogous data or the use of exposure models.
- Exposure estimates should be developed by collecting all necessary information (including that obtained from analogous situations or models), evaluating the information (in terms of its quality, reliability, etc.), and thus enabling reasoned estimates of exposure to be derived. These estimates should preferably be supported by a description of any uncertainties relevant to the estimate.
- In carrying out the exposure assessment the risk reduction/control measures that are already in place should be taken into account. Consideration should be given to the possibility that, for one or more of the defined populations, risk reduction/control measures which are required or appropriate in one use scenario may not be required or appropriate in another (i.e., there might be subpopulations legitimately using different patterns of control which could lead to different exposure levels).

**Biomonitoring of exposure** (Chapter 4.3) is the best tool to determine actual individual exposure by determination of the chemical or its metabolites in blood, critical organs, urine or exhaled air. It allows determination of:

- the amount of a chemical taken into the organism by all routes (aggregate exposure)
- the metabolic fate of the chemical, its persistence in the organism, its rate of elimination, and from that the total body burden at the time of measurement
- the amount of the chemical and/or metabolites that reaches the target organs.

This procedure is also helpful to evaluate whether an environmental exposure such as increased indoor air concentrations or contaminated dust or soil, which might be ingested by children, actually leads to an increased body burden.

Reactive compounds may react with macromolecules like proteins or DNA. The latter does not automatically lead to a genotoxic effect because mutations usually occur at much higher doses. Thus, DNA adducts are markers of exposure and do not necessarily indicate effects.

**Biomonitoring of effects** determines the changes in a cellular function such as enzyme activity.
1.2.4 Risk Characterization

The sensitivity of analytical chemistry has advanced to the point where infinitesimally small amounts of chemicals can be detected and identified in the various media that characterize our environment. Detection of a chemical *per se* does not mandate that a toxicological effect in exposed people will be observed. Since the dose determines the poisonous effect, effects only occur when exposure exceeds the NOAEL.

The risk assessment process requires differentiation between reversible and irreversible effects. The dose–response curves for chemicals that induce reversible effects display a region below which no effects are observed. The highest dose at which no effects are seen is called the no observable adverse effects level (NOAEL). The point at which effects become observable is called the lowest observable adverse effect level (LOAEL). The term “adverse” requires evaluation whether the effect identified is adaptation or actually adverse. Note that a threshold is not the equivalent of an NOAEL since it describes concentration or exposure where the slope of the dose–response curves changes.

If damage is not repaired the effect persists and accumulates upon repeated exposure. In such cases a NOAEL cannot be determined and every exposure can be related to a defined risk. Reversibility depends on the regenerative and repair capacity of cells, subcellular structures, and macromolecules during and after exposure. Epithelial cells of the intestinal tract or the liver have a high regenerating capacity and rapidly replace damaged cells by increased cell replication. The highly specialized cells of the nervous system lose this capacity during natal and postnatal development. Consequently, damaged cells are not replaced, at least in the adult.

For chemicals that induce reversible effects, the NOAEL of the most sensitive endpoint is determined and compared with the human exposure to describe the margin of exposure (MOE) (or margin of safety, MOS). If the NOAEL is derived from animal experiments a MOE of 100 or greater is desirable (see Section 1.3.2).

A MOE of at least 10 is sufficient if the NOAEL is derived from human data (see Figures 1.1 and 1.3). These factors can be modified when data on the toxicokinetics or specific sensitivities indicate that the MOE can be reduced or needs to be increased.

The covalent binding of genotoxic mutagens and carcinogens to DNA is considered an irreversible event despite the availability of repair processes. Although there is increasing knowledge about DNA-repair mechanisms, the role of tumor-suppressor genes and apoptosis, their interactions and dose responses are not sufficiently understood to conclude whether genotoxic effects exhibit a threshold at low exposure or even a NOAEL (see Chapters 2.8 and 2.9). So far, the general agreement remains that the potency of genotoxic carcinogens increases with increasing dose so that the risk at a given exposure is determined by linear extrapolation from the dose–response data obtained from experimental studies in animals or from data obtained from humans (Chapter 4.8).

1.3 Toxicological Evaluation of New and Existing Chemicals

The various toxic effects that chemicals may exert and the different applications for which chemicals are designed require in-depth understanding of the cause and effect relationship, i.e., knowledge of the chemical and the specific organs upon which it impacts. As a result toxicologists tend to focus on specific organs, specific applications (e.g., pesticides or drugs), specific compounds like metals or solvents, or specific effects like carcinogenicity. Chapters in this book are devoted to specific organ toxicity and the specific effects of compounds such as carcinogenicity and mutagenicity. Institutions that document the toxicological, including epidemiological, data for hazard and risk assessment are given in Table 1.1.
Table 1.1  International institutions that publish documentations on chemicals.

