Relevance of reactivity determinants to exposure assessment and biological monitoring of the elements

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1 Introduction

The purpose of this paper is to explore the scope and content of the *Journal of Environmental Monitoring* (*JEM*) in the context of determinant factors judged to be important in the understanding and rationalization of the biological chemistry and toxicity of inorganic compounds of the elements (including organometallics). Our aim is to illustrate how physical, chemical and biological reactivity parameters can influence what is measured in exposure assessment and what variables are selected in biological monitoring. Many of the illustrations chosen will be from human experience. It is not our intent to put any restrictions on the scope of the journal but rather to be expansive, to challenge the reader, to outline current areas of research and to illustrate *JEM*'s interdisciplinary objectives.

2 Determinant factors in the biological chemistry and toxicology of the elements

2.1 Introductory comment

Nieboer and Fletcher[1] outlined in some detail the determinants of reactivity recognized in coordination chemistry, inorganic biochemistry and toxicology. The salient features described below of the reactivity parameters listed in Table 1 are based on their text. In addition, the analytical techniques employed in the measurement of the determinants will be identified.

2.2 Physical state

Solids are the most commonly encountered forms of metals and metalloids in human respiratory exposures. Particle size determines what can be inhaled and where in the respiratory tract particles penetrate and are deposited, while particle surface activity and solubility influence the extent and rate of absorption of its constituents by the lung or by the gastrointestinal (GI) tract. As shown later, these properties can also mediate the type and degree of tissue injury on contact.

Under standard conditions (25 °C and 101.3 kPa), very few compounds of metals and metalloids are liquids. Exceptions are mercury metal, nickel tetracarbonyl, certain alkyl derivatives of, for example, lead, mercury and tin and chlorides of arsenic and germanium. If not contained, such liquids release vapours, which in most cases are extremely toxic.[2,3] Vapours can also arise from solids by sublimation, of which arsenic trioxide ($\geqslant$193 °C) is an example.

Gases are common forms of compounds that have as components the lighter elements (*e.g.*, N, O, S, halogens) and some elements with high atomic masses such as As, Sb and Se. Obviously the most frequently encountered environmental gaseous pollutants are included in the former group, namely sulfur dioxide, ozone, carbon monoxide, carbon dioxide and nitrogen oxides. Gases penetrate deep into the lungs unless they are extremely soluble in water. Although the main route of absorption occurs by way of the gas-exchange region of the lung (the alveoli), significant uptake into the blood stream can also occur from the nasal and bronchial epithelia.[4]

In the context of exposure, the concept of an aerosol is pertinent. It is a scientific term which applies to any disperse system of liquid or solid particles suspended in a gas, usually air.[5] It is convenient to classify inorganic aerosols as fume, smoke, dust, mist and spray. Detailed definitions may be found in aerosol science textbooks (*e.g.*, Vincent[5]).

2.3 Atomic properties

Cations have specific ionic radii and preferred coordination numbers and geometries, which X-ray crystallography has illustrated to be crucial to their biological activities.[6,7] Although not directly related to exposure assessment and biological monitoring, non-isomorphous replacement of naturally occurring metal ions by others is an important toxicological principle;[8–11] it is of mechanistic relevance. Antagonistic relationships do also exist for anions, such as between arsenate and phosphate.[12]

Two other atomic properties should also be considered, namely charge and oxidation state. Metal-ion complex formation gradually increases with increasing charge-to-size ratios of metal ions and oxidation state can dominate the essential

Table 1 Determinants in the biological chemistry and toxicity of the elements (adapted from Nieboer and Fletcher[1])

Physical state—
- Solids
- Liquids
- Vapours and gases

Atomic properties—
- Ion size
- Geometry
- Oxidation state
- Electronegativity

Reactivity—
- Ionic and covalent bonding tendencies
- Donor-atom preference
- Complex formation and stability
- Kinetic aspects
- Solubility
- Radical formation
- Particle size and shape
- Physico-chemical properties of solid surfaces

Biological phenomena—
- Compartmentalization
- Respiratory tract deposition and clearance
- Bioavailability and uptake
- Biological turnover time and bioaccumulation
- Tolerance and susceptibility

functions of elements. Many essential biological processes such as respiration, photosynthesis and nitrogen fixation depend on metalloproteins or metalloenzymes in which the metal centre can assume various oxidation states. Iron in haemoglobin and cytochromes, copper in amine oxidase and superoxide dismutase, manganese in photosystem II, molybdenum in nitrogenase, cobalt in vitamin B_{12} coenzyme and nickel in bacterial dehydrogenases are examples.[6,7,13] Oxidation state can also direct the toxicity of elements. For example, the toxic effects of mercuric salts are distinct from those induced by mercury vapour or alkylmercury compounds and, in most situations, chromium(III) compounds tend to be considerably less toxic than chromates in which chromium is present as chromium(VI). In monitoring workers exposed to chromium, it is therefore important to quantify exposure to both chromium(VI) and total chromium.[14] The chromate ion serves as an effective vehicle for delivering chromium across biological membranes and this is believed to contribute to this differential toxicity. However, the generation of active dioxygen species and other radicals (see below) concomitant with the reduction of chromate to Cr^{3+} is also considered to be a contributing factor.[1,14,15] Replacement of an essential metal ion with redox function with an ion with fixed oxidation state can obviously also lead to inhibition and toxic consequences. The antagonistic action of cadmium in copper or iron metabolism illustrates this point.[16]

Electron spectroscopy, electron paramagnetic resonance (EPR), Augur electron spectrometry, X-ray induced spectrometry, Mössbauer spectrometry, redox titrations and reduction potential measurements are some of the techniques employed to determine the oxidation states of elements in compounds or solids.[6,17] Cyclic voltammetry is an effective tool for assessing the reversibility of redox couples (*e.g.*, Fe^{3+}/Fe^{2+}, Ni^{3+}/Ni^{2+}).

Electronegativity (χ) is an important atomic property in understanding the relative contributions of ionic and covalent bonding in metal ion-donor atom interactions. In simple terms, electronegativity (χ) may be defined as the electron attracting capability of an atom or ion in a molecule.[8,18] Since valence orbital energy is a measure of the ability to accept electrons, and thus form covalent bonds, it is related to χ. Representative values of χ are $\chi(O)=3.44$ (divalent), $\chi(S)=2.58$ (divalent), $\chi(N)=3.04$ (trivalent), $\chi(F)=3.98$ (monovalent), $\chi(Cl)=3.16$ (monovalent), $\chi(H^+)=2.20$, $\chi(K^+)=0.82$, $\chi(Mg^{2+})=1.31$, $\chi(Ca^{2+})=1.00$, $\chi(Fe^{2+})=1.83$, $\chi(Fe^{3+})=1.96$, $\chi(Zn^{2+})=1.65$, $\chi(Cd^{2+})=1.69$, $\chi(Hg^{2+})=2.00$, $\chi(Al^{3+})=1.61$, $\chi(Tl^{3+})=2.04$ and $\chi(Tl^+)=1.62$. For small to moderate differences in χ between bond partners (<1.8), the ionic character of a bond increases steadily with increasing magnitude of $\Delta\chi$. The χ values quoted are Pauling electronegativities, which are calculated from thermodynamic data, specifically bond energies or heats of formation.[18,19]

2.4 Determinants of reactivity

2.4.1 Ionic and covalent bonding tendencies and donor-atom preference. A useful measure employed in classifying metal ions according to their ability to form covalent bonds is the index $(\chi_m)^2 r$, where χ_m is the Pauling electronegativity and r the ionic radius corresponding to the most common coordination number.[8] $(\chi_m)^2 r$ is interpreted as a quotient comparing valence orbital energy with ionic energy and thus reflects the relative ability to form covalent *versus* ionic bonds. Effectively, it may be taken as a covalent index. By contrast, z^2/r, where z is the ion charge and r is defined as above, is accepted as an effective measure of ionic interactions.[18,20,21] Nieboer and Richardson[8] separated metal and metalloid ions into three groups by plotting the covalent index $(\chi_m)^2 r$ *versus* the ionic index z^2/r. This classification reproduced the traditional groupings of Class A ('hard' acids), Borderline (intermediate) and Class B ('soft acids') metal ions,[7,18,22,23] as illustrated in Fig. 1. A perusal of the X-ray crystallographic structures of metalloproteins and metalloenzymes permitted the further classifi-

