Bioaccumulation modeling of organic chemicals and metals based on chemical properties and species characteristics Karin Veltman

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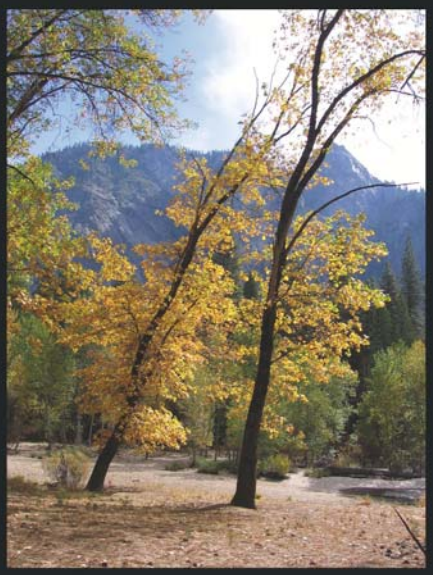

Karin Veltman

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Hierbij nodig ik u uit om de verdediging bij te wonen van mijn promotie getiteld:

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BIOACCUMULATION MODELING OF ORGANIC CHEMICALS AND METALS BASED ON CHEMICAL PROPERTIES AND SPECIES CHARACTERISTICS

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Natuurwetenschappen, Wiskunde en Informatica

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Chapter 1.

General introduction

1.1 History

Since World War II there has been an enormous increase in industrial (chemical) production, global trade of products, and use of manufactured goods, which has resulted in the emission of thousands of man-made chemicals into the environment. Although these chemicals are essential for maintaining a high standard of living, and many have proved effective in pest and disease control, some substances have had unforeseen adverse effects on human health and the environment. The widespread concern about possible ecological effects of man-made chemicals developed during the 1950s and 1960s, when some agricultural pesticides, such as DDT, were found to have adverse effects on wild-life (Carson, 1962). Shortly after, Jensen (1966) discovered polychlorinated biphenyls (PCBs) in fish throughout Sweden. This study showed that industrial chemicals designed for use in closed-systems were also entering the abiotic environment and accumulating to significant concentrations in fish (Jensen, 1966). During the 1970s, evidence that persistent organochlorines, as PCBs and DDT, were ubiquitous environmental pollutants continued to build (Nelson et al., 1972; Clayton et al., 1977). Since then, PCBs and DDT have been detected in a myriad of wild-life species including aquatic organisms, as zooplankton, mussels, fish, seals and whales, and terrestrial species, as earthworms, small mammals and birds (Van den Berg et al., 1987; Boon et al. 1989; Norstrom and Muir, 1994, Hop et al., 2002; Wolkers et al., 2007). This includes species inhabiting remote areas in Arctic regions, such as polar bears, killer whales and glaucous gulls (Norstrom and Muir, 1994; Hop et al., 2002; Wolkers et al., 2007), illustrating that persistent organochlorines may be transported over long-distances and end up in ecosystems where there have been no production and/or emission sources. These monitoring studies revealed that chemical concentrations in organisms are often far in excess of the concentrations in the surrounding environment, i.e. these chemicals have a high bioaccumulation potential. Additionally, these chemicals can biomagnify, which implies that internal concentrations increase from organism to organism in food chains, resulting in highest levels in top-predators. This may threaten the health of these animals or of those who consume them. For example, PCB transfer through the water–zooplankton–fish–bird food chain has probably been responsible for the drastic, worldwide decline of several piscivorous bird populations in the 1950s – 1960s (Ratcliffe, 1970; Koeman et al., 1972; Fry, 1995). Beginning in the 1970s, many organochlorine pesticides, such as DDT, were banned in North America, Western Europe and Japan, and use of PCBs was restricted (Muir and Howard, 2004). As a result of these regulations the current quality of air and surface waters has

significantly improved as compared to the situation in the 1960s and some threatened wild-life populations are slowly recovering (Bowerman et al., 1995; Hendriks and Enserink, 1996).

Lessons from the past on bioaccumulation of persistent organic pollutants, as DDT and PCBs, have taught us that even small concentrations of chemicals in the environment can find their way into organisms in dosages high enough to cause problems. For a valuable risk assessment of chemicals, it is therefore essential to be able to predict this bioaccumulation potential. Nowadays, bioaccumulation has become an important criterion in chemical regulation worldwide. In the new chemical legislation of the European Union, REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals), bioaccumulation, along with persistency and toxicity, is used to prioritize substances for regulatory action and for evaluation of chemical risks (EC, 2006). Additionally, bioaccumulation has been used to develop water quality criteria for chemicals and to set remediation objectives for contaminated soils and sediments (EU-WFD 2000; OSPAR, 2004; UNEP, 2006).