<table>
<thead>
<tr>
<th>Institution</th>
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<tr>
<td>Agency for Toxic Substances and Disease Registry (ATSDR)</td>
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</tr>
<tr>
<td>American Conference of Governmental Industrial Hygienists (ACGIH)</td>
<td>ACGIH Threshold Limit Values (TLVs) - ChemSafetyPro.COM</td>
</tr>
<tr>
<td>Canadian Centre for Occupational Health and Safety</td>
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</tr>
<tr>
<td>Dutch Expert Committee on Occupational Standards (DECOS)</td>
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<tr>
<td>European Centre for Ecotoxicology and Toxicology of Chemicals</td>
<td><a href="http://www.ecetoc.org/">http://www.ecetoc.org/</a></td>
</tr>
<tr>
<td>Health and Safety Executive (HSE)</td>
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<tr>
<td>SCOEL – EC Scientific Committee on Occupational Exposure Limits</td>
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<td>The Nordic Expert Group</td>
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</table>

1.3.1  General Requirements for Hazard Identification and Risk Assessment

Toxicological evaluation of chemicals requires knowledge of the health consequences of acute, subchronic and chronic exposure via routes relevant to the common use of the chemical. Therefore, all elements of risk assessment (hazard identification, dose response, exposure, and the risk) have to be evaluated.

Organ specificity and other relevant endpoints like fertility, pre- and postnatal toxicity or carcinogenicity, their dose response, and determination of the NOAEL can only be identified by appropriate repeated dose studies in animals. The use of *in vitro* testing can contribute important pieces of information, but so far cannot replace whole animal experimentation.

To obtain sufficient information on the hazardous properties of a chemical requires investigation of:
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• acute, subchronic, and chronic toxicity (oral, inhalation, dermal)
• irritation (skin, mucous membranes, eye) and phototoxicity
• sensitization and photosensitization
• genotoxicity (*in vitro* and *in vivo* methods)
• carcinogenicity (lifetime studies)
• reproductive toxicity
• toxicokinetics
• mode and mechanism of action.

In all studies information on the dose response of effects is essential to identify the slope of the dose–response curves, possible thresholds, NOAEL, LOAEL, and maximal tolerated dose (MTD). For most of the relevant tests guidelines have been proposed (e.g., see the Organization for Economic Cooperation and Development (OECD) Guidelines, Chapter 4.1).

**Acute Toxicity, Subchronic Toxicity, and Chronic Toxicity**

**Acute toxicity studies** describe toxic effects assessed after a single administration of the chemical to rodents and are primarily aimed at establishing a range of doses in which the chemical is likely to produce lethality. After dosing, the animals are observed over a period of one to two weeks to determine immediate or delayed effects. It is possible to plan studies in which other endpoints are examined as well.

Having established the lethal dose range the chemical may be examined for effects produced upon repeated administration. The common practice in such **repeated dose studies** is to treat animals each day for a few weeks or months. These studies usually include rodents, but larger species such as dogs, and in the case of new drugs, monkeys or apes, may be employed. The animals must be observed for effects on general, as well as specific, organ toxicity. At the termination of these studies the animals are usually examined for gross and microscopic pathology.

**Chronic studies**, usually in rodents, involve treatment of animals for several months up to a lifetime. Their intent is to examine the likelihood of the development of pathology after long-term exposure to low levels of chemicals and in the case of lifetime studies are focused on cancer.

There is an ongoing discussion regarding the extent to which *in vitro* studies and consideration of structure–activity relationships provide sufficient information to waive repeated *in vivo* exposure studies (Chapter 4.5). From a toxicological point of view it has to be stressed that this discussion is primarily concerned with cost reduction and protection of animals. It is necessary to ensure that in this climate protection of human health and the environment do not become secondary considerations.

**In vitro** studies allow identification of hazardous properties of substances, but only those that can be detected by the specific test system. When the dose response in the *in vitro* test system is known, toxicokinetic modeling may predict the dose response at the specific target *in vivo*. Even when the test system has a metabolic capacity, its appropriateness must be verified in intact organisms. Consequently, identification of all relevant endpoints, their dose response, thresholds, and NOAELs can only be determined in the intact animal by repeated dose studies. In the absence of such information hazard identification is incomplete and there is no basis for appropriate assessment of the risk of human exposure.

