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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).

Fundamental to understanding Toxicology are the definitions of hazard, exposure, and risk:

Hazard: Intrinsic toxic properties [Expression of hazard depends upon conditions of use or exposure.]

Dose: Concentration of the chemical and the time of exposure

Risk: Likelihood of an adverse effect resulting from a given exposure

Since humans or organisms in the environment can be exposed via inhalation, skin contact, or oral intake, the concentrations in the different environmental compartments, which result in human or environmental exposure, must be determined. It is obvious from this that risk characterization comprises the following elements:

- Hazard identification, i.e. a description of the agent's toxic potential.
- Dose–response, including information on the concentration above which the agent induces toxic effects to identify the no-observable-effect level (NOEL).
- Exposure assessment, in which the concentration of the agent in the relevant medium and time of exposure are evaluated.

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 that characterize our environment. Detection of a chemical does not mandate that a toxicological effect in exposed people will be observed. Since the dose makes the poison, effects only occur when exposure exceeds the NOEL.

People 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.

It is necessary to establish toxicological profiles of each chemical, either pre-existing or newly developed, to insure 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 is used starting from simple *in vitro* and *in vivo* short-term tests. Depending on the hazardous potential of the agent, studies can be extended to evaluate long-term effects by repeated dose studies, toxicokinetics, and toxic mode of action. For existing chemicals the available information can be collected and a risk assessment, based on exposure data, knowledge of the dose–response relationship, and the mode of action, can be performed.

The parameters which 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.

1.2 The Risk-Assessment Process

1.2.1 Hazard Identification

Acids or bases can be direct-acting agents which cause irritation or corrosion at the site of exposure. 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 and the result is persistent accumulated damage. 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. 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 is also considered to require reaching a threshold dose, even if this dose is very low, and the NOELs 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 any genotoxic event is considered irreversible. [A more detailed discussion of this concept appears below.]

The Toxic Potential

Depending on reactivity, solubility, and metabolism, the chemical, or its metabolites, can reach critical target organs. Irritation or corrosion may occur when the reactant comes into contact with the skin or mucous membranes of the eye, the gastrointestinal tract, or the respiratory system. Distribution and metabolism of the chemical can result in various systemic effects upon interaction at targets in the critical organ. The organ-specific effects are described in the various chapters on organ toxicity, e.g. liver, kidney, the central and peripheral nervous system.

Historically, 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’ have significantly improved the understanding of alterations in cellular and sub-cellular function and the reaction of the cell to toxic insults. The result is a better understanding of toxic mechanisms, species differences, and the consequences of exposures at high and low concentrations for different times.

1.2.2 Dose–Response and Toxic Potency

The Paracelsian admonition teaches us that the occurrence and intensity of toxic effects are dose dependent. His 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. in 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. Absorption rates via the different routes can vary significantly, although oral and inhalation exposure usually lead to the highest internal dose. For

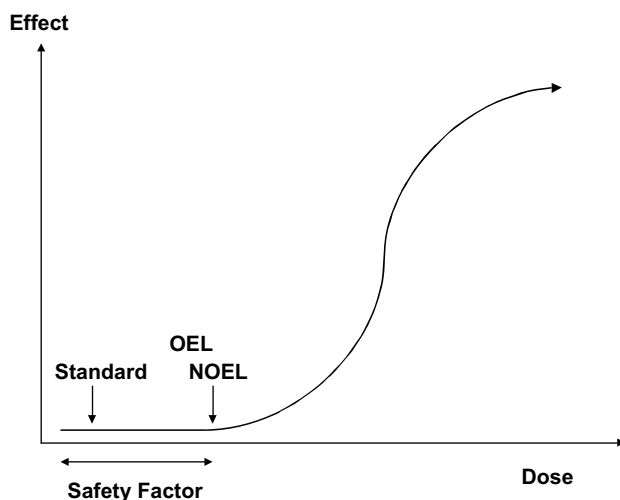


Figure 1.1 Dose-response curve showing log dose on the X-axis and % response (Effect) on the Y-axis. The figure illustrates the location of regulatory values such as the NOEL, Occupational Exposure Levels (OELs), or environmental standards such as Acceptable Daily Intake (ADI). Note that a doubling of dose in the lower or upper part of the S-shaped curve results in small increases of effects, whereas it is much more prominent in the steep part [Modified from Greim and Deml, *Toxikologie*, Ch.1, Abt. 1-2. Copyright (1996), with permission from Wiley-VCH].