However, chemical evaluation and risk assessment is seriously impaired by a lack of relevant bioaccumulation-data (EEA, 2004). Compared to the variety of chemicals currently used in commerce, measured bioaccumulation data, from laboratory or field studies, are quite limited (Arnot and Gobas, 2006; Weisbrod et al., 2007). Bioaccumulation studies have historically focussed on the approximately 100 known persistent organic pollutants (POPs) (Hendriks and Van de Guchte, 1997; Weisbrod et al., 2007). Yet, several other non-organic contaminants have also been classified as “hazardous” because of their bioaccumulation potential. This includes organometallic compounds such as methylmercury and tributyltin and various heavy metals (OSPAR, 2004; UNEP, 2006; Helcom, 1992). Although use and/or production of several persistent organochlorines has been banned for a decade, these chemicals continue to be emitted into the environment from diffuse sources (AMAP, 2000; Muir et al., 2000), requiring attention of chemical management. While still dealing with emissions from the past, new chemicals are produced and discarded continuously as well. These relatively unknown chemicals can have a large contribution to water and sediment toxicity (Hendriks et al., 1994; Lahr et al., 2003). Recently, there has been concern on the bioaccumulation potential and global distribution of so-called “novel” POPs as perfluorinated alkyl compounds, in particular perfluoro-octane sulfonate (PFOS) (Giesy and Kannan, 2001; Martin et al., 2004) and brominated flame retardants (De Boer et al., 1998; Hites, 2004; Jenssen et al., 2007). Finally, chemical bioaccumulation is relatively well studied in various fish species, yet, measured bioaccumulation data for other species are largely

absent. Recent studies have shown that biomagnification factors are much higher for air-breathing organisms, as terrestrial species and marine mammals, than for water-ventilating, aquatic organisms (Hendriks et al., 2001; Gobas et al., 2003; Kelly et al., 2007). Additionally, some chemicals that do not accumulate in fish appear to have a high bioaccumulation potential in terrestrial species (Hendriks et al., 2001; Gobas et al., 2003; Czub and MacLachlan, 2004; Kelly et al., 2007). Chemical risk assessment therefore requires a broader taxonomic assessment, including terrestrial organisms.

Obviously, most of the thousands of substances and species that are of interest for environmental management will not be monitored at all relevant locations and periods, because of financial, practical and ethical constraints. To allow risk assessment for many substances and species at different locations and periods, environmental management requires quantitative tools that can reliably estimate the bioaccumulation potential of various chemicals for a range of species.

1.2 Bioaccumulation

Chemical bioaccumulation in organisms is a dynamic process resulting from exposure through multiple routes involving air, water, soil or sediment, and food, and elimination via several mechanisms of loss. Bioaccumulation occurs when an organism eliminates a chemical at a rate lower than at which the substance is taken up, as a result of biological “storage” and a lack of efficient biotransformation processes. For persistent nonpolar organic chemicals, lipid tissues are the primary storage compartment. The chemical affinity for lipid tissues can be characterized by the octanol-water partition coefficient (K_{ow}), which is a measure for hydrophobicity. An increasing K_{ow} ($\log K_{ow} > 5$) leads to an increasing propensity of a chemical to bioaccumulate, as a result of decreasing elimination rates (Gobas et al., 1986; Sijm and Van der Linde, 1995). Additionally, the percentage of lipid tissues is a key factor determining the bioaccumulation of organic pollutants.

For metals, several, distinct “storage” compartments exist: first, metals may bind to low molecular weight proteins, such as phytochelatins in plants and metallothioneins in animals (Klaassen et al., 1999; Hendriks and Heikens, 2001). Second, metals may be sequestered in inclusion bodies as vacuoles and granules (Vijver et al., 2004; Bonneris et al., 2005; Rainbow, 2007). Finally, metals may be incorporated in hard tissues with a support and cover function, such as shell, chitin, bones, feathers and fur (Hendriks and Heikens, 2001). Unlike accumulation kinetics of organic compounds, metal affinity for these “storage” compartments has not been linked to a metal-specific property, although it has been suggested that metal-bioaccumulation may be related to binding of metals to specific chemical

functional groups in the organism (e.g. the metal-binding protein metallothionein) (Hendriks and Heikens, 2001; Paquin et al., 2003).