**Irritation and Phototoxicity**

Dermal irritation of compounds is evaluated by studies in animals and humans prior to testing for sensitization. These are usually performed by using a single occluded patch under the
same conditions as applied when testing skin sensitization. Phototoxicity and photoallergic reactions have to be expected when compounds show significant absorption in the ultraviolet range (290–400 nm). Using the test strategy for irritation, an additional patch site is irradiated immediately after application of the test substance or after patch removal. Phototoxicity can also be tested by validated *in vitro* tests, such as uptake of Neutral Red by 3T3 cells. If such a test is negative further *in vivo* testing may not be necessary.

**Sensitization and Photosensitization**

For detection of the sensitizing potential of products the choice of a relevant animal is crucial (Chapter 3.9). However, in many cases animal models may be inappropriate for detection of a sensitizing potential so most dermatologists prefer studies in humans. An acceptable alternative may be studies with non-human primate species like cynomologus or rhesus monkeys. Generally, the Buehler guinea pig test and the local lymph node assay (LLNA) in mice are used in the preclinical testing program. The LLNA received great attention because it is the only reliable test for screening compounds that cause sensitization via routes other than the skin. So far the test has been successfully applied to determine relative potencies of contact allergens and has been reported to closely correlate with NOAELs established from human repeat patch testing. When the animal data indicate a weak contact sensitizing potential human skin sensitizing testing is conducted, usually by a human repeated-insult patch test (HRIPT). In any case, detection of antibodies in the serum during the studies using specific ELISA methods or bioassays to measure antibodies may be appropriate.

**Genotoxicity**

Test systems and test strategies to evaluate possible genotoxicity of a compound are described in detail in Sections 4.2.1 and 4.2.2. Generally, a bacterial mutation assay and an *in vitro* cytogenetic assay are performed. The results are usually verified by the mouse bone marrow micronucleus test, a reliable and widely used test system that detects aneugens as well as clastogens. Chemicals that yield positive responses to these tests frequently do not undergo further development. However, those which appear to lack genotoxicity may be carried forward and evaluated in lifetime carcinogenicity studies in rodents.

**Carcinogenicity**

The design of carcinogenicity studies is similar to that of chronic studies. At least three adequately spaced doses are tested, the highest dose being the MTD. Usually relatively large doses/concentrations are used to maximize the chance of finding a possible increase in tumor incidence in relatively few animals (50 per sex/dose). If thought necessary additional animals are included for investigations at 12 and/or 18 months. Rats and mice are used because of their relatively short lifespan of about 2 years and available information on their susceptibility to tumor induction, physiology, and pathology. A large historical database on tumor incidence in most strains and tissues exists, which is important in view of the large variability of tumor incidence in untreated animals and among different strains. Although the tumor incidence of the control group of the specific study is of major relevance, the historical control data of the specific strain used in the specific laboratory can be helpful to evaluate the incidences in the controls, especially when they deviate from the expected values. The incidence of spontaneous and substance-induced tumors increases in older animals, so it is necessary to terminate the study after a defined period to avoid the impact of different lifespans on the interpretation of the data.
Since it is difficult to predict the MTD, severe toxicity may occur at this dose. This requires specific consideration when interpreting the results. In such cases the metabolism of the animal may be overwhelmed and/or detoxifying mechanisms, such as GSH levels or DNA repair, may no longer be operative. It should also be recognized that a number of tumors are species-specific and may not be relevant for humans. One example is the α-urinary globulin-induced kidney tumors of the male rat. Moreover, rodents are more sensitive to compounds that disturb thyroid hormone metabolism and are also much more sensitive to compounds that induce peroxisome proliferation in the liver. When such mechanisms have been properly demonstrated the resulting tumors are of low relevance to humans. In any case, the incidence and type of tumors found have to be evaluated by experts and the underlying mechanisms as well as possible high-dose effects, such as overwhelmed metabolism of the test compound, have to be taken into account in the final judgment of whether a substance is considered to be carcinogenic to humans.

Toxicity for Reproduction and Development

Studies to evaluate reproductive and developmental effects may only be needed if there are indications that the chemical, or critical metabolites, can reach the embryo and/or fetus and could cause teratological, feto-toxic, or developmental effects. In such cases tests such as a reproduction/developmental toxicity screening test (OECD 421), a combined repeated dose toxicity study with a reproduction/developmental toxicity screening test (OECD 422), or the appropriate standard tests to evaluate effects on reproduction (one-generation reproduction toxicity, OECD 415) and prenatal developmental study (OECD 414) may be performed.