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 that reaches the cellular target over a given time period that results in the toxicological response. The dose that defines the toxic potency of a chemical is the product of the interrelated external, internal, and target doses. No toxic effects will be seen if the dose is below the NOEL, whereas effects increase with increasing exposure. The dose-response curve may be expressed using a variety of mathematical formulas. Using the semi-logarithmic 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 of 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.

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. Workplace exposure may occur via dermal contact, by inhalation, even inadvertently, by oral ingestion. Permissible levels of occupational exposure are determined for an 8-hour work day over a 5-day work week extrapolated to an estimated working lifetime of 40 years. Monitoring of exposure usually requires measurement of the chemicals in the air of the workplace and/or the use of personal monitoring equipment. Whereas occupational exposure is regular and repetitive, exposure to consumer products is more difficult to assess. These methods are not usually applied in the home or the ambient environment. Exposure in the home can be estimated by the use of appropriate modeling techniques. Data useful for the determination of exposure to chemicals in consumer products include frequency, duration and site of exposure, concentration and weight of substance in the product, and the amount of product used per contact. Children represent a special case of exposure. For example, they may be exposed to chemicals that are released from toys during mouthing or via skin contact. Accurate exposure determinations for children are difficult to achieve. 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 influence the 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. This external exposure may not necessarily correlate with internal exposure.

Biomonitoring of the compound or its reaction products in the exposed individuals provides the most reliable estimate of internal exposure. However, dose–response curves usually provide a correlation between external dose and effects. Therefore, risk assessment of an internal exposure requires either knowledge of the dose–response of internal exposure versus adverse effects or information to which extent external and internal doses correlate. The estimation of exposure is more complicated when mixtures of chemicals are the source of exposure.

1.2.4 Risk Characterization

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 can be observed. The highest dose at which no effects are seen is called the ‘no-observable-effects level’ (NOEL). The point at which effects become observable is called the ‘lowest-observable-effect level’ (LOEL). A threshold is not the equivalent of an NOEL, since it describes concentration or exposure at which 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 NOEL cannot be determined and every exposure can be related to a defined risk. Reversibility depends on the regenerative and repair capacity of cells, sub-cellular 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 have lost this capacity during natal and postnatal development. Consequently, damaged cells are not replaced, at least in the adult.

For chemicals which induce reversible effects the NOEL 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 NOEL is derived from animal experiments an MOE of 100 or greater is desirable. An MOE of at least 10 is sufficient if the NOEL is derived from human data (see Figure 1.4).

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 allow us to conclude whether genotoxic effects exhibit a threshold at low exposure or even a NOEL. So far, the general agreement remains that the potency of genotoxic carcinogens increases with increasing dose so that the risk at a given exposure needs to be estimated by linear extrapolation from the dose-response data obtained from experimental studies in animals or from data obtained from studies in humans.

1.3 Toxicological Evaluation of New and Existing Chemicals

The various toxic effects which 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 specific effects of compounds such as carcinogenicity and mutagenicity.

1.3.1 General Requirements for Hazard Identification and Risk Assessment

Toxicological evaluation of chemicals requires knowledge on 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 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 NOEL 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:

- acute, sub-chronic 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, and the NOEL, LOEL, and the maximal tolerated dose (MTD).

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. Common practice of 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. 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 insure 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 which can be detected by the specific test system. 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 NOELs can be determined in the intact animal only by repeated-dose studies. In the absence of such information hazard identification is incomplete and without information of the dose–response relationship obtained from these studies 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 sensitizing potential of products the choice of a relevant animal is crucial. However, in many cases animal models may be inappropriate for detection of a sensitizing potential so that most dermatologists prefer studies in humans. An acceptable alternative may be studies with nonhuman primate species like cynomolgus or rhesus monkeys. Generally the Buehler guinea pig test and the popliteal lymph node assay (PLNA) in mice are used in the preclinical testing program. The PLNA 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 NOELs 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 (Carcinogenicity)

Test systems and test strategies to evaluate possible genotoxicity of a compound are described in detail in Chapter 4.3.1–3. Generally, a bacterial mutation assay and an *in vitro* cytogenetic assay are performed. The results are often verified by the mouse bone marrow micronucleus test, a reliable and widely used test system, which detects aneugens as well as clastogens. Chemicals that yield positive responses to these tests frequently do not undergo further development. However, those that appear to lack genotoxicity may be carried forward and evaluated in lifetime carcinogenicity studies in rodents.