Biomagnification is a phenomenon that is in particular important for persistent nonpolar organic pollutants. In numerous field studies it has been observed that lipid-based concentrations of very hydrophobic ($\log K_{ow}>5$) nonpolar, organic chemicals increase in a food chain, reaching highest concentrations in top-predators (Thomann and Connolly, 1984; Connolly and Pedersen, 1988; Russell et al., 1999; Hendriks et al., 2001; Hop et al., 2002). There are at least two factors responsible for the biomagnification of persistent organic substances: firstly, there is a decreasing ability to eliminate very hydrophobic chemicals across respiratory surfaces (gill, lung) or excrete these chemicals with urine, as a result of increasing affinity for lipid tissues (Gobas et al., 1986; Sijm and Van der Linde, 1995; Hendriks et al., 2001). And secondly, there is an ability to maintain high dietary diffusion gradients in the gut, as a direct consequence of lipid depletion due to food digestion and the assimilation of digestion by-products (Gobas et al, 1993; Gobas et al., 1999; Barber, 2008).
Although trophic transfer is observed for metals, controversy remains on their biomagnification potential (Beyer, 1986; Reinfelder et al., 1998; Croteau et al., 2005). It is generally assumed that metals do not biomagnify, with exception of some organometallic forms as methylmercury (Reinfelder et al., 1998; Hendriks and Heikens, 2001). Several studies have shown, however, that uptake via food is an important exposure route, even for aquatic organisms (Luoma and Rainbow, 2005; Meyer et al., 2005; Croteau and Luoma, 2008). This implies that predator-prey relationships have to be taken into account when assessing metal bioaccumulation.

The terms “bioconcentration”, “bioaccumulation” and “biomagnification” have distinct meanings and it is therefore important to define these terms. The most commonly accepted definitions are those presented by Gobas and Morrison (2000): “Bioconcentration is the net process by which the chemical concentration in an (aquatic) organism achieves a level exceeding that in water as a result of chemical uptake through respiratory and dermal surfaces from the surrounding water-phase”. The bioconcentration factor (BCF) represents an equilibrium concentration ratio and can be expressed as the chemical concentration in an organism divided by the dissolved chemical concentration in water (Eqn. 1.1). Bioconcentration factors usually apply only to controlled laboratory conditions, in which dietary intake of the chemical is deliberately not included (Arnot and Gobas, 2006). BCFs are less applicable to field data, if other uptake routes, i.e. ingestion of

food or inhalation of air, contribute substantially to the total chemical concentration in an organism.

$$BCF_{i,x} = \frac{C_{i,x}}{C_{0w,x}}$$ Equation 1.1

$C_{i,x}$ = chemical (x) concentration in biota (i) in [$\mu g \cdot kg^{-1}$ lipid wt] for organic chemicals or [$\mu g \cdot kg^{-1}$ dry weight] for metals

$C_{0w,x}$ = total dissolved chemical concentration in water in [$\mu g \cdot L^{-1}$]

Bioaccumulation is defined as "the net process by which the chemical concentration in an organism achieves a level exceeding that in water or organic solids as a result of chemical uptake through all possible routes of exposure (inhalation, absorption, ingestion and dermal diffusion) from all possible sources (water, sediment, soil, food and air) and elimination via all possible routes" (Gobas and Morrison, 2000). The potential of a chemical to bioaccumulate can be expressed as the bioaccumulation factor (BSAF or BAF), i.e the concentration in an organism divided by the exposure concentration in an abiotic medium (soil, sediment, suspended solids or dissolved in water) (Eqn. 1.2).

$$BAF_{i,x} = \frac{C_{i,x}}{C_{0s(w),x}}$$ Equation 1.2

$BAF_{i,x}$ = Bioaccumulation factor in [$\mu g \cdot kg^{-1}$ lipid wt./ $\mu g \cdot kg^{-1}$ organic dry wt] for organic compounds or [$\mu g \cdot kg^{-1}$ dry wt / $\mu g \cdot kg^{-1}$ organic dry wt] for metals

$C_{0s(w),x}$ = Chemical concentration in suspended solids, sediment or soils (s) in [$\mu g \cdot kg^{-1}$ organic dry weight] for organic compounds and [$\mu g \cdot kg^{-1}$ dry weight] for metals or dissolved chemical concentration in water (w) in [$\mu g \cdot L^{-1}$] for both organic compounds and metals

Generally, the bioaccumulation factor represents a non-equilibrium ratio between the chemical concentration in biota and the chemical concentration in the abiotic medium. Field BAFs of organic substances that are known to biomagnify in food webs, can be up to almost 2 orders of magnitude greater than the BCFs from laboratory experiments that do not include dietary exposure (Arnot and Gobas, 2006).