Toxicokinetics

Toxicokinetics describe the absorption, distribution, metabolism, and elimination (ADME) of a chemical in humans, experimental animals or cellular systems. Of specific importance for the interpretation of animal studies and for the extrapolation of hazards between species is the comparative information on the exposure and the dose that reaches the critical target (Chapter 2.2).

A chemical may enter the body via food, air or the skin. The amount absorbed depends on the concentration in the different media, on physical-chemical parameters such as solubility in water and fat, stability, and the route of exposure (Figure 1.2).

Upon inhalation or skin penetration the compound directly enters the circulation and is distributed into the organs. When absorbed from the gastrointestinal tract the chemical enters the liver via the portal vein. The epithelial cells of the gut wall and the liver demonstrate a large capacity for metabolizing chemicals so that a compound may be extensively metabolized by this “first-pass effect” before entering the (cardiovascular) systemic circulation. Larger molecules, e.g. the glucuronosyl-conjugates, can be excreted via the biliary system into the duodenum, where the conjugates may be hydrolyzed so that the original compound is reabsorbed and re-enters the liver. This process is defined as enterohepatic circulation. Inhalation or dermal exposure to a chemical or intravenous or intraperitoneal injection may result in different effects than after oral exposure because of the first-pass effect.

After entering the cardiovascular system the chemical or its metabolites distribute to the organs, where they can accumulate, such as in fat or bones, or are further metabolized. Reactive metabolites will interact with tissue components and may induce cellular damage. This “tissue dose”, i.e. the concentration of a chemical or its metabolite at the critical target over a given time, is
Figure 1.2  Routes of exposure and systemic distribution of a compound within the organism. After oral ingestion the compound reaches the liver, where it can be extensively metabolized. Upon inhalation or dermal exposure and intravenous application the compounds reach the circulation without major metabolism.

an important factor that helps to explain the correlation between internal exposure and external (environmental) exposure in relation to toxicity. By comparing tissue doses in different species at similar exposures it also helps us to understand species differences in the sensitivity to chemicals, as well as interindividual variations.

The chemical or its more water-soluble metabolites are primarily excreted via the kidneys or the biliary system. Volatile compounds may be exhaled. The great variety of processes observed during absorption, metabolism, distribution, and excretion cannot be predicted by modeling or by in vitro experiments without confirmatory data from animals and humans.

Mode and/or Mechanism of Action

Identification of the modes or mechanisms by which a chemical induces toxicity and the dose–response relationship are essential to understand species specificities, species differences, sensitive populations or the interpretation of data regarding threshold or non-threshold effects. They also help to evaluate the relevance of the toxic effects derived from experimental animals for humans. Whereas a toxic mechanism is often not known in detail, modes of action, which can be described in a less restrictive manner, are undergoing consideration for inclusion in the risk assessment process.

There is an array of mechanisms by which chemicals, or any other stressors like heat or radiation, can lead to toxicity. They may be differentiated as follows:

**Physiological changes** are modifications to the physiology and/or response of cells, tissues, and organs. These include mitogenesis, compensatory cell division, escape from apoptosis and/or senescence, inflammation, hyperplasia, metaplasia and/or preneoplasia, angiogenesis,
alterations in cellular adhesion, changes in steroidal estrogens and/or androgens, and changes in immune surveillance.

**Functional changes** include alterations in cellular signaling pathways that manage critical cellular processes such as modified activities for enzymes involved in the metabolism of chemicals such as dose-dependent alterations in Phase I and Phase II enzyme activities, depletion of cofactors and their regenerative capacity, alterations in the expression of genes that regulate key functions of the cell, e.g. DNA repair, cell cycle progression, post-translational modifications of proteins, regulatory factors that determine rate of apoptosis, secretion of factors related to the stimulation of DNA replication and transcription, or gap–junction-mediated intercellular communication.

**Molecular changes** include reversibility or irreversibility of changes in cellular structures at the molecular level, including genotoxicity. These may be formation of DNA adducts and DNA strand breaks, mutations in genes, chromosomal aberrations, aneuploidy, and changes in DNA methylation patterns.

As indicated in Chapter 4.6, data derived from gene expression microarrays or high-throughput testing of agents for a single endpoint become increasingly available and need to be evaluated for suitability for use in the hazard and risk assessment process. As long as the information is not related to functional changes their relevance is poor and there is the possibility of overinterpreting the effects observed. High-throughput data on specific endpoints may aid in the identification of common mechanisms of multiple agents.

