Chelation Therapy in the Treatment of Metal Intoxication

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Preface

The idea of writing an interdisciplinary book on the clinical uses of chelating agents in genetic diseases and various metal overexposures was conceived around 2011–13 and developed during the 10th Nordic Trace Element Society Conference in Loen, Norway in 2013. The author group organized symposia on metal chelation during this conference, as well as during the conferences in Belek, Turkey, in 2011, and in Dubrovnik, Croatia, in 2015, both of which were organized by ISTERH (The International Society of Trace Element Research in Humans).

The history of chelating agents was initiated during World War II. And during the subsequent decades important advances in chemistry, molecular biology, and the molecular understanding of roles of metals in health and diseases have taken place. During World War II, BAL (2,3-dimercaptopropanol) was developed as an antidote to the war gas dichlorovinyl arsine (Lewisite). Lewisite was, however, never used, so the first clinical use of BAL was to treat intoxications due to the use of organic drugs against syphilis. Later, BAL was recommended as a therapeutic antidote against inorganic mercury, lead, and copper. And during a five-year-period from 1951 intramuscular injections of BAL was even used in the treatment of Wilson's disease.

The next chelator to come into clinical use was calcium-EDTA (ethylenediaminetetraacetic acid), initially to combat lead intoxication and for decorporation of radionuclides, the latter role presently played more efficiently by the calcium–sodium and zinc–sodium salts of diethylenetriaminepentaacetic acid (DTPA). Military, industrial, and medicinal production and uses of radionuclides also gave a boost to studies from the viewpoint of assessing hazards, protection, and decorporation of radionuclides, the classical chelators here being DTPA and Prussian blue (PB).

An important development in chelation treatment was the introduction of desferal (desferrioxamine, DFO) for treatment of transfusional iron overload in thalassemias and sickle cell anemia, preventing disability and early death for hundreds of thousands of individuals in Southern Europe, Africa, and Asia. DFO has also saved the lives of numerous children acutely poisoned by ingesting their mothers' iron supplements. In recent years, the development of deferiprone and deferasirox as orally active chelating agents has extensively eased the treatment of pathological iron deposits resulting from blood transfusions and hemolytic processes accompanying thalassemia and sickle cell anemia, resulting in better treatment compliance and improved life quality for these patients.

As is well known for our readers, from basic lessons in biochemistry, iron as Fe(II) or Fe(III) is an oxygen-seeking or oxygen-carrying metal, with affinity to nitrogen also, as is illustrated by the function and structure of heme in hemoglobin. And the therapeutic iron chelators, as well, bind and detoxify Fe-cations from tissue deposits by use of the same ligand groups, oxygen, and nitrogen.

In contrast, several toxic heavy metals, such as arsenic, mercury, copper, and lead may be referred to as sulfur-seekers, having higher affinity to endogenous sulfur than oxygen groups. These metal cations may be bound and inactivated by the two vicinal thiol groups on the therapeutic agent BAL. However, today, the clinical use of BAL is limited due to its own high toxicity. Its less toxic derivatives, meso-2,3-dimercaptosuccinic acid (DMSA) and D,L-2,3-dimercapto-1-propanesulfonic acid (DMPS), have now entered the clinical arena and superseded dimercaprol in most cases of heavy metal poisonings. These latter dithiols are nowadays available for oral administration, as tablets, as well as for parenteral administration.

The present book also gives guidelines for clinicians who are responsible for diagnosis and treatment of metal poisonings and overload diseases. In addition, some guidelines for further research are precipitated in the last chapter.

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December 2015

List of Abbreviations

Recommended name	IUPAC name	Recommended acronym	Further names	Trade name
D-penicillamine	(2S)-2-amino-3-methyl-3-sulfanylbutanoic acid	DPA	Dimethyl-cysteine, H_2Pen, PSH	Cuprimine
DMSA	*meso*-2,3-Dimercaptosuccinic acid	DMSA	Succimer	Chemet
DMPS	D,L-2,3-dimercapto-1-propanesulfonic acid	DMPS		Unithiol, Unitiol, Dimaval
Deferiprone	3-Hydroxy-1,2-dimethylpyridin-4(1H)-one	DFP	L1	Ferriprox
Deferoxamine	*N*′-{5-[Acetyl(hydroxy)amino]pentyl}-*N*-[5-({4-[(5-aminopentyl)(hydroxy)amino]-4-oxobutanoyl}amino)pentyl]-*N*-hydroxysuccinamide	DFO	Desferri-oxamine, DFOA	Desferal
Deferasirox	4-[(3Z,5E)-3,5-bis(6-oxo-1-cyclohexa-2,4-dienylidene)-1,2,4-triazolidin-1-yl]benzoic acid	DFX		Exjade
Trientine hydrochloride	*N*,*N*′-Bis(2-aminoethyl)ethane-1,2-diamine	Trien	Trieth-ylene tetramine, TETA	Syprine
BAL	2,3-Dimercaptopropan-1-ol	BAL		Dimercap-rol
Calcium diso-dium edetate	calciumdisodium-2-({2-[Bis(carboxymethyl)amino]ethyl}(carboxymethyl)amino)acetate	$CaNa_2$EDTA		Calcium-disodium-versenate