For organic chemicals, it is important to express BCFs and BAFs using lipid-normalized concentrations in tissue, the organic carbon normalized concentration

in sediment or soils, and the dissolved chemical concentration in water, as these expressions facilitate comparison of accumulation ratios across ecosystems. As metals generally accumulate in dry tissues of organisms, the chemical concentration in biota can be expressed on a dry tissue basis. The metal concentration in suspended solids and sediments is corrected for the organic dry weight percentage of the soils. Similar to organic compounds the total dissolved metal concentration in water is often assumed to be bioavailable (Hendriks and Heikens, 2001).

Biomagnification is the process by which the chemical concentration in an organism achieves a level exceeding that in the organism's diet through dietary uptake and retention (Eqn. 1.3). The test for food-chain biomagnification is an increase in the concentration on a lipid weight basis, not on a wet weight basis. Although this definition of biomagnification is useful, it is not very practical when comparing modeled BMFs (BioMagnification Factor) to field BMFs, as in reality organisms take up chemicals from all possible routes of exposure. Therefore, in the present study modeled and measured BMFs represent the concentration in biota divided by the concentration in food, as a result of uptake from all possible exposure pathways and elimination via all possible pathways of loss. Biomagnification factors for metals are seldom derived, but these may be expressed on a dry weight basis.

$$BMF_{i,x} = \frac{C_{i,x}}{C_{i-1,x}} \qquad \text{Equation 1.3}$$

$BMF_{i,x}$ = biomagnification factor in [$\mu g \cdot kg^{-1}$ lipid weight / $\mu g \cdot kg^{-1}$ lipid weight] for organic compounds

$C_{i,x}$ = concentration in biota (i) [$\mu g \cdot kg^{-1}$ lipid weight]

$C_{i-1,x}$ = concentration in food (i-1) [$\mu g \cdot kg^{-1}$ lipid weight]

For the quantification of bioaccumulation, it is of utmost importance to understand the species-specific and chemical-specific, uptake and loss processes causing concentration amplification. The complexity of bioaccumulation kinetics and our lack of data and knowledge limit our ability to quantify all processes involved in the uptake and elimination of chemicals. Also, including all processes would yield a model that is too complex to understand and requires too many (unknown) input parameters. Therefore, the approach to model development is to simplify chemical bioaccumulation kinetics as much as possible and include only key processes and parameters that govern bioaccumulation. In the next section a (more) qualitative

description of these key environmental, physiological and chemical parameters is provided for both nonpolar, organic pollutants and metals.

1.3 Exposure media

1.3.1 Relevant exposure media

Obviously, the exposure concentration is an important parameter determining the total amount of chemical uptake by an organism. A bioaccumulation assessment often begins with determining the concentration of contaminants in relevant exposure media, i.e. air, soil or sediment, (pore) water and food. The exposure media that should be taken into account depend on species-characteristics as whether an animal respires oxygen from air or water, the animal's habitat and habitat use and feeding preferences.

Feeding relationships play a crucial role in chemical exposure of organisms (Connolly and Pedersen, 1988; Russell et al., 1999). A practical way to conceptualize predator-prey relationships and energy flow within an ecosystem is to organize ecosystems into trophic levels. Several trophic levels can be distinguished depending on the ecosystem studied: primary producers, detritivores, herbivores, primary carnivores and secondary carnivores. One should realize that although some organisms are specialized feeders, many species are omnivorous opportunists because of fluctuations in food availability. Feeding preferences may change during the year because of prey availability and migration, possibly resulting in varying exposure concentrations. Often such detailed information on predator-prey relationships is not available for the ecosystem of interest.

1.3.2 Bioavailability

Not all the contaminants present in abiotic exposure media are available for uptake by organisms. For example, organic compounds present in water may be sequestered in sorbed form in dissolved and particulate organic matter. It is assumed that this sorbed chemical fraction is not bioavailable for aquatic organisms, as the chemical complexes formed are too large to permeate biological membranes (Gobas, 1993). Only the truly dissolved chemical fraction is assumed to be available for uptake by aquatic organisms. Similar phenomena exist in sediments and soils, and in the atmosphere where chemicals can be sorbed to aerosols.