Mechanistic information is most relevant for the evaluation and classification of carcinogens. If the carcinogenic effect is induced by a specific mechanism that does not involve direct genotoxicity, such as hormonal deregulation, immune suppression or cytotoxicity, the detailed search for the underlying mode of action may allow identification of a NOAEL. This can also be considered for materials such as poorly soluble fibers, dusts and particles, which induce persistent inflammatory reactions as a result of their long-term physical presence that ultimately lead to cancer.

### 1.3.2 General Approach for Hazard Identification and Risk Assessment (for details see Chapter 2.1)

Before starting any evaluation, structural alerts and physical-chemical parameters like water/lipid solubility and volatility need to be identified as well as the purpose of the hazard identification. To screen for specific effects such as relative cytotoxicity, mutagenicity or hormonal effects simple *in vitro* tests may be appropriate. This allows identification of specific wanted or unwanted effects and by that selection of useful compounds for further studies or their elimination.

For a more detailed evaluation the stepwise procedure usually starts with the determination of the LD$_{50}$ to determine acute toxicity and the evaluation of genotoxicity by an *in vitro* bacterial test system (Ames test) and cytogenicity in mammalian cells. Positive results are verified *in vivo*, usually by the mouse bone marrow micronucleus test. For structural alerts or questionable results the compound needs further evaluation by additional tests, including studies on toxicokinetics or potential genotoxic mechanisms.

The information so far collected provides information on the reactivity of the test compound, its absorption and distribution in the organism, and possibly on critical targets. This allows a decision on whether or not the database is appropriate for further testing by repeated dose studies in animals for 28 and 90 days, which depending on the outcome and intended use of the chemical are followed by a 6-month or lifetime study to evaluate potential effects upon long-term exposure, including carcinogenicity.
Figure 1.3  Assessment of the acceptable daily intake (ADI)/tolerable daily intake (TDI) and limit values for drinking water, food and air.

This information finally allows appropriate risk assessment for potential human exposure or the setting of acceptable exposure limits for risk management. For example, when the detailed toxicological evaluation can exclude genotoxic and carcinogenic effects the NOAEL in long-term studies in experimental animals can be determined. This NOAEL is the starting point to set the acceptable daily intake (ADI), which is usually 100-fold below the NOAEL (Figure 1.3). This factor considers a 10-fold difference between the sensitivity of the experimental animals and humans, and another factor of 10 to take into account possible interindividual differences among the human population. If the NOAEL is derived from studies in humans the interspecies factor is not necessary and the factor of 10 to cover possible individual differences within the population is applicable only. These factors can be reduced if specific information is available to conclude that the species–species or intraspecies differences are less than 10. From the ADI permissible concentrations in food, drinking water, consumer products, indoor and outdoor air, and other environmental compartments may be established.

1.3.3  Toxicological Issues related to Specific Chemical Classes

Jurisdictions and regulatory agencies around the world have established a variety of guidelines for risk assessment and permissible exposure standards for chemicals in the workplace, the home, and the general environment. Regulatory decision-making depends upon the estimation of health risks from chemical exposure.

Health risks of chemicals designed for specific applications, e.g. consumer products, drugs or pesticides, must be assessed when people are exposed in the many types of environment in which people can be found. Therefore all elements of risk assessment (hazard identification, dose response, exposure, and the risk) have to be thoroughly evaluated.

Data requirements for new and existing chemicals usually depend on annual production rate and the extent of human exposure. When there is considerable exposure regulatory requirements demand an extensive toxicological evaluation of the potential adverse effects of the specific chemical and the likelihood of their expression under the conditions of use or exposure and the definition of the MOE or the health risk under defined conditions of exposure (Chapters 5.2 and 5.3).

For drugs special emphasis must be placed on efficacy, therapeutic index, potential side effects, and the effects of overdosage (Chapter 5.1).
For pesticides the relative impacts of the chemical on the target versus on people is a critical requirement. Thus, the NOAEL for people must be established and an acceptable daily intake (ADI) must be determined because of the possibility of contamination of food and other consumer products with the pesticide, and the margin of safety needs to be established (Chapter 6.10).

Exposures to chemicals in the workplace is, accordingly to law, controlled by the Occupational Safety and Health Administration (OSHA) in the United States and by the Chemicals Law Act (1992) in Europe. Various governmental and non-governmental institutions are involved in setting occupational exposure standards. Since the institutions publish the complete toxicologically relevant information and a justification for the proposed limit value, these documentations are valuable sources for the toxicological database of the compounds. Institutions that publish these documents are listed in Table 1.1.