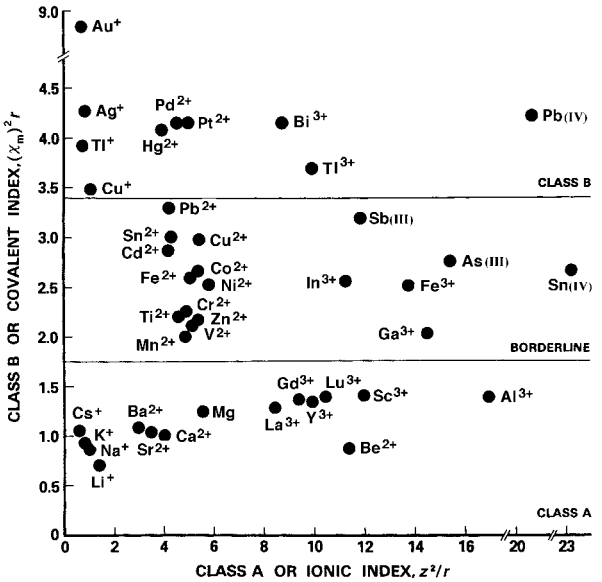

Fig. 1 Separation of metal ions and the metalloid ions As(III) and Sb(III) into three categories: Class A (oxygen-seeking), Class B (nitrogen/sulfur seeking) and Borderline (intermediate or ambivalent). The Class B or covalent-bonding index $(\chi_m)^2 r$ is plotted for each ion against the Class A or ionic-bonding index z^2/r. In these expressions, χ_m is the metal-ion Pauling electronegativity, r the ionic radius corresponding to the most common coordination number and z the formal charge on the ion. Oxidation states given by Roman numerals imply that simple cations do not exist, even in acidic aqueous solutions. Reproduced with permission from Nieboer and Richardson.[8]

cation of Class A ions as oxygen donor-atom seekers, Class B ions as nitrogen/sulfur seekers and Borderline (or Intermediate) as displaying ambivalent affinity for all three donor-atom types. Generally, for a fixed value of the ionic index, toxicity increases with increasing magnitude of covalent index; conversely, for a fixed value of the covalent index, toxicity increases with increasing magnitude of the ionic index. Nieboer and Richardson[8] pointed out that metal or metalloid ions forming water-stable organometallic compounds (*i.e.*, those with a direct bond to carbon) fall along an outer arc across the upper portion of Fig. 1 [*e.g.*, Bi(III), Hg(II), Pb(IV), Sn(IV), Tl(III)]. Selected applications in environmental and toxicological studies of the Nieboer and Richardson classification are summarized in Table 2. Clearly, donor preference is an important reactivity principle that has implications for environmental and biological monitoring.

2.4.2 Metal-ion complex formation and stability. Natural waters, extracellular and intracellular fluids, soils and sediments provide a wide range of complexing agents or ligands which favour metal-ion complex formation. Not surprisingly, and as will be shown later, metal–ligand interactions are central to understanding contaminant pathways (uptake, metabolism and excretion) and bioavailability, in addition to being relevant to metal measurement (*e.g.*, speciation methods). In parallel with the general trends in toxicity stated in the previous section, for fixed values of the covalent index $(\chi_m)^2 r$ complex stability with nearly all ligands correlates positively with the ionic index. Conversely, for a fixed z^2/r value, stability increases with increasing $(\chi_m)^2 r$. For example, endogenous ions such as Mg^{2+}, Ca^{2+}, Mn^{2+} and Zn^{2+} are readily displaced by Cd^{2+} or Pb^{2+}, which have comparable values of z^2/r but substantially larger $(\chi_m)^2 r$ values. Similarly, Be^{2+}, Al^{3+} and the trivalent lanthanide ions have similar $(\chi_m)^2 r$ indices to Mg^{2+} and Ca^{2+}, but larger z^2/r values, which favours the displacement of the endogenous cations. Increased complex formation, such as changes in the size and geometry of the metal site, is often associated with spatial distortion and inhibition of function. Complex stability also determines the free ion concentration, which in many instances determines the toxic response.[48]

In vivo distribution of metal ions involves transport as complexes of amino acids, peptides, proteins or other natural ligands such as citrate.[49,50] Examples are Ni^{2+} and Cu^{2+} bound to the protein human-serum albumin, the amino acid L-histidine and a ternary complex involving both ligands. Complexes of glutathione or cysteine appear to be involved in the biliary excretion of methylmercury or its uptake into the brain or kidney, respectively.[51] Interestingly, the Fe^{3+} ion bound to the protein transferrin gains entry into cells by receptor-mediated endocytosis in which the transferrin–receptor unit is internalized, and the receptor and iron-free apotransferrin are recycled.[52] Transferrin also binds other trivalent cations such as Cr^{3+}, Al^{3+}, In^{3+}, Ga^{3+} and Co^{3+}. Furthermore, it is well established that complex formation influences GI uptake of metal ions.[53] The facilitation of Al^{3+} uptake by citrate is a well known example.[54] Competition between cations is also important. Diets rich in Ca^{2+} reduce the GI uptake of Pb^{2+} in humans and test animals and low iron status potentiates lead toxicity.[55,56] It is unclear whether the latter is due to enhanced Pb^{2+} absorption.[56]

Because it is impracticable to measure activities of all species participating in metal ion–ligand equilibria, stoichiometric stability constants (or concentration quotients) are most often measured rather than thermodynamic constants.[57] Potentiometry is still the most widely used technique for the measurement of stability constants, although other approaches are utilized, including calorimetry, UV/VIS spectrophotometry, nuclear magnetic resonance (NMR) spectrometry, voltammetry, ion exchange, conductance, solvent extraction and solubility measurements.[57] The majority of computational programs employed to estimate formation constants involve some variant of the non-linear least-squares algorithm.[58] The compilations of stability constants provided by Stumm and Morgan[59] constitute a convenient directory to available data.

2.4.3 Kinetic aspects. Strictly, living systems are dynamic and are far removed from equilibrium.[60] Nevertheless, equilibrium arguments are often applicable if 'steady state' conditions are assumed to prevail.[61] While equilibrium constants can be used to ascertain whether reactions or processes are thermodynamically feasible, they cannot render judgements about the time frame in which they occur. Inorganic kinetics display a wide range of time-scales. Simple ligand substitutions are about 10-fold slower than water-exchange rates, which range from the diffusion-controlled limit of about 1 ns (*e.g.*, Na^+, K^+, Pb^{2+}, Cu^{2+}, Hg^{2+}) to near 1 μs (*e.g.*, Fe^{2+}, Mg^{2+}, Ni^{2+}), about 1 ms (*e.g.*, Fe^{3+}, Ga^{3+}, Be^{2+}), approximately 1 s (*e.g.*, Ru^{2+}, Al^{3+}) and to as slow as 1 d (*e.g.*, Cr^{3+}, Ru^{3+}).[62,63] For trivalent ions (*e.g.*, Fe^{3+}, Al^{3+} and Cr^{3+}),

Table 2 Selected applications of the donor-preference classification of metal and metalloid ions according to Nieboer and Richardson[8] in the interpretation of their chemistries, relative toxicities, environmental monitoring or related observations