Sorption of nonpolar organic contaminants to sediments, suspended particles and soils is generally considered to follow a linear equilibrium partitioning process between the octanol-equivalent fraction in solids and the dissolved concentration in (pore) water. This dissolved chemical concentration can be estimated as a

function of the substance K_{ow} and the organic carbon fraction in soil or sediment (Karickhoff, 1979; Sabljic et al., 1995).

In the abiotic environment metals occur in several forms and species. In the solid phase metals exists as precipitates, absorbed on reactive soil surfaces and occluded or bound into soil minerals (Allen, 1993; Peijnenburg et al., 1997). Metals in the (pore) water-phase can exist as free metal ion or as species bound to organic and inorganic matrices, as clay minerals, aluminium hydroxide and silicates (Allen, 1993). Metal speciation is a function of environmental conditions such as pH and the organic matter content of soil (Sauvé et al., 2001) and the acid volatile sulphide (AVS) content of sediments (Allen, 1993).
It is well established that the speciation of a metal has an important impact on its uptake in biological systems (Campbell, 1995; Vijver et al., 2007; van der Welle et al., 2007). It is generally assumed that the "free" metal cation (Me^{+}) is bioavailable (Luoma, 1983; Allen, 1993; Paquin et al., 2003). However, no consensus exists on the possible availability of certain other (dissolved) metal-complexes in addition to the free metal ion. For example, Chuang and Wang (2006) showed that several dissolved metal-complexes are available for uptake by bivalves as well, although uptake rate constants likely vary between the different complexes.

The bioavailable chemical concentration in food is largely determined by the species-specific food assimilation efficiency. These food assimilation efficiencies vary in relation to dietary composition. Efficiency is less in low digestible diets that are low in fat and protein content and high in fiber content (Fisk et al., 1998). Lipids are assimilated more efficiently from the gut than proteins and co-transport of nonpolar organic compounds would lead to a more efficient chemical uptake in high-lipid diets. Generally, detritivores, herbivores and carnivores digest about 20, 40 and 80% of the ingested food, respectively (Hendriks, 1999). The metal assimilation efficiency of the predator is affected by the metal biochemistry in prey / food and the physiology of the predator (Luoma, 1983). Metal subcellular partitioning in prey plays a major role in trophic transfer of metals to predators. Recent studies suggest that subcellular distribution of metals may influence metal bioavailability, with cytosolic metals tending to be highly bioavailable (Wallace et al., 2003; Wang and Rainbow, 2006), whereas metals sequestered in granules are possibly less bioavailable (Nott and Nicolaideau, 1990). Generally, metal assimilation efficiency is low compared to the assimilation efficiency of organic chemicals, and depends on several conditions, such as pH in the gastro-intestinal tract, metal speciation and available biological ligands in food, and stability of these

metal complexes in the food matrix (Luoma, 1983; Zalups and Ahmad, 2003; Andersen et al., 2004).

1.4 Uptake and elimination kinetics

1.4.1 Mechanisms of uptake and loss

The densities of organisms and their food are determined by metabolic flows at rate constants for absorption and excretion of water, ingestion of food and egestion of feces, (re)production, respiration and mortality of tissues (Hendriks et al., 2001). Each of these metabolic flows may carry a chemical contaminant into and out of the organism. Apart from dermal passage, three possible uptake mechanisms of a chemical contaminant by an organism exist (Hendriks et al., 2001):

1. Inhalation of air
2. Absorption of water (including drinking, ventilation and filtration)
3. Ingestion of food

And six loss mechanisms exist (Hendriks et al., 2001):

1. Exhalation of air
2. Excretion with urine and elimination via ventilation and filtration
3. Egestion with feces
4. Biotransformation
5. Production (animal growth) and reproductive loss (birth, egg laying, lactation)
6. Death of tissues (cells, feathers, skin etc.)

In order for a chemical contaminant to reach storage tissues within an organism, the chemical must be delivered from the exposure medium to the absorbing epithelium (e.g the gill, the lung, or gastro-intestinal tract), move through several diffusion layers (a.o. mucus, lipid membrane, unstirred water layer) into the blood and be transported with blood to a “storage” compartment. Chemicals that are “stored” are not easily eliminated via excretion with urine, egestion with feces or exhalation with air. Hence, these chemicals can only be removed via growth dilution (including animal growth and reproduction) and loss of dead tissues.