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 either teratological, fetotoxic, or developmental effects. In such cases tests such as a reproduction/developmental toxicity screening test (OECD 421), a combined repeated dose toxicity study with the 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 Absorption, Distribution, Metabolism, and Elimination (ADME) of a chemical in humans, experimental animals, or cellular systems. Of specific importance for interpretation of animal studies and for extrapolation of hazards between species is the comparative information on the exposure and the dose that reaches the critical target.

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, on its stability, and on the route of exposure (Figure 1.2).

Upon inhalation or skin penetration the compound directly enters the circulation and distributes 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 general circulation. Larger molecules, e.g. the glucuronosyl-conjugates, can be excreted via the biliary system into the duodenum where the conjugates may be hydrolysed so that the original compound is reabsorbed and reenters the liver. This process is defined as **enterohepatic circulation**. Inhalation or dermal exposure to a chemical, or its intravenous or

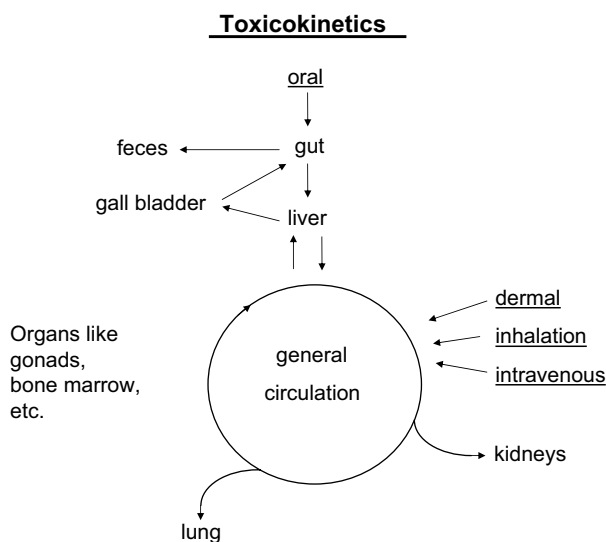


Figure 1.2 Routes of exposure and systemic distribution of a compound within the organism. After oral ingestion the compound reaches the liver, where it can be extensively metabolized. Upon inhalation or dermal exposure and intravenous application the compound reaches the circulation without major metabolism [Modified from Greim and Deml, *Toxikologie*, Ch.1, Fig. 1-1. Copyright (1996), with permission from Wiley-VCH].

intraperitoneal injection, may result in different effects than after oral exposure because of the 'first-pass effect.'

After entering the general circulation the chemical or its metabolites distribute to the organs where they can accumulate in organs such as 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 an important factor that helps us to understand 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 inter-individual 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 man.

Mode and/or Mechanism of Action

Identification of the possible modes or mechanisms by which a chemical induces toxicity and the dose–response relationship are essential to our understanding of species specificities, species differences, sensitive populations, or the interpretation of data regarding threshold or nonthreshold effects. They also help us to evaluate the relevance of the toxic effects to humans when the data are derived from experimental animals. Whereas the 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 (e.g. 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 the chapter on Toxicogenomics, data derived from gene expression microarrays or from high-throughput testing of agents for a single endpoint will 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 applicability is poor and there is the possibility to overinterpret the effects observed, although they might be useful in assessing mechanisms. 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, a detailed search for the underlying mode of action may allow identification of an NOEL. 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, which ultimately lead to cancer.

1.3.2 General Approach for Hazard Identification and Risk Assessment

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 (Figure 1.3) the stepwise procedure usually starts with the determination of the dose range for Lethality and the evaluation of genotoxicity by an *in vitro* bacterial test system (Ames test) and cytogenicity in mammalian cells. In the case of positive results the results are verified *in vivo* usually by the mouse bone marrow micronucleus test. In the case of structural alerts or questionable results the compound needs further evaluation by additional tests, including studies on toxicokinetics or potential genotoxic mechanisms.