Application	Ref.
Sea-water speciation, biological function and toxicity	21,24
Acute toxicity in mice	25
Interaction with algae	26
Ionoregulation in fish	27
Speciation in soil leachates	28
Inhibition of plant enzymes	29
Renal toxicity in rats	30
Toxicity of mercury in bacteria	31
Assimilation in marine copepods and mussels	32,33
Binding to fish gills	34
Phytotoxicity in wheat seedlings	35
Adsorption by fungi	36
Mechanisms of lead toxicity (including the Pb^{2+}/Ca^{2+} axis)	37
Cd^{2+}/Ca^{2+} competition or axis	34,38,39
Blocking of Class A sites in fish gills by Al^{3+}	40–42
Induction of conformational changes in synthetic DNA	43
Chemistry and environmental fate in aquatic ecosystems	44
Biomonitoring in the marine environment	45
Predicting relative toxicities in bacteria	46
Interaction of Mn^{2+} and Al^{3+} in plant cultures	47

coordination of the hydroxide ion significantly labilizes the remaining water molecules, facilitating complex formation. Rate constants for complex formation for ions such as Cu^{2+} can decrease by as much as 10 log units as one proceeds from water to monodentate, bidentate, linear polydentate and flexible, to rigid macrocyclic ligands.[64] For interactions at solid surfaces, of which soil particles and hydrous iron oxide are examples, surface binding sites tend to be considerably more labile and reversible than interior sites, presumably because the latter involve diffusion from or to the colloid interior.[59,60] Not unrelated appears to be the observation that the dissolution of a mineral is often limited by the rate of chemical reactions at its surface.[59] This includes chemical weathering.

Flow injection, continuous-flow and stopped-flow reactors with UV/VIS or IR spectrometric detection, voltammetry and NMR are some of the techniques permitting the measurements of reaction rates in coordination chemistry and geochemistry.[59,65]

2.4.4 Solubility. Whether a compound is soluble in aqueous or lipid phases is important in the toxicology of the elements and also in the design of environmental and biological monitoring strategies. Solubility in water influences bioavailability and therefore uptake (see below), toxicity and in some cases detoxification. This is readily illustrated. In aquatic systems, release of metal ions from sediments is an important parameter in geochemical and toxicological considerations.[21,24] The urinary and plasma nickel data summarized in Table 3 clearly imply that aerosols of dissolved nickel salts are more readily absorbed than relatively insoluble particulates by the respiratory tract. This will be discussed in greater detail in subsequent sections. A number of natural ligands such as phytate (hexaphosphoinositol), phosphates and polyphenols (*e.g.*, tannins) inhibit the GI uptake of metal ions by forming insoluble complexes.[53,68,69] In plants, phytochelatins are involved in vacuolar deposition of metals,[70] and in invertebrates granule inclusion occurs intracellularly or extracellularly as inorganic (*e.g.*, as phosphates) or organic deposits (*e.g.*, containing protein, oxalate, phytate or nucleotides).[11] Aquatic insects also feature metal-containing granules.[71] Presumably, sequestration, storage, excretion and/or detoxification are major reasons for the formation of such granules. Intracellular deposits of protein complexes of lead known as inclusion bodies have been identified in animal and human kidney cells as a result of heavy chronic exposure.[72–74] Interestingly, most of the chronic lead body burden in humans is located in the mineral matrix of bone, which illustrates the Pb^{2+}/Ca^{2+} axis mentioned earlier (Table 2).[75] This is the basis for the measurement of lead in bone *in vivo* by using γ-rays from a ^{109}Cd source to excite lead K X-rays.[76]

Finally, lipid solubility has a drastic effect on uptake, distribution, route of excretion and the nature of toxic effects. Toxicity in marine organisms, cultured cells, rodents and mammals (including humans) generally correlates with lipophilicity or hydrophobicity of alkyltin compounds.[77–80] Lipid solubility promotes accumulation in lipid-rich compartments such as the brain. Mercury and lead compounds provide good illustrations. Chronic exposures to the lipid-soluble forms of Hg^0 (elemental mercury) and CH_3Hg^+ (methylmercury) perturb neuromotor, behavioural and cognitive functions.[81–83] Acute exposures cause severe damage primarily to the lung (Hg^0) or brain (CH_3Hg^+). By comparison, the toxicity of mercury(II) salts, which are water soluble, is more systemic, disrupting all tissues they contact. Demarcations in uptake, distribution, excretion and specific toxic effects (including neurotoxicity) are also known between organolead and inorganic lead compounds.[84–86]

2.4.5 Speciation of the elements. The comprehensive treatment of the determinants of reactivity in metal biochemistry and toxicology outlined in this paper is consistent with the definition of speciation of the elements formulated in 1991 and endorsed in 1994 and 1997 at the First, Second and Third International Symposia on Speciation of Elements in Toxicology and in Environmental and Biological Sciences.[87–89] This definition is not limited to a strict chemical perspective:

> *Speciation is the occurrence of an element in separate, identifiable forms (i.e., chemical, physical or morphological state).*

It is evident from our earlier deliberations that certain compounds of the elements can occur in different physical states, namely solid, gas, liquid, vapour or aerosol. Each of these states can influence or determine the nature of an exposure (both route and extent), the uptake or absorption and the toxicological consequences. The reasons for including particle morphology (*i.e.*, form and structure) and other solid-state properties as species-distinguishing features are pursued in subsequent sections. Here we will focus on chemical speciation.

As we have indicated, oxidation state changes and metal-ion complex formation constitute ways of generating chemical species of the elements. Formation of organometallic derivatives is another. A brief review of some of the more common applications of analytical techniques available to conduct chemical speciation will serve to reinforce its relevance to environmental and biological monitoring.

Standard biochemical separation techniques (*e.g.*, dialysis, chromatography, electrophoresis and ultracentrifugation) have been used to isolate and characterize metal-ion complexes with ligands of low molecular mass (*e.g.*, amino acids) and high molecular mass (*e.g.*, proteins and DNA).[90,91] For example, they have permitted the most abundant complexes in serum of essential metals (*e.g.*, Fe, Cu, Zn)[49] and toxic metals (*e.g.*, Al, Cr, Ni)[14,49,54,92] to be identified. Interestingly, sophisticated computer-aided species-distribution calculations using available formation constants have been less helpful in this task than first expected.[93] More recently, the classical separation

Table 3 Linear relationships between ambient and body fluid nickel levels[a]

Equation[b]	Occupational exposure detail
$U_{Ni}=21A_{Ni}+10$ or $P_{Ni}=2.1A_{Ni}+3$	Nickel matte crushing[c]
$U_{Ni}=85A_{Ni}+2$ or $P_{Ni}=11A_{Ni}+2$	Exposure to nickel metal or particulates composed primarily of nickel oxides, nickel sulfides and mixed metal oxides and sulfides
$U_{Ni}=700A_{Ni}+2$ or $P_{Ni}=60A_{Ni}+2$	Aqueous aerosols of nickel sulfate or nickel chloride

[a]Based on data compiled by Nieboer *et al.*;[66] see also Thomassen *et al.*[67] Data are for nickel refinery workers chronically exposed in the late 1970s. [b]Urinary nickel (U_{Ni}) and serum or plasma nickel (P_{Ni}) concentrations are in μg L^{-1}; air levels of nickel (A_{Ni}) are in mg m^{-3}. [c]Nickel matte is a mixture of nickel, copper and sulfur, with Ni_3S_2, Cu_2S and a nickel–copper metallic phase as major components.

techniques indicated or their modifications have been combined with element-specific detectors such as atomic absorption, atomic emission or mass spectrometers. The separation of inorganic arsenic, monomethylarsonic acid and dimethylarsinic acid by ion-exchange chromatography, followed by direct atomic absorption analysis after hydride reduction is an example.[94] However, concerns have been expressed about the stability of biomolecules containing the trace-element analyte during the separation stage[95,96] in applications to complicated biological matrices, including foods and tissues.[97–100]

Gas chromatography (GC) interfaced with atomic absorption spectrometry (AAS), atomic emission spectrometry (AES) or inductively coupled plasma mass spectrometry (ICP-MS) provides versatile tools for identifying and quantifying volatile metal or metalloid species. For example, alkyllead compounds have been determined in wine, polar snow and ice,[101,102] alkyl tin compounds in sediments and fish tissues,[103] organomercury compounds in environmental matrices[104] and volatile alkyl and hydride species of antimony, tin and bismuth in landfill and fermentation gases.[105] The separation (mostly by high-performance liquid chromatography) and selective detection of arsenic species in foods, urine and environmental samples have contributed significantly to our understanding of the exposure, metabolism and toxicity of arsenic compounds.[94,106–108] Similar applications have been reported for selenium.[109,110] Vela *et al.*[111] and Tomlinson *et al.*[112] summarized applications of coupled techniques to the speciation of other elements.