1.4.2 Metabolic rates

Metabolic rates play a key role in toxicant accumulation kinetics as they largely determine the quantity of chemical uptake and elimination. These metabolic rates generally decrease with increasing species weight and can be predicted following so-called allometric relationships (Peters, 1987; West et al., 1997; Hendriks, 1999; Hendriks, 2007). The classic allometric scaling relationship relating metabolic rate

(B) to adult body mass (w) (Eqn. 1.4) was formulated first for mammals and birds by Kleiber in the 1930s (Kleiber, 1932).

$$B = B_0 \cdot w^{-1/4}$$ Equation 1.4

B	=	Metabolic rate (e.g., respiration, food ingestion, ventilation)	[$kg \cdot kg^{-1} \cdot d^{-1}$]
B_0	=	Coefficient	[$kg^{1/4} \cdot d^{-1}$]
w	=	Adult mass	[kg]

For each metabolic rate, the value of B_0 varies among broad taxonomic or functional groups (warm-blooded vs.cold-blooded; air-breathing vs. water-ventilating). The value of the scaling exponent is, however, invariably close to -¼ (Peters, 1987; West et al., 1997; Hendriks, 2007). This implies that generic allometric relationships can be derived for each metabolic rate by categorization of organisms in functional groups and setting the scaling exponent value at -0.25 (Figure 1.1) (Hendriks, 2007). These allometric relationships exist for ingestion rates, ventilation rates, filtration rates, production rates and respiration rates, suggesting that adult mass of species is a key parameter in explaining differences in chemical uptake and elimination rates between species.

Two important variations in metabolic rates are identified in Figure 1.1. Firstly, water fluxes (ventilation rates, filtration rates, absorption rates) are approximately a factor of 1000 higher in aquatic organisms than in terrestrial animals, as aquatic species respire oxygen from water instead of air, resulting in a large water flux (Hendriks, 1999). For terrestrial organisms water fluxes consists of drinking, excretion of urine and transpiration via the skin. Secondly, basal metabolic rates are highly dependent on body-temperature and generally these rates are higher in warm-blooded species compared to cold-blooded species. Cold-blooded vertebrates (fish, reptiles and amphibians) take on the temperature of their surroundings (Norstrom, 2002). Warm-blooded organisms (mammals, birds) have a high basal metabolic rate to maintain body temperature (Norstrom, 2002). For animals of the same body size, average food consumption of warm-blooded animals is approximately a factor of 10 higher than that of cold-blooded animals (Hendriks, 1999). Field observations have shown that lipid-corrected biomagnification factors of neutral organic compounds are substantially higher in warm-blooded organisms compared to cold-blooded animals living in the Arctic, which is mainly related to higher energy requirements, but also to higher trophic

position and longer life-span of warm-blooded species (Fisk et al., 2001; Hop et al., 2002).

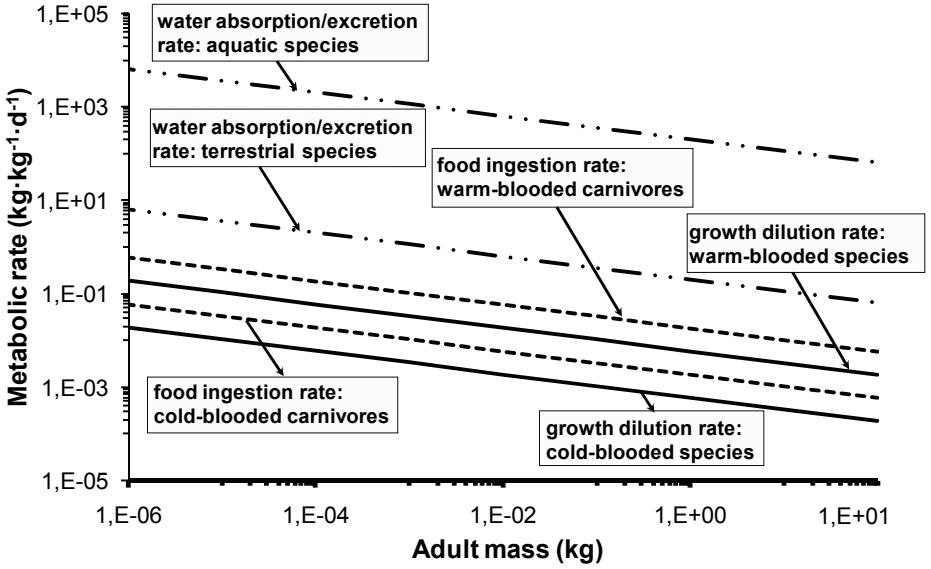

Figure 1.1: Allometric relationships for various metabolic rates based on data from Hendriks (1999) and Hendriks (2007)