(*Continued*)

Recommended name	IUPAC name	Recommended acronym	Further names	Trade name
Calcium diethylenetriaminepentaacetate	2-[Bis[2-[bis(carboxymethyl)amino]ethyl]amino]acetic acid, calcium trisodium salt	Ca-DTPA	Calcium trisodium pentetate	Ditripentat-Heyl
Zinc diethylenetriaminepentaacetate	2-[Bis[2-[bis(carboxymethyl)amino]ethyl]amino]acetic acid, zinc trisodium salt	Zn-DTPA	Zinc trisodium pentetate	
Prussian blue	Iron(II,III) hexacyanoferrate(II,III)	PB		Radiogardase, Antidot Thallii-Heyl
Disodium edatate	disodium-2-({2-[Bis (carboxymethyl)amino]ethyl} (carboxymethyl)amino) acetate	Na-EDTA		Endrate Chelest B Komplexon III

Note: The abbreviations used are also explained in each chapter.

Chapter 1

General Chemistry of Metal Toxicity and Basis for Metal Complexation

Jan Aaseth, Lars Gerhardsson, Marit Aralt Skaug, Jan Alexander

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1.1 GENERAL CHEMISTRY OF METALS

About 60% of the adult human body is water, and most of the biochemical interactions take place in the aqueous environment, either extra- or intracellularly. In small children, the amount of water is larger, about 75% of the body weight.

A useful definition of metals from a biological or toxicological viewpoint is based on the properties of their ions in aqueous solutions, for example, in the human body. A metal is an element, which under biologically significant

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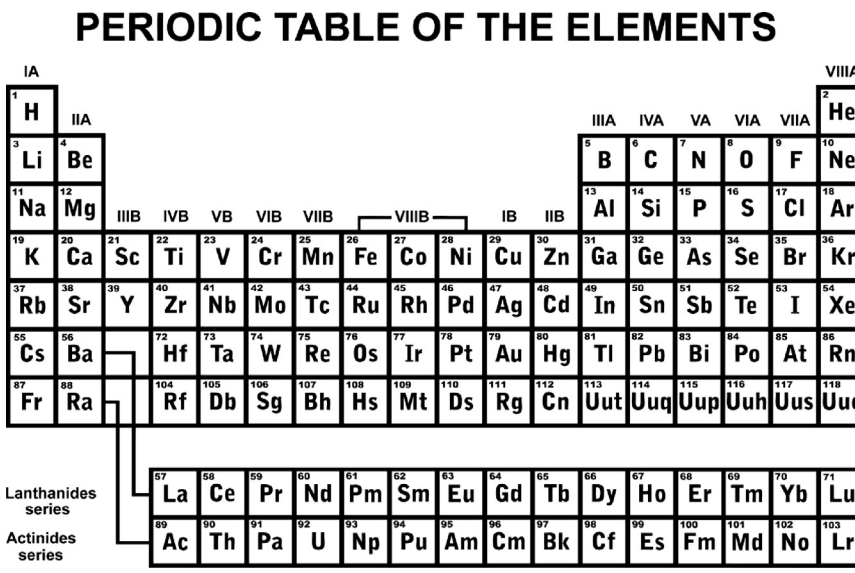