Voltammetric methods of speciation are also common and have found wide application for the characterization of the elemental components in natural waters.[113–115] Additional species-specific analytical approaches to those already alluded to are reviewed in books on the analytical aspects of element speciation.[115,116] Included are spectrometry (UV, VIS, fluorescence), potentiometry employing ion-specific electrodes, selective or sequential extraction and neutron activation.[115,116]

Few methodologies have been developed for the chemical speciation of metals in workroom air. For nickel aerosol speciation, a sequential leaching scheme has been developed for the determination of the four nickel fractions, namely water-soluble, sulfidic, oxidic and metallic.[67] It constitutes an operational definition and determination of nickel speciation. Briefly, it involves the preparation of the following leachates in sequence: (i) the soluble fraction with 0.1 M ammonium citrate (pH 4.4) for 90 min at room temperature; (ii) the sulfidic fraction with 0.067 M ammonium citrate–10% m/m hydrogen peroxide for 60 min at room temperature; (iii) the metallic fraction with 2% v/v bromine in anhydrous methanol (of the order of seconds) at room temperature; and (iv) the oxidic fraction by hot-plate digestion in concentrated nitric and perchloric acid.

2.4.6 Radical formation. Free radicals are chemical species with one or more unpaired electrons. Because of the Pauli exclusion principle, two-electron reductions of free radicals by a diamagnetic ('singlet') reactant is spin-forbidden.[117] It is for this reason that reactions of molecular oxygen (O_2), which is in a 'triplet' electronic ground state, tend to be relatively sluggish unless the electron donor is a radical. By virtue of unpaired d-electron configurations, transition metals are good reactants for O_2. In higher animals, the redox-active metals are Fe, Cu, Mn, Co and Mo, and Ni and V have redox roles in bacteria, fungi, algae, plants or invertebrates.[6,7,13,118,119]

Free radicals in excess are capable of damaging tissues. Molecular oxygen and its reduced derivatives (*i.e.*, superoxide anion, $O_2^{-\cdot}$; hydrogen peroxide, H_2O_2, which is not a radical; the hydroxyl radical, $^{\cdot}OH$; and lipid hydroperoxy radicals, lipid–$^{\cdot}O_2$) are of special concern.[120] It is important to emphasize that aerobic systems are inherently dependent on active oxygen species as electron acceptors and reactive metabolites in many essential anabolic, catabolic and immunological processes.[7,121,122] In addition, organic radicals generated in reactions involving vitamin B_{12}-dependent enzymes permit unique molecular rearrangements and synthesis.[7] Necessarily, defences against oxidative damage are intrinsic to biological systems. Examples are superoxide dismutase and catalase, which are effective scavengers of the superoxide anion and hydrogen peroxide, respectively. Low molecular mass radical scavengers such as vitamin C, vitamin E, glutathione and uric acid also abound and systems that repair damaged proteins, lipids or DNA exist.[123] Additionally, there is some evidence that the inducible sulfhydryl-rich protein metallothionein has a free-radical scavenging capability.[124]

Oxidative damage is considered a determinant in a number of diseases.[121,125] Suspected radical-mediated processes or disorders include ageing,[123] cardiovascular disease,[126,127] cancer,[128] inflammation, rheumatoid arthritis and oxygen toxicity,[121] Lou Gehrig's disease (amyotrophic lateral sclerosis, ALS) and iron-overload diseases.[125,129,130]

Perturbations of active oxygen metabolism due to environmental/occupational exposures to substances capable of inducing unusual fluxes of radicals are of concern. For example, the formation of dioxygen and other radicals is postulated to accompany the *in vivo* reduction of chromate (CrO_4^{2-}).[1,15,131] Another example is the potential for redox cycling involving the Ni^{3+}/Ni^{2+} couple in nickel(II) complexes of peptides. Such compounds have been shown to participate in active oxygen biochemistry and toxicity.[132–134] Both chromium(VI) and nickel(II) compounds are genotoxic and are recognized as human carcinogens.[131,135–138]

Free radicals can be identified and/or quantified by EPR, spin-trapping, UV/VIS spectrophotometry, monitoring of species-specific chromophores, often in the presence of enzymes, molecular oxygen consumption/release, lipid peroxidation assays or radical scavenging assays.[132,134]

2.4.7 Particle size and shape. Submicron (<1 μm in diameter) mineral colloidal matter, such as oxyhydroxides of iron, are strongly implicated in modulating the bioavailability and mode of dispersion of both metal contaminants and nutrients.[139,140] Within the context of environmental monitoring of industrial activities, lichens and mosses have been shown to trap micrometre-sized particles extracellularly at surface sites and interstitial spaces within the plant body.[141,142] In human exposures by inhalation, three size-dependent particulate fractions are defined, which determine where penetration and deposition occur in the respiratory tract and a response is elicited.[143–145] The *inhalable fraction* (aerodynamic diameter, $d_{ae} \leqslant 100$ μm; d_{ae} is defined below) is the fraction of total airborne particles that enters the body through the nose and/or mouth during breathing; it is relevant to health effects anywhere in the respiratory tract such as rhinitis, nasal cancer and systemic effects. The *thoracic fraction* [corresponding to mass fractions of total aerosol of 50% at $d_{ae} = 10$ μm (the PM_{10} fraction) and of 1% at $d_{ae} = 28$ μm] is the inhaled particle component which penetrates into the lung (*i.e.*, the whole region below the larynx) and is important for asthma, bronchitis and lung cancer. The *respirable fraction* [corresponding to mass fractions of total aerosol of 50% at $d_{ae} = 4$ μm (the PM_4 fraction) and of 1% at $d_{ae} = 10$ μm] constitutes the inhaled particles that penetrate to the alveolar region of the lung (*i.e.*, includes the respiratory bronchioles, the alveolar ducts and sacs) and is pertinent to the development of such chronic diseases as pneumoconiosis and emphysema. This aerosol classification is especially useful to the industries employing pyrometallurgical refining techniques such as in the nickel-producing industry in which lung and nasal cancers have been consistently reported.[67,136–138] Particulate air pollutants corre-

sponding to the $PM_{2.5}$ and PM_{10} fractions appear to be linked to increased mortality in the general population from lung cancer, cardiopulmonary disease and other respiratory causes. These effects were strongly correlated with suspended sulfates and contributions from metals are also suspected.[146–148]

The shape of a particle can have a significant impact on how it behaves in air and after deposition in the respiratory tract.[5] Particles can be spherical (*e.g.*, mists, sprays), angular (non-spherical or isometric, *e.g.*, those generated in the mining and refining of metals), flat (*e.g.*, mica-bearing rocks) or fibrous (*e.g.*, asbestos, glass-wool). For non-fibrous particles, the d_{ae} best describes particle motion. It permits a comparison of differently shaped particles through the operational definition: d_{ae} is the diameter of a spherical particle of unit density which has the same falling speed in air as the particle in question.[5,149] For fibres, both length and diameter need to be defined; their ratio is referred to as fibre aspect. Animal studies suggest that asbestos fibres longer than 5 μm are poorly cleared from the lung by scavenger cells (see below) and appear to be more fibrogenic.[149] Movement of the shorter asbestos fibres into the interstitium, the space between the basement membranes of the alveolar epithelial and the blood capillary endothelial cell layers, enhances cell infiltration and inflammation as well as the fibrotic processes of asbestosis that decrease lung function.[149] Translocation from the interstitium to the hilar nodes or pleura also occurs. Fibrous particles appear to have a special ability to accumulate in the pulmonary pleural cavity.[150] Pleural fibrosis and cancer (mesothelioma), and also bronchogenic carcinoma, are also observed among asbestos workers.[149]