1.4.3 Transport across biological barriers

Regardless of the exposure route, a chemical contaminant must cross a biological barrier, consisting of a membrane and an aqueous diffusion layer, before entering the systemic circulation. Membranes consist of lipid layers with proteins embedded in them. It is generally assumed that nonpolar organic compounds are taken up from the exposure medium through the lipid membrane following passive diffusion along a concentration gradient (McKim et al., 1985; Gobas et al., 1986; Gobas and MacKay, 1987; Hendriks et al., 2001; Barber, 2003). The diffusion through the lipid layer membrane and the unstirred aqueous layer can be predicted according Fick's law of diffusion, as a function of the exchange surface area (gill, lung, skin), the thickness of the diffusion layers and the chemical diffusivity in the water- and lipid-phase (McKim et al., 1985; Gobas et al., 1986; Gobas and MacKay, 1987). The rate of transport across the lipid membrane is related to the chemical's octanol-water partition coefficient (DeVito, 2000). For nonpolar organic compounds, membrane transport may be hindered if the substance is poorly soluble in octanol (lipid), resulting in slow transport over the membrane (Gobas et al., 1986). Additionally, some researchers have suggested restricted uptake due to steric hindrance, such as

a large molecular size (i.e. > 9.5 Å) (Opperhuizen et al., 1985; Muir et al., 1985; Opperhuizen and Sijm, 1990).

The exact mechanism of contaminant transfer across the gut membrane is not yet known. It is believed to occur as a thermodynamically driven molecular diffusion process, similar to transport across gill and lung epithelia. The diffusion gradient is a direct consequence of lipid depletion in the gut due to food digestion and the assimilation of digestion by-products (Gobas et al. 1993; Gobas et al., 1998; Barber, 2008). However, other alternative modes of diffusion have been proposed, which include micelle-mediated and fat-flush diffusion (Kelly et al., 2004).

Metals can cross lipid membranes following passive diffusion, facilitated diffusion via membrane transporter proteins, active transport or endocytosis (Simkiss and Taylor, 1995). Passive diffusion across membranes is only possible for neutral, lipid-soluble metal species as organotins, methylmercury and tetraethyllead. Most metal-ions are extremely hydrophilic and are taken up via three types of membrane transport proteins, called channels or pumps if they span the whole-membrane and carriers if they shuttle between two sites (Hendriks and Heikens, 2001). These transport mechanisms are designed for essential nutrients as sodium, potassium, calcium, zinc and copper. Non-essential metals as cadmium, mercury and lead can also adventitiously enter by ionic mimicry of essential analogues. Membrane transport proteins may base their selectivity on metal charge and size and on coordination- and ligand-preferences (Bell et al., 2002; Handy and Eddy, 2004).

The uptake of metals largely depends on the presence of transport systems that provide biological gateways for the metal to cross the membrane. This implies that at high external metal concentrations, availability of these carriers may become more limited and uptake rate constants will decrease as a function of exposure concentrations (Hendriks and Heikens, 2001).

Additionally, both the amount and affinity of transport proteins is not constant and can be regulated by organisms, in particular for the uptake of essential nutrients (Schlekat et al., 2007). Some animals may activate physiologically-based feedback mechanisms that result in changes in the affinity of transport proteins or the relative number of particular proteins available for uptake within a specific membrane system (Niyogi and Wood, 2003; Schlekat et al., 2007).

1.4.4 Accumulation

The primary storage compartment for nonpolar organic compounds is fatty tissues. The affinity for lipid tissues is generally considered to be linearly related to the octanol-water partition coefficient (K_{ow}) of the chemical. It is often assumed that

the accumulation of organic compounds can be characterized solely by the K_{ow} and the lipid fraction ($p_{CH2,i}$) of the organism. This is generally a good approximation, because lipid is the organism component with the highest affinity for nonpolar organic chemicals. However, for lean organisms and lean tissues, such as muscle, kidney and liver, proteins and other non-lipid components, contribute substantially to chemical partitioning (Bertelsen, 1998; Hendriks et al., 2005; deBruyn and Gobas, 2007). For a reliable estimation of chemical accumulation in organisms and tissues with low lipid content, it is important to consider partitioning to non-lipid constituents (Hendriks et al., 2005; deBruyn and Gobas, 2007)