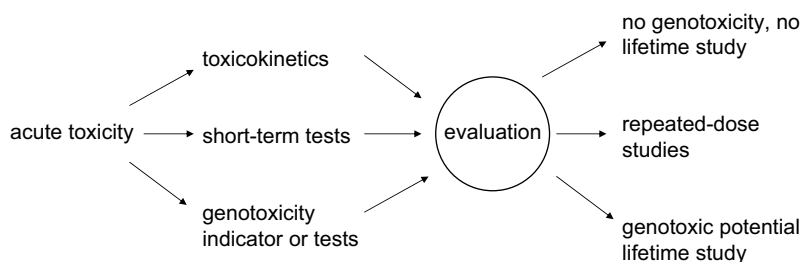


Figure 1.3 Stepwise procedure to evaluate the toxic potential of a chemical [Modified from Greim and Deml, *Toxikologie*, Ch. 1, Fig. 1-3. Copyright (1996), with permission from Wiley-VCH].

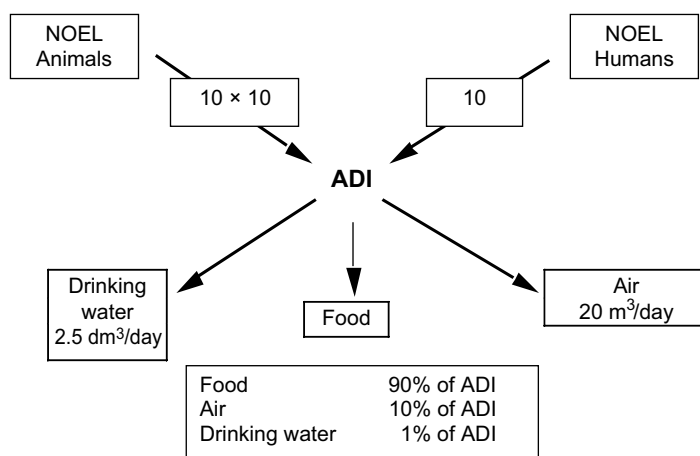


Figure 1.4 Acceptable daily intake (ADI) and maximal acceptable concentration in air, food, and drinking water [Modified from Greim and Deml, *Toxikologie*, Ch. 25, Abt. 25-1. Copyright (1996), with permission from Wiley-VCH].

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 the decision to be made as to whether the database is appropriate for further testing by repeated-dose studies in animals for 28 and 90 days, which, depending on their outcome and the intended use of the chemical, are followed by a 6-months or lifetime study to evaluate potential effects upon long-term exposure, including carcinogenicity.

This information finally allows appropriate risk assessment for a potential human exposure or to set acceptable exposure limits for risk management. For example, when the detailed toxicological evaluation can exclude genotoxic and carcinogenic effects the NOEL in long-term studies in experimental animals can be determined. This NOEL is the starting point to set the ADI, which is usually 100-fold below the NOEL (Figure 1.4). 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 inter-individual differences among the human population. If the NOEL is derived from studies in man a factor of 10 is used to cover possible individual differences. These factors can be reduced if specific information is available to conclude that the species-species or intra-species 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.

For **drugs** special emphasis must be placed on efficacy, therapeutic index, potential side effects, and the effects of overdosage.

For **pesticides** the relative impacts of the chemical on the target versus on people is a critical requirement. Thus, the NOEL for people must be established, and an 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.

Exposure to **chemicals at the workplace** is, according 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 nongovernmental 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 are in use. They are produced in quantities ranging from less than a ton to several million tons per year. Except for drugs and pesticides, toxicity data requirements for existing or new chemicals have 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 US the Environmental Protection Agency (EPA) has initiated an HVP (High Production Volume) program. In an international cooperation the Organization for Economic Cooperation and Development (OECD) has launched the 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 the REACH regulation (see below) will be set into action by 2008. A list of institutions that evaluate toxicity of chemicals and publish the results in comprehensive English documentations is given in Table 1.2.

REACH

REACH (Registration, Evaluation and Authorization of Chemicals) of the European Union is to identify hazardous properties of substances and to evaluate the risks of human and environmental exposure. The regulation will become effective by 2008. It is the responsibility of the producer or downstream user to provide the necessary information to the 'Agency'. As indicated in Table 1.3, the extent of toxicological information largely depends on the annual production rate of a chemical. As long as there is no indication

Table 1.1 The new DFG/MAK criteria for the classification of carcinogenic chemicals compared with those from international bodies.