FIGURE 1.1 Elements in the periodic table. The groups from IA to VIIIA may also be numbered successively from 1 to 18, including the 10 elements in the intermediate B-series. It should be noted that most of the transition metals in the first row (Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}) have electronegativities in the range 1.6–1.8 on the Pauling scale, and these cations are classified as "intermediate" according to the theory of Pearson (1963) implying that they have high affinity to electron donor groups containing nitrogen. This is illustrated by the structures of hemoglobin and cobalamine, where Fe^{2+} and Co^{2+}, respectively, are coordinated between four nitrogens. These elements might also bind oxygen, for example, the oxygen-carrying function of hemoglobin. Metals on the left side of the periodic table, for example, Be^{2+}, Ca^{2+}, and Sr^{2+} have lower electronegativities on the Pauling scale, and will consequently be prone to form ionic bonds, for example, to oxygen in the phosphate or carboxylic groups. These elements are often referred to as "hard" or oxygen seeking. On the other hand, metals on the right side of the periodic table, for example, Ag^{+}, Au^{+}, Hg^{2+}, CH_3Hg^{+}, Pd^{2+}, Cd^{2+}, Pt^{2+}, and As^{3+} are "soft" sulfur-seeking metals and will more easily establish bonds to thiols or selenol groups.

conditions may exist as a solvated cation (M), that is, the element has lost one or more electrons. Elements with electronegativity below 2.0–2.5 on the Pauling scale may loose electrons and exist as cations in aqueous solutions and may thus be classified as metals (Aaseth, Skaug, Cao, & Andersen, 2015). The electronegativities of the transition metals (vanadium, chromium, manganese, iron, and copper) are about 1.6–1.8, whereas mercury, lead, and arsenic have higher electronegativities, namely around 2.0. Elements on the left side in the periodic table (Fig. 1.1) have lower electronegativities and are classified as metals. It should be noted, however, that the distinction between metals and nonmetals is not a sharp one.

In some groups of the periodic table, such as in group IVA (alternatively numbered group 14), there is a gradual transition of properties from nonmetals to metals as we descend from the lighter to the heavier elements, in the order

C, Si, Ge, Sn, and Pb. Borderline elements such as As, Ge, Sb, Se, and Te are sometimes referred to as metalloids.

1.2 ESSENTIAL AND NONESSENTIAL ELEMENTS

At present, 20 of the elements in the periodic table are defined as essential for humans, with certainty. First, these are the four organic elements H, C, N, and O. In addition seven "macro-minerals" are essential, namely Na, K, Ca, Mg, Cl, P, and S.

Furthermore, nine trace elements are defined as essential, namely Fe, Mn, Cu, Zn, Se, Co, Ni, Mo, and I. At present, some other elements are under discussion to be included in the category as essential, such as F, B, Si, and As.

To be categorized as an essential, however, an element must satisfy all of the following conditions:

1. It must be present in the human tissues.
2. It's dietary deficiency must result in a reduction of a biological function from optimal to suboptimal.
3. The reduction in physiological function can be normalized by appropriate supplementation of the element (Mertz, 1974).

Oxygen and hydrogen: A human body of 70 kg contains about 46 and 7 kg, respectively, of oxygen and hydrogen. These elements are predominantly bound in water, which makes up 60–65% of the body weight of an adult individual. While intracellular water makes up about two-third of this amount of water, the extracellular compartment makes up the remaining one-third. In aerobic organisms, continuous supply of molecular oxygen is a prerequisite for the controlled combustion to generate chemical energy in mitochondria.

Carbon: This element makes up the principal organic constituent of endogenous molecules of living organisms, for example, carbohydrates and fat as well as proteins. The content of carbon in an adult human body is about 13 kg, since elemental carbon cannot be utilized by the human body, it must be ingested as organic carbon in reduced form in carbohydrates, fat, and/or proteins.

Nitrogen: This element is also essential in organic form. It is particularly found in amino acids, in proteins, and as constituents of nucleic acids. The amount of nitrogen in an adult human body is almost 2 kg.

Calcium: This is the most abundant inorganic constituent of the human body, accounting for about 1.2 kg of the body weight. As hydroxyapatite, $Ca_5(PO_4)_3(OH)_2$, calcium is a major component of normal bone and teeth. Hydroxyapatite makes up the bone mineral and the matrix of teeth, and this calcium compound gives bones and teeth their rigidity.

Calcium is cofactor for numerous enzymes and is also important for intracellular functions as a messenger in cascade signaling reactions, for example, muscle and nerve function, and for blood coagulation. The blood plasma levels of total calcium are kept fairly constant, within narrow limits, 2.2–2.6 mmol/L

(9–10.5 mg/dL). However, about 50% of this blood plasma calcium is bound to albumin, and measurements of "ionized" calcium (1.1–1.4 mmol/L or 4.5–5.6 mg/dL) may be the recommended analysis, since the amount of total calcium varies with the level of albumin.