At the cellular level, particle size is very important and relates to uptake by phagocytosis and subsequently toxicity. Animal cells have diameters of 10–30 μm and thus for particles to be endocytosed ('eaten' or phagocytosed), they must be considerably smaller (<10 μm in diameter). Costa *et al.*[151] have shown for Chinese hamster ovary (CHO) cells that the mean particle size of crystalline α-NiS correlated inversely with uptake and cytotoxicity as measured by colony formation in the range 1–6 μm. Some unicellular organisms such as protozoa obtain food phagotropically. In cell culture, many mammalian cells are phagocytes, whereas in multicellular organisms the 'professional' phagocytes are macrophages and neutrophils and few other cells are able to ingest large particles.[152] Since human alveolar macrophages have larger diameters than those in experimental animals [*e.g.*, 20 μm *versus* 13 μm (rat), 14 μm (hamster), or 16 μm (monkey)],[153] the mentioned 5 μm particle-size restriction on the clearance from the lung of asbestos fibres may be less severe in humans. Little is known about whether, for example, epithelial cells lining the human lung have such a facility, although by analogy with asbestos, fine particles ($d_{ae}<10$ μm) are hypothesized to enter the alveolar interstitium.[154] Interstitialization of such particles is believed to enhance inflammatory responses that may lead to the respiratory and cardiovascular diseases mentioned.[155]

Direct-reading instruments with optical, electrical, resonance oscillation or β-radiation attenuation are available for measuring ambient concentrations and in some cases size distribution of aerosols.[148,156] Estimates of the coarse aerosol fraction for both the general and workplace environments are achieved by collecting particulates on filters employing stationary and personal samplers.[144,148] Recent developments aim to standardize such quantification of airborne particulates so that air sampling approximates inhalability by estimating the inhalable fraction as defined above.[143–145] Aerosol spectrometers are also available for indoor and outdoor uses that sample a number of size fractions such that the inhalable, thoracic and respirable fractions can be measured.[144,148] Cascade impactors are the most commonly used devices and are now available for personal (individual) sampling.[144,157]

Characterization of the collected particles can be achieved in numerous ways: bulk elemental composition [*e.g.*, X-ray fluorescence (XRF), AAS, AES]; particle size and shape (scanning and transmission electron microscopy; SEM, TEM); crystallinity (X-ray diffraction spectrometry); elemental composition of individual particles (SEM plus energy dispersive XRF; electron probe microanalysis in combination with energy or wavelength dispersive XRF).[17,67] Fibre counting, sizing and identification can be achieved by phase-contrast optical microscopy, SEM or TEM.[158]

2.4.8 Physico-chemical properties of solid surfaces. Because solid metal compounds have exposed cationic or anion sites on their surfaces with reduced coordination number, hydrolysis or chemisorption results in surface-active functional groups such as hydroxyl groups.[10,59,159] Examples are silanol (–SiOH) groups on hydrated silica and the pH-dependent surface charge on silicates.[59] Ion exchange occurs when a sorbed ion on the mineral surface exchanges with another of the same charge.[150] Neutral or charged organic molecules are also known to adsorb on solid surfaces,[59] adsorption of polycyclic aromatic hydrocarbons on asbestos fibres being an example. It has been postulated that this contributed to the enhanced lung cancer risk for asbestos workers who smoked.[149]

The protein adsorption data compiled in Table 4 illustrate that particulate nickel compounds of comparable size ($\leqslant 10$ μm) exhibit different surface properties. The crystalline compounds, and also 5 μm Ni powder, which had a very smooth exterior by electron microscopy, were found to have low protein adsorption capacities, with the colloidal $Ni(OH)_2$ and amorphous NiS exhibiting very high values. Recent work (E. Nieboer and G. G. Fletcher, unpublished work) indicates that the magnitude of desorption constants (in μg protein per mg Ni) for nickel particles with a thin oxide coating is dependent on the protein employed, but appears not to be strongly influenced by the relative molecular mass or by the net charge of the protein. A comparable ranking to that observed in Table 4 is achieved when haemolysis of human red blood cells is the outcome measurement (Table 5). Perhaps surprisingly, the non-crystalline compounds are the least carcinogenic in animals: colloidal $Ni(OH)_2$ is non-carcinogenic and amorphous NiS weakly so. For rats, Sunderman[162] established the carcinogenicity sequence α-Ni_3S_2, β-NiS > NiO ≫ Ni powder ⋙ amorphous NiS (material of median particle size <2 μm was injected intramuscularly). Dried, crystalline $Ni(OH)_2$ is also known to be carcinogenic.[163] A review of the available laboratory studies[136,151,162,164–170] suggests that surface passivity of solids, namely smooth exterior, crystallinity,

Table 4 Ranking of nickel compounds according to relative protein adsorption capacity (source: Nieboer *et al.*[160] and Maxwell[161])

Nickel compound[a]	Protein adsorption capacity[b]/μg mg^{-1}	Rank
Colloidal $Ni(OH)_2$	570 ± 10	1
Amorphous NiS	(300)[c]	2
NiO	8 ± 0.5	3
Ni (1 μm)	4.3 ± 0.4	4
α-, β-NiS[d]	3.4 ± 0.2[e]	5,6
Dried $Ni(OH)_2$[d]	2.9 ± 0.1[f,g]	7
α-Ni_3S_2[d]	2.2 ± 0.4[h]	8
α-NiS (source 2)[d]	1.4 ± 0.1	9
Ni (5 μm)	0.4 ± 0.1	10

[a]Other than colloidal $Ni(OH)_2$, the mean particle size by electron microscopy was <10 μm unless indicated otherwise. [b]Values correspond to slopes of the initial linear segments of plots of the amount of human serum albumin adsorbed *versus* the amount of particulate nickel compound (in 0.04 M TRIS-buffered saline, 0.15 M, pH 7.4). [c]Single determination. [d]Crystalline solid by X-ray diffraction analysis. [e,f,g,h]Not significantly different from each other ($p > 0.025$).

Table 5 Ranking of nickel compounds according to haemolytic ability (source: Maxwell[161])

Nickel compound[a]	Specific haemolytic index[b]/% mg^{-1}	Rank
Colloidal $Ni(OH)_2$	40 ± 2	1
NiO	2.05 ± 0.07	2
β-NiS[c]	1.3 ± 0.1	3
Ni (1 μm)	1.00 ± 0.07	4
α-NiS (source 1)[c]	0.72 ± 0.02	5
α-NiS (source 2)[c]	0.61 ± 0.01	6
α-Ni_3S_2[c]	0.26 ± 0.07[d]	7
Dried $Ni(OH)_2$[c]	0.22 ± 0.02[d]	8
Ni (5 μm)	0.09 ± 0.01	9

[a]Other than colloidal $Ni(OH)_2$, the mean particle size by electron microscopy was <10 μm unless indicated otherwise. [b]Values correspond to slopes of the initial linear segments of plots of % induced haemolysis of human erythrocytes *versus* the amount of particulate nickel compound (in 0.004 M veronal buffered saline, 0.15 M, pH 7.2). [c]Crystalline solid by X-ray diffraction analysis. [d]Not significantly different from each other ($p > 0.025$).

low surface charge, low surface activity with respect to protein absorption and cell lysing ability and moderate solubility appear to be a predisposition to experimental carcinogenicity.[160] These properties parallel the ability of particles to be phagocytosed, which as indicated constitutes an effective uptake mechanism.[171] Observations of this type constitute strong evidence that the structure, composition and surface properties of mineral species influence toxicity. Interestingly, exposure to particulates composed primarily of nickel oxides, nickel sulfides and mixed metal oxides and sulfides figure prominently in the epidemiology of occupational nickel-related lung and nasal cancer.[67,137,170]