EU	DFG/MAK	ACGIH/TLV	IARC
1 Substances known to be carcinogenic to man	1 Substances which cause cancer in man	A1 Confirmed human carcinogen	1 The agent is carcinogenic to humans
2 Substances which should be regarded as if they are carcinogenic to man	2 Substances which are considered to be carcinogenic for man	A2 Suspected human carcinogen	2A The agent is probably carcinogenic to humans
3 Substances which cause concern for man owing to possible carcinogenic effects	3 Substances which cause concern that they could be carcinogenic for man but which cannot be assessed conclusively because of lack of data	A3 Animal carcinogen	2B The agent is possibly carcinogenic to humans
3b Substances which are insufficiently investigated			
3a Substances which are well investigated	4 Substances with carcinogenic potential for which genotoxicity plays no or at most a minor role. No significant contribution to human cancer risk is expected, provided that the MAK value is observed.	A4 Not classifiable as a human carcinogen	3 The agent is not classifiable as to its carcinogenicity to humans
	5 Substances with carcinogenic and genotoxic potential, the potency of which is considered to be so low that, provided that the MAK value is observed, no significant contribution to human cancer risk is to be expected		
		A5 Not suspected as a human carcinogen	4 The agent is probably not carcinogenic to humans

Abbreviations: DFG/MAK: Deutsche Forschungsgemeinschaft (German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area); EU: European Union (EU); IARC: International Agency for Research on Cancer (IARC); ACGIH/TLV: American Conference of Governmental Industrial Hygienists / Threshold Limit Values Committee (ACGIH). For contacts see Table 1.2.

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 to chemicals which show bioaccumulation, persistence, and toxicity (BPT compounds) in the environment. The specific use of such compounds needs to be authorized. Within the first 3 years after implementation the >1000 t/a chemicals need to be submitted, during the 2nd three-year phase the <100 t/a, and within the 3rd phase the 10–100 t/a chemicals.

Table 1.2 International institutions that publish documentations on chemicals.

Institution	Contact
ACGIH (American Conference of Governmental Industrial Hygienists)	http://www.acgih.org/TLV/
ATSDR (The Agency for Toxic Substances and Disease Registry)	http://www.atsdr.cdc.gov/
BUA – Advisory Committee on Existing Chemicals (of the GDCh, the German Chemical Society)	http://www.gdch.de/taetigkeiten/bua__e.htm ; http://www.gdch.de/taetigkeiten/bua/berichte__e.htm
The Canadian Centre for Occupational Health and Safety	http://www.ccohs.ca/
Dutch Expert Committee on Occupational Standards (DECOS),	http://www.gr.nl/adviezen.php?jaar=2006
Environmental Protection Agency (EPA)	http://www.epa.gov/
European Centre for Ecotoxicology and Toxicology of Chemicals	http://www.ecetoc.org/
HSE (UK Health and Safety Executive)	http://www.hse.gov.uk/
International Programme on Chemical Safety	http://www.inchem.org/ International Agency for Research on Cancer (IARC) Summaries and Evaluations Concise International Chemical Assessment Documents (CICADS) Environmental Health Criteria (EHC) monographs Health and Safety Guides (HSG) JECFA (Joint Expert Committee on Food Additives) - monographs and evaluations JMPR (Joint Meeting on Pesticide Residues) - monographs and evaluations. OECD Screening Information Data Sets (SIDS)
The Japanese Association of Industrial Health	http://joh.med.uoeh-u.ac.jp
MAK Commission (German Research Foundation)	http://www.mak-collection.com
NIOSH	http://www.dfg.de/mak http://www.cdc.gov/niosh/homepage.html
The Nordic Expert Group	http://www.nordicexpertgroup.org/
OSHA	http://www.osha.gov/
SCOEL - EC Scientific Committee on Occupational Exposure Limits	http://ec.europa.eu/employment_social/health_safety/docs_en.htm

The Globally Harmonized System (GHS) contains classification criteria and hazard communication elements and is applicable for REACH (http://www.unece.org/trans/danger/publi/ghs/ghs_rev00/00files_e.html).

Table 1.3 Use- [tons of annual production(t/a) + import] dependent data requirements of the European REACH regulation. LLNA: local lymph node assay.