If the diet provides insufficient amounts of this element, the organism will mobilize calcium from bone, through a process that is brought about by increased circulating levels of the parathyroid hormone (PTH).

Hypercalcemia: It is a disorder commonly encountered by primary care physicians. The diagnosis often is made incidentally in asymptomatic patients. Clinical manifestations affect the neuromuscular, gastrointestinal, renal, skeletal, and cardiovascular systems. The most common causes of hypercalcemia are primary hyperparathyroidism and malignancy. Some other important causes of hypercalcemia include overdoses of vitamin D. An initial diagnostic workup should include measurement of intact PTH, and any medications that are likely to be causative should be discontinued. PTH is suppressed in malignancy-associated hypercalcemia and elevated in primary hyperparathyroidism. It is essential to exclude other causes before considering parathyroid surgery, and patients should be referred for parathyroidectomy only if they meet certain criteria. Many patients with primary hyperparathyroidism have a benign course and do not need surgery. Hypercalcemic crisis with total Ca above 14 mg/dL (or above 3.5 mmol/L) is a life-threatening emergency, often precipitated by malignancy. Aggressive intravenous rehydration is the mainstay of management in severe hypercalcemia, and an intravenously administered bisphosphonate (pamidronate or zoledronate) can usually alleviate the clinical manifestations of hypercalcemic disorders. Whereas bisphosphonates have Ca-chelating properties, the previous use of another chelator, disodium-EDTA, in hypercalcemia is considered obsolete today. In hypercalcemia mediated by vitamin D and in hematologic malignancies, for example, myeloma, glucocorticoids may be the first line of therapy after fluids.

Hypocalcemia: It may occur due to hypoparathyroidism, acute or chronic kidney failure, low vitamin D intake, genetic anomalies, or iatrogenic causes related to some antiosteoporosis or chelation drugs. In chronic hypocalcemia bone mineralization may be compromised, whereas acute cases may present by convulsions, tetany, or numbness.

Phosphorus: This essential element exists in the human body as phosphate groups (PO_4^{3-}), not only in bone and blood, but also in organic compounds such as ATP and in DNA and other nucleotides (Fig. 1.2). Phosphorus has important regulatory functions in intracellular processes via kinase-catalyzed phosphorylation and dephosphorylations that activate and deactivate a large number of key enzymes in internal metabolism. Chemically, arsenate and phosphate have similarities, and arsenic compounds may interfere with the organic binding of phosphates in DNA. The total amount of phosphorus in a human body is about 700 g. At physiological pH, phosphate in blood exists predominantly as a mixture of the buffering anion pair HPO_4^{2-} and $H_2PO_4^{-}$. Average

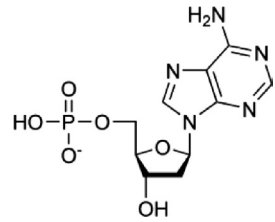

FIGURE 1.2 A nucleotide containing the sugar deoxyribose covalently bond to adenine and a phosphate group. This structure is named a deoxyribonucleotide, and is a constituent of DNA. The oxygen in the phosphate group can bind magnesium and other metals with low electronegativity by complexation through a predominantly ionic bond.

phosphate levels in blood plasma are 0.8−1.5 mmol/L. Increased phosphate levels in blood may result from renal insufficiency. Patients with end-stage renal disease in dialysis with blood phosphate values above 1.8 mmol/L should be treated with a phosphate binder. Nowadays, lanthanum carbonate (Fosrenol, Shire Pharmaceutical) is a commonly used phosphate binder. Whereas dietary phosphate restriction and removal of phosphate by dialysis is often insufficient to prevent hyperphosphatemia, administration of lanthanum carbonate as chewable 500, 750, or 1000 mg tablets, three times a day, combined with dialysis, is usually efficient to avoid severe hyperphosphatemia with secondary hyperparathyroidism.

Sulfur: This essential element for the human body must be ingested in an organic form. The sulfur amino acid methionine is the sulfur species classified as essential in the human diet. Another important sulfur amino acid, cysteine, can be synthesized by the human body if sufficient quantities of methionine are available. The sulfur in cysteine exists as a thiol group. Due to the ability of thiols to undergo redox reactions, cysteine has antioxidant properties. These properties are typically expressed in the tripeptide glutathione, which occurs intracellularly in millimolar concentrations in humans as well as in other organisms. The bioavailability of orally given glutathione (GSH) as such is negligible; it is degraded in the intestine so it must be synthesized intracellularly from its constituent amino acids, namely cysteine, glycine, and glutamic acid. GSH is an important endogenous detoxifying agent both for reactive organic electrophilic compounds and for metals. It is a necessary cofactor of the selenium-enzyme family of glutathione peroxidases that detoxifies intracellular peroxides. Cysteine and methionine play important roles in protein structure. The thiol group also has a high affinity for heavy metals, so that proteins containing cysteine may be targets in heavy metal poisonings. The low molecular weight thiol-rich protein, metallothionein, has a particularly high ability to bind metals such as zinc, copper, mercury, lead, and cadmium.