### 1.3.4 Existing Chemicals

In 1992 the European Commission estimated that about 100,000 chemicals were in use. They are produced in quantities ranging from less than a ton to several million tons produced per year. Except for drugs and pesticides, the data requirement for existing or new chemicals has not been regulated. Although it is the responsibility of the producer and downstream user to release safe products, there are high-volume products with a relatively small database. Several programs have been launched to obtain knowledge at least for compounds with high annual production rates. In the United States the Environmental Protection Agency (EPA) has initiated an HVP program. In an international cooperation the OECD has launched the International Council of Chemical Associations (ICCA) program, which evaluates and documents the available information on environmental and human health hazards and risks for about 1000 chemicals. In Europe, Risk Assessment Reports under the Existing Chemical Program of about 150 compounds are being produced and Registration, Evaluation and Authorization of Chemicals (REACH) regulation started in 2008.

### REACH (Chapter 5.3)

REACH regulation in the European Union identifies substances of hazardous properties and evaluates the risks of human and environmental exposure. The regulation became effective by 2008. It is the responsibility of the producer or downstream user to provide the necessary information to the relevant agency. The extent of toxicological information largely depends on the annual production rate of a chemical. As long as there is no indication of a specific risk the chemicals will be registered for the intended use. Special attention will be paid to carcinogens, mutagens, and reproductive toxins (CMR compounds) and to other serious toxic effects as well as chemicals that show bioaccumulation, persistence, and toxicity (BPT compounds) in the environment. The specific use of such compounds needs to be authorized.

### 1.3.5 Test Guidelines (Chapter 4.1)

For reproducibility and acceptance by regulatory agencies standardized study protocols for each test have been developed. Moreover, only qualified laboratories are accredited to perform tests for regulatory purposes and they must adhere to good laboratory practice (GLP) guidelines. These guidelines describe how to report and archive laboratory data and records. GLP guidelines also require standard operating procedures (SOPs), statistical procedures for data evaluation, instrumentation validation, materials certification, personnel qualification, proper animal care, and
independent quality assurance (QA). GLP regulations have been developed by the US Food and Drug Administration (FDA) and by the OECD.

REACH requires the use of test methods as described in the Commission Regulation (EC) No. 440/2008 or any methods based on internationally recognized scientific principles. Use of the data for classification and labeling of chemicals according to the Globally Harmonized System (GHS) is described in the European Chemicals Agency (ECHA) Guidance on the Application of the classification, labelling and packaging (CLP) of substances and mixtures, Version 5.0 (July 2017). For pharmaceuticals the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has produced a comprehensive set of safety guidelines to uncover potential risks (www.ich.org, Safety Guidelines). These include repeated dose toxicity testing, toxicokinetics and pharmacokinetics, genotoxicity studies, carcinogenicity studies, and immunotoxicology studies. They refer in part to OECD guidelines for test descriptions and include guidance for result interpretation. Guidelines prepared by the US FDA or the European Medicines Agency (EMA) are generally in agreement with the ICH guidelines.

1.3.6 Alternatives to Animal Experiments (Chapter 4.5)

In recent years the 3R (refinement, reduction, and replacement) strategy for substituting animal experiments has received increasing attention. This intends to preferentially use in vitro studies and consideration of structure–activity relationships to waive and reduce studies in laboratory animals. Most success in the development of alternative methods is in local toxicity and acute toxicity testing, when the effects of the chemical, not its possible metabolites, are determined. The standardized in vitro mutagenicity/genotoxicity tests include some metabolizing capacity. Since these tests are frequently afflicted by false-positive results there is a need to verify their outcome by appropriate in vivo testing. In vitro studies allow identification of hazardous properties of substances, but only those that can be detected by the specific test system. Even when the test system has a metabolizable capacity, its appropriateness must be verified in intact organisms. As indicated before, toxicokinetic modeling may predict the external or internal dose response in an intact organism. Consequently, identification of all relevant endpoints, their dose response, thresholds, and NOAELs can only be determined in the intact animal by repeated dose studies. In the absence of such information hazard identification is incomplete and without information on the dose–response relationship obtained from these studies there is no basis for appropriate assessment of the risk of human exposure. Thus, in case of the methodologies for long-term testing for systemic and reproductive toxicity and carcinogenicity, which consume the highest number of animals, validated alternatives are lacking. In spite of this Regulation (EC) No. 1223/2009 requires validated alternative methods, in particular in vitro replacement methods for the safety evaluation of cosmetic substances and products.