Phagocyte recruitment and activation result in the generation of the active oxygen species identified earlier ($O_2^{-\cdot}$, H_2O_2, $^{\cdot}OH$) and others such as nitric oxide ($NO^{\cdot}$), peroxynitrite ($ONOO^-$) and hypochlorous acid ($HOCl$).[120,121,152,172,173] These reactive species are directed to the intracellular or extracellular destruction of invading microorganisms such as bacteria or parasites. In the absence of phagocytic cells, certain particles (*e.g.*, coal, quartz, PM_{10} particles collected from ambient air) or fibres (*e.g.*, asbestos, glass) can generate radicals from molecular oxygen or catalyse free radical reactions.[150,174] The exact mechanisms are not completely understood, but appear to involve internal redox reactions and diffusion to and from the surface of electrons, or redox cycling by surface constituents or contaminants (*e.g.*, Fe^{3+}/Fe^{2+}).[150,174,175] Such reactivity has the potential for generating tissue-damaging radicals, producing detrimental changes in the radical fluxes emanating from phagocytic cells or reducing the pool of natural scavenging agents such as vitamin C, vitamin E, glutathione or uric acid.[123,132]

The diversity in biological responses induced by particulate nickel compounds, even among those with identical chemical composition and X-ray diffraction patterns but different pyrometallurgical histories,[164] is a warning that the physicochemical properties of metal compounds used in laboratory toxicological assessments must be well characterized for the meaningful interpretation of experimental results. These observations provide the rationale for the need to characterize ambient air particulates in exposure assessments.

2.5 Biological factors

2.5.1 Compartmentalization. In our discussion of solubility as a determinant of reactivity, the extracellular and intracellular inclusion of metal compounds in invertebrates was mentioned. Whatever the exact purpose or function of such localized deposits or granules is, they do permit organisms such as barnacles, molluscs and amphipods to be used in the biomonitoring of the aquatic environment (see Table 2).[32,33,44,45] In humans, compartmentalization of metals in specific organs or tissues, such as lead in bone or red blood cells, strongly influences not only toxicity but also the selection of suitable biological indices of exposure. As indicated, the measurement of lead in bone permits an assessment of body burden, while lead in whole blood is a suitable index of current exposure. About 99% of the blood lead resides in the red blood cells.[86] As explored further in a subsequent section, movement in and out of compartments determines what parameter to measure in biological monitoring and when.

The role of Ca^{2+} as a second messenger or trigger illustrates the inherent importance of compartmentalization in biology. The release of Ca^{2+} from extracellular or intracellular stores into the cell cytosol leads to such complicated processes as muscle contraction or exocytosis of, for example, neurotransmitters.[7] Further, the electrophysiology underlying nerve conduction requires gradients of K^+ and Na^+ across cell membranes and the production of ATP depends on gradients of protons in mitochondria.[7]

Compartmentalization of toxic metals is suggested to be a significant factor at the cellular level. This is illustrated in a study by Fletcher *et al.*[168] in which the uptake, cytoxicity and mutagenicity of three water-soluble nickel salts (chloride, sulfate and acetate), six water-insoluble nickel compounds (three oxides and three sulfides) of particle diameter $\leqslant 5$ μm and two relatively insoluble nickel compounds (carbonate and hydroxide) of the same particle size were evaluated in AS52 CHO cells. The composition of all particulate compounds was verified by chemical analysis and X-ray diffraction analyses; for the salts, the suppliers' documentation was accepted. LC_{50} values, corresponding to the concentration at which the survival (assessed by colony forming ability) of exposed cells is 50% of that of non-exposed control cells, ranged from 2 to 130 μg Ni mL^{-1} for particulate nickel compounds and from 45 to 60 μg Ni mL^{-1} for the water-soluble salts in 24 h exposures. While the administered LC_{50} doses differed by a factor of 75 for the 11 nickel compounds, the spread for both the cytosolic and nuclear concentrations of dissolved (non-particulate) nickel was about a factor of 10. The most toxic compounds exhibited the highest nuclear and cytosolic levels. Interestingly, the particulate compounds delivered dissolved nickel to both compartments, whereas the water-soluble salts loaded the cytosol but very little nickel reached the nucleus. Presumably this reflects the different modes of uptake, *i.e.*, phagocytosis (particles) and diffusion (water-soluble salts). Whereas the cytosolic dissolved nickel concentrations for the 11 compounds did not correlate with the LC_{50} values, the nuclear levels did ($p < 0.05$). All the compounds tested were weakly mutagenic. Mutant profile analyses involving a specific gene locus were compound specific and suggested different mutagenic mechanisms for the small number of water-soluble and particulate compounds examined in this part of the study. These results were interpreted to reflect the different intracellular compartmentalization patterns of the nickel ion described.[176]

Organ-specific toxicity usually involves compartmentalization of the toxicant. A good example is industrial exposure by inhalation for which the nasal passages and lungs are the tissues most often affected by cancer. Exposures to chromate, nickel compounds and perhaps also stainless-steel welding fumes are good examples.[136] Analysis of lung and nasal tissues of deceased nickel refinery workers has confirmed considerable residual deposits (presumably particulate and interstitial).[177] The accumulation of asbestos in the alveolar interstitium discussed earlier is also pertinent. Direct exposure of the eyes and skin are other illustrations. Skin contact with chromium or nickel compounds and the subsequent dermal penetration of nickel or chromium species are a major cause of contact

dermatitis.[178–180] By contrast, toxic effects related to preferential compartmentalization in target organs after absorption into the blood compartment also occur and include renal damage by cadmium,[74,181,182] perturbation of renal function associated with the occurrence of lead inclusion bodies in individuals chronically exposed to high levels of lead[73,86] and repercussions on bone cell function of the preferential accumulation of lead, as 95% of the body burden of lead is located in the bones.[75,76,183] Accumulation in a biological compartment need not necessarily have toxicological consequences. For example, even though high levels of aluminium have been found in most of the organs of groups of patients with chronic renal failure, only the central nervous system and bone were apparently affected.[54]

By analogy with organ-specific toxicity, molecule-specific toxicity also seems possible. However, unlike simply inherited disorders (*i.e.*, transmission of a single mutant gene; see Section 2.5.5), most of the known toxic effects of metals and metalloids do not have a single molecular mechanism. The development of cancer[138,184,185] and Pb^{2+} neurotoxicity[37] are good examples. Similarly, the repression of haem synthesis in the bone marrow compartment by Pb^{2+}, which results in anaemia, involves not only the inhibition of δ-aminolaevulinic acid dehydratase (ALAD) and ferrochelatase, but also at least three other enzymes.[37] Interestingly, binding of Pb^{2+} to critical sulfhydryl groups appears to occur in all of the haem-biosynthesis enzymes affected.

2.5.2 Respiratory tract deposition and clearance. Clearance of a toxic substance before it exerts its toxic effect or is absorbed can be distinguished from removal after absorption. In the latter case, normal elimination of metals from the body by way of the kidney, liver or secondary organs (*e.g.*, skin, pancreatic juices) occurs; this is addressed in Section 2.5.4. Although tearing of eyes or washing and shedding of the skin can remove toxicants, the major inherent clearance mechanisms operative in humans occur in the respiratory tract. Even though particle size strongly influences where in the respiratory tract aerosols are deposited, it is the anatomical structures that impose the size selectivity described earlier. Descriptions of the structure and function of the nasopharyngeal, tracheobronchial and air-exchange portions of the respiratory tract are available in standard texts[149,186,187] and are limited here to the components involved in clearance pathways.