Potassium: This cation occurs predominantly intracellularly and contributes significantly to the intracellular osmolality. The body contains about 105 g of potassium. The electrochemical potential in nerves depends on the physiological

presence of potassium, and thus it is of importance for the signaling in nerves. In the intracardial pathways of signaling and regulation of heart rhythm, it is of particular significance. Some other elements such as lithium, cesium, and thallium have chemical similarities with potassium, and may displace potassium from important intracellular locations.

Sodium: This is the extracellular counterpart of potassium. It regulates the amount of water in the extracellular space via osmotic homeostatic processes together with other electrolytes and macromolecules, and together with potassium it regulates the total amount of water in the body. In nerves it is fundamental for the electrical signaling. Unphysiologically high intakes of sodium as table salt may increase the blood pressure. Ordinarily, the body contains about 90 g of sodium.

Chlorine: In the form of the chloride anion this element is important for balancing the cations in the body, in particular the sodium cation extracellularly and the potassium cation intracellularly. The human body contains about 115 g of chlorine. Extracellularly, the important anions are chloride (about 100 mmol/L) and bicarbonate (normally about 25 mmol/L). Since the physiological extracellular amount of cations (sodium plus potassium) is about 140 mmol/L, there are a so called "anion gap" of about 140–125 mmol/L, that predominantly is made up of negatively charged proteins.

Magnesium: It is important in more than 300 metabolic reactions, many of these are related to energy production and consumption. A crucial substrate in these reactions is the Mg-ATP complex. One example of a magnesium-dependent energy-consuming process is the import of potassium into cells that is coupled to the export of sodium out of cells and catalyzed by the Na-K-ATPase. Magnesium is also important in the structure of skeleton and muscles. The amount of magnesium in an adult human body is about 30 g.

Iron: It is implied in at least hundred enzymatic reactions, and Fe^{2+} represents the oxygen-carrying core of hemoglobin. The extracellular amounts of the toxic "ionized iron" are negligible, since the plasma protein transferrin has extremely high affinity for Fe^{3+}. Extracellular hemoglobin may also act as a prooxidant, but intracellularly it is shielded not only by the red cell membrane, but also by intracellular glutathione (about 3 mmol/L) and the antioxidative enzyme glutathione peroxidase. In sickle cell anemia, thalassemia, and/or transfusional siderosis, toxic amounts of iron are deposited in liver, heart, and other organs.

Zinc: It takes part in the enzymatic action of more than 300 proteins and has important functions in organizing the tertiary structure of proteins via zinc fingers. Many zinc finger proteins function via interactions with nucleic acids, for example, regulation of gene expression by transcription factors interacting with DNA responsive elements through zinc fingers. Zinc deficiency in developing countries leads to decreased resistance against infection, particularly in children, and in severe cases, it may lead to hypogonadism and dwarfism. Abundant intakes of zinc induce synthesis of a metal-binding protein, metallothionein, also in gut mucosal cells, and may thereby protect against toxic actions of copper, for example, in Wilson's disease.

Copper: It is important in various enzymatic reactions, particularly as an electron donor. In the respiratory chain in mitochondria, the copper enzyme cytochrome c oxidase operates as an electron transporter. High intakes of copper may lead to toxic effects. In the hereditary defect in copper excretion known as Wilson's disease, physiological intakes are also toxic.

Iodine: It is required for the biosynthesis of the thyroid hormones, thyroxin, and triiodothyronine. Iodine deficiency is an important health problem throughout the world, leading to goiter, decreased synthesis of thyroid hormones, hypothyreosis, and children with impaired brain development and cretinism.

Selenium: It is essential for a variety of enzymes including several antioxidants. Unlike sulfur that has to be ingested in organic form, predominantly as methionine, inorganic selenium as selenite is incorporated into the amino acid selenocysteine and further into selenoenzymes by a specific genetic machinery that is unique and different from that of "ordinary" amino acids in the human organism.