1.3.7 Evaluation of Mixtures (Chapter 2.7)

Humans and their environments are exposed to a wide variety of substances. The potentially adverse effects of substances when present simultaneously have been analyzed in several reviews and documentations. Most recently the available scientific literature has been analyzed by SCCS/SCHER/SCENIHR 2011. The general conclusions are that chemicals with common modes of action will act jointly to produce combination effects that are larger than the effects of each mixture component applied singly (dose/concentration addition). However, effects only occur when the concentrations of the individual compounds are near or above their zero effect
levels (NOAELs). For chemicals with different modes of action (independently acting), no robust evidence is available that exposure to a mixture of such substances is of health or environmental concern if the individual chemicals are present at or below their zero effect levels. If no mode of action information is available, the dose/concentration addition method should be preferred. Prediction of possible interaction requires expert judgement and hence needs to be considered on a case-by-case basis.

1.3.8 Evaluation of Uncertainties

Derivation of the ADI, derived no effects level (DNEL), NOAEL, and risk assessments includes uncertainties. For example, the NOAEL may not be a real NOAEL for statistical reasons in that too few animals have been used in the specific experiment. Alternatively, the NOAEL may be rather conservative because the next higher dose, which determines the LOAEL of a weak adverse effect, is 10-fold higher. Usually this uncertainty is covered by applying assessment factors that build in a margin of error to be protective of the population at risk. In case of ADIs or DNELs the uncertainty factor of 100 covers the uncertainties of inter- and intra-individual differences unless toxicodynamic and/or toxicokinetic information allows its reduction. Whereas the experts who have performed the risk assessment are usually aware of uncertainties, the risk manager tends to use the numbers as such, with the consequence that any exposure even slightly higher than the ADI or DNEL is not considered to be acceptable. To acknowledge these uncertainties statistical analysis may be used to characterize and weight the different assumptions from various components of the risk assessment process, such as dose response, emissions, concentrations, exposure, and valuation. This will improve understanding of the uncertainties, thereby allowing better mean estimates of risk and of the risk for different individuals and populations. For uncertainty analysis see European Food and Safety Authority (EFSA, 2006) and ECHA (2010).

1.3.9 The Precautionary Principle

The precautionary principle is a measure to enable rapid response in the case of a possible danger to human, animal or plant health, or to protect the environment. In particular, where scientific data do not permit a complete evaluation of the risk this principle may, for example, be used to stop distribution or order withdrawal from the market products likely to be hazardous. Since this description allows various interpretations a more precise definition is given in the Communication from the Commission of 2 February 2000. There it is outlined that the precautionary principle may be invoked when a phenomenon, product or process may have a dangerous effect, identified by a scientific and objective evaluation, if this evaluation does not allow the risk to be determined with sufficient certainty. The Commission specifically stresses that the precautionary principle may only be invoked in the event of a potential risk and that it can never justify arbitrary decisions.

Other states use slightly different definitions, for example the Canada definition is as follows:

“The precautionary principle is an approach to risk management that has been developed in circumstances of scientific uncertainty, reflecting the need to take prudent action in the face of potentially serious risk without having to await the completion of further scientific research.”

1.3.10 The TTC Concept

The threshold of toxicological concern (TTC) is a concept to establish a level of exposure for chemicals, regardless their chemical-specific toxicity data, below which there is no appreciable risk to human health. The concept is based on knowledge of structural alerts, the amount of a specific chemical in a product, and the daily human exposure. The TTC as applied to foods is defined as a nominal oral dose which poses no or negligible risk to human health after a daily lifetime exposure. At a mean dietary intake below the level of the TTC, toxicology safety testing is not necessary or warranted. By that, the TTC concept can contribute to a reduction in the use of animals for safety tests. The TTC concept may also represent an appropriate tool to evaluate or prioritize the need for toxicological testing. There is ongoing discussion on its general applicability for the safety assessment of substances that are present at low levels in consumer products such as cosmetics or for impurities or degradation products.

1.3.11 Classification and Labeling of Chemicals

Criteria for the classification and labeling (C&L) of chemicals have been developed by several national and international agencies. For the most recent guidance see ECHA (2017). It is based on the Globally Harmonized System (GHS) of Classification, Labelling and Packaging of Chemicals, which has been developed for worldwide harmonization in the evaluation of chemicals. In the health hazard part the ECHA guidance addresses all endpoints of toxicological relevance as well as the presence of such chemicals in mixtures. Since the CLP criteria only consider the toxic potential not the potency, consideration of high-dose effects that are irrelevant to humans may lead to unnecessary C&L. For example, the hazard-based concept for classification of carcinogens is based on qualitative criteria and reflects the weight of evidence available from animal studies and epidemiology. The mode of action and potency of a compound are either not taken into account or at best are used as supporting arguments. The advancing knowledge of reaction mechanisms and the different potencies of carcinogens at least triggered a discussion for a re-evaluation of the traditional concept.