Annual production (Years)	1–10 t/a	10–100 t/a (2014–2017)	<100 t/a (2011–2013)	>1000 t/a (2008–2010)
Toxicokinetics		assess	assess	assess
Acute toxicity		oral, dermal, inhalation	oral, dermal, inhalation	oral, dermal, inhalation
Irritation	skin/eye <i>in vitro</i>	skin/eye <i>in vivo</i>	skin/eye <i>in vivo</i>	skin/eye <i>in vivo</i>
Sensitization	LLNA	immunotoxicity	immunotoxicity	immunotoxicity
Repeated dose		28 days	90 days	≥12 mo
Genotoxicity	bacterial test	cytogenetic, gene	<i>in vitro/in vivo</i>	mutagenicity,
Carcinogenicity	<i>in vitro</i>	mutation <i>in vitro</i>	mutagenicity	carcinogenicity
Reproduction		reproduction/ development screening test, developmental toxicity	development 2nd-generation	development 2nd-generation

The TTC Concept

The Threshold of Toxicological Concern (TTC) is a concept to establish a level of exposure for chemicals, regardless of their chemical-specific toxicity data, below which there is no appreciable risk to human health. The concept is based on knowledge of the chemical structure for evaluation of structural alerts, the amount of a specific chemical in a product, and the daily human exposure.

So far the TTC is applied for chemicals in food. It 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 safety assessment of substances that are present at low levels in consumer products such as cosmetics or impurities or of degradation products.

1.3.5 Classification of Carcinogens

The systems for classification of carcinogens used by various national or international institutions were developed in the 1970s. Classification is based on qualitative criteria, and reflects essentially the weight of evidence available from animal studies and epidemiology. 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 (Table 1.3):

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

For classification, 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 has initiated a reevaluation of the traditional concepts.

The International Agency for Research of Cancer (IARC 2006) and the OECD propose to use data on the carcinogenic mechanism and potency in decision-making. Similarly, the General Directorate of Employment of the European Union is currently discussing the application of information on carcinogenic mode of action and potency as criteria for a revised classification. The European Food and Safety Authority has proposed to determine the margin of exposure (MOE) between the effect level obtained from animal studies and human exposure assuming that carcinogenicity is 'of minor concern' below a certain dose. Recently, the US Environmental Protection Agency (EPA) and a committee of the German Research Foundation (Deutsche Forschungsgemeinschaft) recommended consideration of the mode of action and have published modified concepts for classification. These activities in part originate from the recognition that one can distinguish between mechanisms of carcinogenicity caused by nongenotoxic and genotoxic carcinogens. Thus, it is possible to identify a NOEL for nongenotoxic carcinogens, provided that there is sufficient information on the primarily nongenotoxic mechanism. The American Conference of Governmental Industrial Hygienists (ACGIH 1997) has used a concept that considers carcinogenic potency for classification since 1995.

To determine the potency of genotoxic carcinogens and cancer risk at a given exposure a linear or sub-linear extrapolation from the high-dose effects observed in animals to the usually lower human exposure is requested by regulatory agencies. Recently, the European Food and Safety Authority (EFSA) recommended avoiding this extrapolation because of its inherent uncertainties. Instead, the margin of exposure (MOE) between a benchmark dose, or the T25 (dose at which 25% of the animals developed tumors) calculated from a carcinogenicity study in animals and human exposure should be determined. An MOE of 10,000 and more would reduce concern for carcinogenicity to be that of only a minor concern. The advantage is that neither a debatable extrapolation from high to low doses needs to be performed nor are hypothetical cancer cases calculated.

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, exposure, and risk. 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 human environment. When one understands 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 improves the relevance of the findings for man. 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 may aid in the identification of common mechanisms of multiple agents.

Altogether, 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).

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- Paracelsus, *Selected Writings*, ed. J. Jacobi, transl. N. Guterman (1951), p. 95, Princeton University Press, Princeton, NJ.

For Further Reading

- EFSA (European Safety Authority): 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.
http://www.efsa.europa.eu/en/science/sc_committee/sc_opinions/1201.html
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<http://ecb.jrc.it/tgddoc/> Technical Guidance Document, European Chemicals Bureau.
- US EPA Assessing Health Risks from Pesticides (Pesticides: Topical and Chemical Fact Sheets).
<http://www.epa.gov/pesticides/factsheets/riskassess.htm>