The epithelial lining of the bronchi has single-celled exocrine glands that secrete mucus, in addition to ciliated cells. Submucosal glands of the bronchial lining also produce mucus to blanket the bronchial epithelium. The cilia rhythmically move the mucus blanket and any trapped or dissolved toxicant toward the trachea and pharynx, where they are swallowed or expectorated by coughing.[149,186,187] Most of the nasopharyngeal region is also cleared of dissolved substances, foreign particles or microorganisms by the mucociliary pathway. In the alveolar region of the lung, alveolar macrophages ingest particulate foreign material that reaches the alveoli, permitting removal through the lymphatics; transfer of particle-laden macrophages to the mucociliary escalator by a poorly understood mechanism is also suspected.[149] Drainage of exuded fluids upwards *via* the airways and through the lymphatic system also appears to help in scavenging substances (including naked particles) from the air-exchange region (*i.e.*, respiratory bronchioles and alveoli).[149] Removal of uncleared metal-containing materials from nasal and lung tissue appears to be slow (see Section 2.5.4).

Intake by both ingestion and inhalation is important for aerosol substances that after absorption, and by way of the peripheral blood, act on a specific internal organ or have systemic effects. In such cases, the fraction of the inhaled aerosol that is cleared from the respiratory tract by the mechanisms outlined and subsequently ingested constitutes a significant contribution to the total dose. As already indicated (see Section 2.4.7), in these situations the measurement of the inhalable aerosol fraction is preferred in personal exposure assessments.[5,143,149] Traditionally, the 'total aerosol' fraction has been employed to estimate inhalability.[188]

2.5.3 Bioavailability and uptake. A consensus was reached at the Winnipeg Ecotox Workshop (organized by G. R. B. Webster, Department of Social Science, University of Manitoba, Winnipeg, Canada, June 16–19, 1996) in favour of the following definition of bioavailability: *the fraction of a substance that is available for absorption by an organism when considering a specific route of exposure*. This interpretation is consistent with that rendered by the US Agency for Toxic Substances and Disease Registry[189] and Health Canada.[190] Note that this definition does not include any reference to outcome, although the underlying assumption is that absorption is a prerequisite to any response by an organism. Certainly the latter is not necessarily the case for surface effects on skin or interaction with respiratory epithelial cells. Reaching the surface of the target tissue is the pertinent issue in such instances. It seems reasonable to suggest therefore that in certain cases the word absorption might be replaced by other terms such as 'uptake' or 'accumulation' (*e.g.* in case of metal-ion uptake by cells, fish gill surfaces or plants) or even 'deposition' or 'intake' (*e.g.*, inhalation of aerosols). The definition recommend by the IUPAC Commission on Toxicology[191] has a more restricted emphasis and a human focus: 'bioavailability is the extent to which a substance to which the body is exposed (by ingestion, inhalation, injection or skin contact) reaches the systemic circulation, and the rate at which this occurs'. Although this approach appears less widely applicable, it is more amenable to defining an effective dose, namely that which causes a defined magnitude of response in a given system,[191] or to assessing the extent a toxic agent reaches an internal target site. Nevertheless, it seems more practical to adopt a definition of bioavailability that is not tied to a response, adverse or otherwise, as suggested above.

As already indicated (see Sections 2.4.2 and 2.4.4), GI uptake of metal ions is very dependent on the ligands present in the food. Mineral bioavailability is therefore strongly dependent on the type of diet and the nature of the metal-containing components.[192] For example, the ratio of lead absorption from soil and soil-derived dust to lead absorption from average diets is reported as 0.63 and 0.76, respectively, as evaluated by a combination of acid extraction studies and dietary assessments in animals and humans.[193] Attempts to link the extractability of metals such as cadmium from soils with some index of environmental toxicity have also been reported.[194] Bioavailability for non-alimentary tract exposure routes is also relevant, *e.g.*, the release of metal in the form of a cation or anion from objects that come into contact with the skin is essential to the development of allergic contact dermatitis.[195,196] Examples are nickel released from jewellery and chromate from cement.[178–180] For exposures by inhalation, the nature of the toxic substances is crucial. Gases such as nickel tetracarbonyl, carbon monoxide, sulfur dioxide, hydrogen cyanide and arsine are instantaneously bioavailable and nearly completely absorbed. Exposures to aqueous aerosols of, for example, nickel(II) salts (see Table 3) or potassium dichromate are accompanied by significant uptake, perhaps involving only a short delay (hours) in absorption.[14,197] By contrast, most particulate metal compounds of low water solubility are not readily absorbed, as indicated by their long biological residence times (see Section 2.5.4). It is well established by magnetopneumography that chronic deposits of lung-retained contaminants occur in workers exposed to metal-containing particles, such

as welders.[198,199] Further, as already indicated, chemical analysis of lung tissue of deceased nickel refinery workers furnishes similar evidence.[177] Presumably, solubility in biological fluids of the deposited material, and also the surface properties discussed earlier that determine biological processes such as uptake and removal by macrophages, regulate the rate of clearance. In terms of cancer in the nickel and chromium refining industries, it is postulated that the slow release of nickel(II) or chromate ions figures prominently in the development of this disease. Interestingly, workers exposed primarily to aqueous aerosols of nickel chloride and sulfate in an electroplating department had significantly less ($p<0.01$) nickel in autopsied lung tissue than those employed primarily in a roasting and smelting department.[177]

From this brief consideration of bioavailability, it is clear that this parameter is important in exposure characterization, irrespective of the route. Water or lipid solubility appears to be an important determinant of bioavailability. Thomassen *et al.*[67] concluded that the water solubility of nickel-and cobalt-containing inhalable aerosols probably determines absorption, as appears to be the case for the example mentioned above of Pb^{2+} derived from soil and dust. Absorption studies indicate that 7% of inorganic mercury and 95% of methylmercury are absorbed in the GI tract,[81,82] illustrating the importance of lipid solubility. The latter also facilitates dermal and respiratory absorption.

2.5.4 Biological turnover time and bioaccumulation. Metals can be eliminated by way of urinary, biliary, pancreatic and intestinal excretion; secretion into sweat, saliva and tears is also established.[200–202] The urinary pathway is the best defined route for most metals. Less is known with certainty about the remaining excretion routes in humans, since the available data are plagued by analytical uncertainty related to inadvertent contamination during collection, handling, storage and analysis of samples.[1,203] For most metals, concentrations in the blood compartments (whole blood, plasma or serum) and urine reflect exposure.[204,205] These parameters may be employed to assess biological residence or elimination half-times when measured serially over time.

A perusal of the half-times compiled in Table 6 indicates that the turnover of metals is idiosyncratic and depends on the form and route of exposure and also on the compartment of the body monitored. Clearance from the blood compartment is relatively fast for most metals, with an upper limit of about 4 months, corresponding to the half-life of mature red blood cells. The slow release from special compartments reflects unique packing or biochemical processes. Incorporation of Pb^{2+} into the Ca^{2+} mineral matrix explains its slow release from the bone, while the localization of cadmium in the kidney is attributed to the inducible Cd^{2+}-scavenger protein metallothionein. The latter binds Cd^{2+} avidly and preferentially.[181] Further, uncleared particulates trapped in the tissues of the respiratory tract are released slowly. This is believed to increase the risk of chronic diseases such as cancer or pneumoconiosis. Short-term toxic effects of metals may also be exacerbated by unfavourable clearance rates. Chelating agents are administered therapeutically in emergency situations to enhance excretion and to reduce the levels of toxic metals such as lead in the blood and soft tissues compartments.[149,210]

The time-frame involved of movement of an agent into, between or from compartments determines the timing of sample collection after exposure.[204,211] For short uptake and release half-times (minutes to hours), such as seen for the inhalation of vapours and gases and their exhalation, the timing of breath sample collection is critical. Assuming relatively rapid absorption, collection timing becomes progressively less critical as removal half-times increase from 1 d (*e.g.*, nickel in urine), to days to weeks (*e.g.*, lead, mercury, or cadmium in whole blood) or to months or years (lead in bone or cadmium in kidney). The availability of biokinetic (also referred to as toxicokinetic) models such as are available for cadmium,[212] lead[86,213] and nickel[206] is therefore extremely helpful, if not essential, in designing biological-monitoring sampling strategies.