The proposals for restriction and authorization require estimation of cancer risk at a given exposure, which is estimated by a linear or sublinear extrapolation from the high-dose effects observed in animals to the usually lower human exposure. However, the EFSA recommended avoidance of this extrapolation because of the inherent uncertainties. Instead, the MOE between a benchmark dose, or the T25 calculated from a carcinogenicity study in animals, and human exposure should be determined. A MOE of 10,000 or more is considered to be of minor concern. The advantage is that neither a debatable extrapolation from high to low doses is performed nor are hypothetical cancer cases calculated.

The systems for classification of carcinogens used by various national or international institutions were developed in the 1970s. So far classification has been based on qualitative criteria only, and reflects essentially the weight of evidence available from animal studies and epidemiology. More recently some institutions have included mechanistic considerations, dose response, and exposure.

Classification is usually based on the certainty with which a carcinogenic potential for a chemical can be established.
Generally three categories, the definitions of which slightly differ, are used:

- human carcinogens
- animal carcinogens, reasonably anticipated to be human carcinogens
- not classifiable because of inadequate data.

The International Agency for Research of Cancer (IARC, 2006) and the OECD proposed to use data on the carcinogenic mechanism and potency in decision-making on whether carcinogenicity is likely or not likely below a certain dose. The German Committee on occupational exposure limits (the MAK committee) and the Scientific Committee to set Occupational Exposure Limits (SCOEL) of the General Directorate Employment of the European Commission apply information on carcinogenic mechanisms and potency as criteria for a revised classification. The US EPA recommended consideration of the mode of action and has published a modified concept for classification. These activities in part originate from the recognition that one can distinguish between mechanisms of carcinogenicity caused by non-genotoxic and genotoxic carcinogens. Thus, it is possible to identify a NOAEL for non-genotoxic carcinogens, provided there is sufficient information on the primarily non-genotoxic mechanism. The American Conference of Governmental Industrial Hygienists (ACGIH, 1997) has used a concept that considers carcinogenic potency for classification since 1995.

1.4 Summary

Toxicology is charged with describing the adverse effects of chemicals in a qualitative sense, and with evaluating them quantitatively by determining how much of a chemical is required to produce a given response. Fundamental to understanding toxicology are the definitions of hazard, potency, dose response, exposure, and their integration into the risk assessment process. Since humans or organisms in the environment can be exposed via different routes, the concentrations in the different environmental compartments are a prerequisite for appropriate risk assessment. This needs to be specifically recognized, since the sensitivity of measurements in analytical chemistry has advanced to the point where infinitesimally small amounts of chemicals can be detected and identified in the various media of the human environment. In understanding the principles of toxicology it is obvious that the presence of a chemical does not necessarily imply a health hazard. Since the dose makes the poison, effects only occur when exposure exceeds the NOEL. This applies for chemicals that induce reversible effects. If damage is not repaired the effect persists and accumulates upon repeated exposure. In such cases every exposure represents a defined risk, which needs to be quantified.

There is an array of testing procedures to determine the hazardous properties of a chemical, such as acute, subchronic, and chronic toxicity, irritation and phototoxicity, sensitization and photosensitization, genotoxicity, carcinogenicity, or toxicity to reproduction. Information on the toxicokinetics and mechanisms of the toxic effects helps to evaluate the relevance of the findings for humans. More recent methodologies, like toxicogenomics or high-throughput testing of agents for a single endpoint, will become increasingly available and may improve hazard identification and aid in the identification of common mechanisms of multiple agents.

In summary, toxicology describes the intrinsic properties of an agent (hazard identification) by applying conventional and substance-specific test procedures to estimate the amount of the chemical required to produce these effects (risk characterization).
Reference


Further Reading


EFSA 2005: Opinion of the Scientific Committee on a request from EFSA related to a harmonized approach for risk assessment of substances which are both genotoxic and carcinogenic, European Food and Safety Authority: https://www.efsa.europa.eu/de/efsajournal/pub/28.


IARC: Monographs on the evaluation of carcinogenic risks to humans, Geneva.


- SCCS/SCHER/SCENIHR (June 8, 2012) Use of the Threshold of Toxicological Concern (TTC) Approach for Human Safety Assessment of Chemical Substances with focus on Cosmetics and Consumer Products.