Chromium: In its trivalent state, chromium apparently contributes to regulate blood glucose levels and the transport of glucose into cells, presumably by some interaction with the insulin action. The exact mechanism of this interaction is however not fully understood.

Manganese: It is essential in a number of enzymes, of which the manganese superoxide dismutase (MnSOD) is of particular importance, since it protects mitochondria from toxic oxidants. Overexposure to manganese, for example, exposure at the work place, may give rise to "manganism" with symptoms as in Parkinsonism.

Molybdenum: It is considered to have several functions. In the gut microbiome, it is important for the transformation of inorganic nitrogen by nitrogen-fixing bacteria, into organic forms.

Cobalt: It is essential as a component of vitamin B_{12} (cobalamin), that is vital for several biological processes, especially for the transfer of methyl groups, for example, into DNA. Whereas iron can be introduced into the resembling porphyrin ring in the human body by an enzyme ferrochelatase, the entire cobalamin molecule must be supplied by the diet.

1.3 EFFECTS OF TOXIC EXPOSURE OF AN ESSENTIAL OR NONESSENTIAL METAL

1.3.1 Basic Concepts in Chemical Toxicity Testing

Both essential and nonessential metals may exert toxic effects if the dose of ingestion or exposure exceeds certain levels (Mertz, 1981), often referred to as *critical levels*. The effects induced at these levels by a toxic agent may be referred to as critical effects. These effects arises from the so-called *critical organ* (Nordberg, 2004). For example, the central nervous system is the critical organ in cases of elemental mercury vapor exposure. When discussing metal

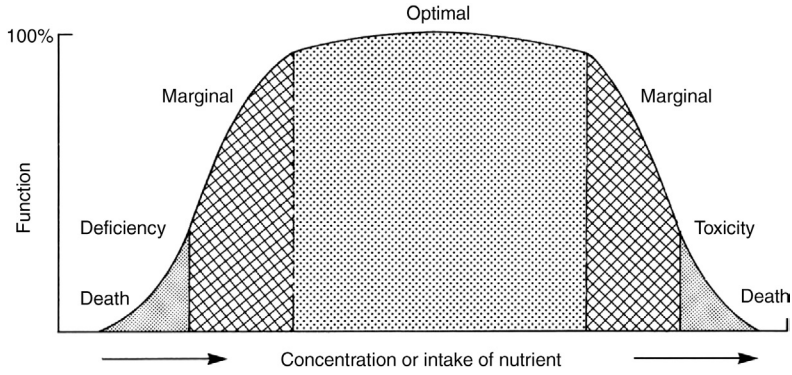

FIGURE 1.3 Schematic picture illustrating an optimal plateau of intake of an essential trace element. Apparently, unphysiologically low or high intakes lead to pathological processes. *(Source: Adapted from Mertz, 1981.)*

toxicity it should be emphasized that not only concentration range, but also speciation and oxidation state are crucial factors that affect the poisoning aspects of a metal in question.

Dose-effect and dose-response relationships are fundamental concepts in toxicology. A dose-effect relationship exists if an increase in the dose of a chemical (here: of a metal compound) causes a quantifiable increase in the toxic effect observed or if additional undesirable effects occur (may be illustrated as in the right half of Fig. 1.3). On the other hand, if an observed effect is not quantifiable in single individuals, but is either present or not present (often called all-or-none effect), a *dose-response* relationship exists if the percentage of a population responding with that effect depends on the dose of the chemical. A schematic dose-response relationship is shown in Fig. 1.4. It is also possible to depict a quantifiable effect on a dose-response curve, by illustrating the percentage of the population with the value of a biomarker above a certain level, for example, beta-2-microglobulin in urine above a certain threshold.

The goal of chemical toxicity testing, and toxicological research is to identify potential adverse health effects that can be caused by low doses of unintentional exposure to environmental toxicants, for example, toxic metal compounds.

One basic principle of the framework provided by National Research Council in the analysis of the dose-response curve (Fig. 1.4) is to define a window of interest in the lower part of the curve (Barnes & Dourson, 1988). This is the window between the lowest observed adverse effect level (LOAEL) and the no observed adverse effect level (NOAEL). Thus, the LOAEL is the lowest dose tested with a statistically significant effect, whereas the NOAEL is identified as the highest dose tested without a statistically significant effect. The LOAEL identifies the more frequently used term “critical dose.” A more frequently used approach nowadays is to model the dose-response relationship