Some toxic agents are not excreted quickly and become stored in parts of the body for long periods of time (*e.g.*, ferrimagnetic particles retained in the lungs of welders; cadmium in the kidney). Continued exposure will result in build-up because the rate of intake exceeds that for removal, and therefore bioaccumulation occurs.[191] Lead in bone and cadmium in kidney can thus serve as suitable measures of body burden due to long-term exposure. As is the case for bone lead, cadmium concentrations in kidney can be measured *in situ*.[182,212] Related to bioaccumulation is biomagnification, which is a process leading to higher concentrations of a substance in an organism than in its food.[191] More formally, it can be defined as a 'sequence of processes in an ecosystem by which higher concentrations are attained in organisms at higher trophic levels (at higher levels in the food web)'.[191] The accumulation of methylmercury in fish is a pertinent example.[81]

2.5.5 Tolerance and susceptibility. Toxicity can be modulated by the development of tolerance or resistance. This is readily illustrated for nickel since a number of terrestrial and aquatic organisms can accumulate enormous amounts of this metal

Table 6 Selected biological turnover times of metals in humans

Substance (compartment)	Half-time[a] ($t_{1/2}$)	Type of exposure	Ref.
Nickel (urine, serum)	1 d	Electroplating/electrorefining or experimental	197,206
Nickel particles (in nasal tissue and lungs)	3.5 y	Pyrometallurgical refining	207
Welding fumes (lung)	3.5 y[b]	Welding	198,199
Aluminium (serum)	4–6 h	Ingestion of $Al(OH)_3$/citrate	54
Aluminium (urine)	20–400 d	Welding	54
Chromium (urine)	15–40 h	Rapid phase in SS welders	14
	14 d to infinity	Slow phase in SS welders	
Lead (blood and soft tissues)	20–30 d	Environmental/occupational	86
Lead (cortical bone)	$\geqslant$10 y	Environmental/occupational	86
Mercury vapour (whole blood)	3 d (fast phase)	Occupational/incidental	82
	30 d (slow phase)		
Methylmercury (whole body, whole blood, hair)	45–70 d	Dietary intake (mostly fish)	81,208
Methylmercury (movement between blood and hair)	5.6 d	Accidental poisoning	208
Cadmium (kidney)	$\geqslant$10 y	Occupational/smoking	181
Cadmium (whole blood)	40–90 d	Occupational/smoking	181,209

[a]d, Day; h, hour; y, year; a good source of values for inorganic, organometallic and organic compounds is the book by Lauwerys and Hoet.[205] [b]Determined by magnetopneumography.

without apparent ill-effect.[214] As discussed earlier, vacuolar deposition as metal complexes is one mechanism at the cellular level;[70,215] another observed in aquatic organisms is exclusion by extracellular complexation/deposition or by reducing cell-membrane permeability.[11,215] Recently, tobacco and papaya plants have been made tolerant to aluminium toxicity by genetically engineering them to pump out citrate from their roots, which through complex formation prevents Al^{3+} uptake.[216] Additional cellular defence mechanisms are possible: cellular exports, biotransformation (of organometallics), extracellular scavenging and/or conversion to non-toxic forms, development of target tolerance and repair of incurred damage.[11,217–219] For example, inducible metal-efflux systems for Borderline ions (*e.g.*, Cd^{2+}, Co^{2+}, Zn^{2+}), and also arsenate, occur in bacteria.[11] Furthermore, induction of metallothionein (MT) by Borderline ions (*e.g.*, Cd^{2+}, Zn^{2+}) and Class B ions (*e.g.*, Ag^{+}, Cu^{+}, Hg^{2+}, Bi^{3+}) provides intracellular protection from their toxic effects in numerous cell systems, animals and humans.[219] MT constitutes a non-toxic reservoir or buffer. Paradoxically, it has been demonstrated in mice that the uptake of the Cd–MT complex by the kidney damaged the proximal tubules of this organ.[181] It appears that the lysosomal degradation of Cd–MT releases Cd^{2+} into the cytosol of these epithelial absorptive cells.

In bacteria, resistance to toxic metals occurs primarily by regulation through plasmid-encoded processes. Hg, Cd, As, Cr and Cu resistance are examples.[11,220] By contrast, bacterial uptake of essential nutrients (*e.g.*, phosphate, sulfate, Mn, Ni, Zn and in part Cu) usually are chromosomally encoded. This is also true for Cu uptake in yeast.[221] Presumably, sensitivity to toxic metals is conferred by gene mutation. Interestingly, mutations in two closely related genes which encode proteins involved in copper transport in humans have been shown to be responsible for either Menkes disease (copper deficiency) or Wilson disease (copper toxicity due to excess).[222] Genetic factors influencing susceptibility of humans to the toxic effects of metals have also been postulated.[223] For example, might the inheritance of genes coding for versions of enzymes that are especially sensitive to lead inhibition result in enhanced sensitivity to this metal?[224] Similarly, do familial blood disorders which have anaemia as one consequence[225] increase one's predisposition to lead, which can also induce this state? Susceptibility to cancer affords another illustration. Since clinical manifestations of this disease involve a number of events at the gene level,[185] can one conclude that the inheritance or somatic acquisition (*e.g.*, through smoking[226]) of a single genetic risk factor increases the probability of developing cancer due to the occupational exposure to nickel, chromium(VI) or arsenic compounds? The detection of such inherited or acquired risk factors is now technically feasible through gene-specific genetic screening techniques.[184,227] The measurement of genetic markers of susceptibility is attracting increasing attention by environmental and occupational health specialists because of the Human Genome project.[184,225,228–230] The latter has as its goal to map (*i.e.*, assign to a specific chromosomal locus) and sequence every gene in the entire genome.[231] Further, immunological tolerance has also been recognized for some time in humans in the context of allergic contact dermatitis due to metals.[178,179,232] Genetic susceptibility to asthma is another recent example.[233,234] The hypothesis in this case is that such inherited sensitivity will exacerbate the effect of chemical respiratory allergens, which include metal compounds such as those of nickel[180] and chromium(VI).[178]

Thus far we have identified two major types of biological markers, namely of exposure and susceptibility. For the sake of completeness, a third possibility needs to be mentioned. It may be designated as a marker of effect or outcome.[230,235,236] The following are illustrative of applications in the monitoring of humans environmentally or occupationally exposed: low and high molecular mass proteins, enzymes and amino acids in urine due to cadmium;[209,212] lead-related urinary excretion of lysosomal enzymes and intermediate metabolites of haem synthesis;[86,205,237] and chromosomal damage in human peripheral lymphocytes in workers exposed to chromium or nickel compounds.[136,238] Outcomes such as those described are often without impairment of any functional capacity for mild exposure and tend to be reversible. They are therefore useful parameters in biological monitoring programmes as indices of exposure.

3 Conclusion

This paper demonstrates that a host of factors are involved in determining toxicological responses to the elements and their inorganic compounds. Not only are atomic/molecular, physical and physico-chemical properties and factors that influence chemical reactivity important, but also anatomical structures, biological processes and genetic determinants clearly modulate toxic outcomes. Because of this complexity, exposure characterization and assessment activities will undoubtedly benefit from interdisciplinary collaboration involving chemists, biologists, biochemists, geochemists, toxicologists, physicians and specialists in medical sciences, occupational hygiene and health and environmental health. We hope that we have succeeded in demonstrating the relevance and importance of incorporating the basic reactivity concepts outlined in the design of effective environmental and biological monitoring programmes. Most of the principles described also have relevance to our understanding of exposures to and the toxicological consequences of substances other than inorganic compounds of the elements. However, biotransformation (mostly by the liver) of organic compounds figures much more prominently in their distribution, clearance, excretion and toxicity.[239]

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