Two major accumulation compartments for metals are (1) binding of metals to proteins as metallothioneins (MT) and / or (2) incorporating metals in non-soluble physico-chemical forms, as granules (Hendriks and Heikens, 2001; Vijver et al., 2004; Rainbow, 2007). Metallothioneins are low molecular weight, cysteine rich proteins with strong affinity for group IB and IIB transition metals, among which Zn^{2+}, Cd^{2+}, Cu^{+}, Pb^{2+}, Hg^{2+}, and Ag^{+} (Miles et al., 2000). These proteins are found throughout the animal kingdom, in higher plants, in eukaryotic microorganisms, and in many prokaryotes (Klaassen et al., 1999). These metallothioneins tend to be concentrated in the liver (or equivalent organs in invertebrates), kidney, gills and intestines of an organism (Paquin et al., 2003). Granules have been identified in various species as isopods, earthworms, and various (aquatic) crustaceans, and are used to incorporate several metals as Zn, Cd, Cu, Hg, Ag and Fe (Vijver et al., 2004; Rainbow, 2007). Both binding to MT and inclusion in granules is related to the metal-detoxification capacity of the organism. This is an active process regulated by the organism, resulting in species- and metal-specific accumulation. Metals may also be bound in soft tissues with high molecular weight proteins and polysaccharids or incorporated in hard tissues with a support and cover function, such as shells, chitin, bones, feathers and fur (Hendriks and Heikens, 2001).

1.4.5 Biotransformation

Biotransformation is an enzyme-mediated conversion of the (lipophilic) parent compound into a usually less toxic and a more hydrophilic metabolite. As a result of the lower hydrophobicity the metabolite is generally more easily eliminated than the parent compound, and total elimination rates increase (Van der Linde et al., 2001). Biotransformation is dependent on both the chemical structure and the xenobiotic transformation capacity of the organism. For organic compounds biotransformation is typically mediated via cytochrome P450 monooxygenase. This diverse class of enzymes exhibits broad but overlapping substrate and product

specificities that may be species- and gender specific (Parkinson, 2001). Compared with mammals, fish have a low biotransformation capacity, ostensibly because they can eliminate xenobiotics unchanged via the gills (Parkinson, 2001; Van der Linde et al., 2001).

The faster the rate of parent biotransformation, the less likely it is that the chemical will bioaccumulate, with the influence of biotransformation on the overall elimination and BAF-value more pronounced for hydrophobic chemicals (de Wolf et al., 1992). Metals are infinitely persistent and therefore not susceptible to biotransformation. However, metals can be converted into different forms, which may affect their elimination kinetics.

1.5 Bioaccumulation assessment methods

The last decade there have been numerous incentives to predict bioaccumulation of nonpolar organic chemicals and metals. At present, there are two approaches for quantifying bioaccumulation phenomena:

1) empirical regression-based approach, in which bioaccumulation factors, derived from lab or field studies, are related to key chemical properties as the K_{ow} for organic pollutants
2) mass-balance model approach, in which chemical accumulation is quantified based on concentrations in exposure media and a set of first-order uptake and elimination rate constants. These rate constants are either "empirical" rate constants, i.e. based on chemical- and species-specific measurements, or "mechanistic" rate constants, i.e. estimated based on chemical specific properties and physiological processes.

1.5.1 Empirical regressions

The earliest approaches to quantify bioaccumulation of organic pollutants were developed in the 1970s when Neely et al. (1974) showed that the logarithm of bioconcentration factors (logBCF) of organic chemicals in rainbow trout is approximately linearly related to the $\log K_{ow}$ of the compound. This relationship is of the general form:

$$logBCF = a \cdot logK_{OW} + b \qquad \text{Equation 1.5}$$

where a and b are empirical parameters obtained by regression analysis of a K_{ow}-BCF dataset for a range of chemicals and one organism. Since then many $\log K_{ow}$-

logBCF-regressions have been developed for different sets of nonpolar, organic chemicals and various species, including fish (Veith et al., 1979; Veith et al., 1980; Mackay, 1982; Oliver and Niimi, 1983; Isnard and Lambert, 1988; Meylan et al., 1999) and other aquatic organisms, as mussels, algae and crustaceans (Geyer et al., 1991; Arnot and Gobas, 2006). Additionally, $logK_{ow}$-logBCF-regressions have been developed for some terrestrial species, as earthworms, assuming that pore-water mediated dermal uptake is the predominant exposure route (Van Gestel and Ma, 1988; Jager, 1998).

There are two important assumptions made in the development and use of these regressions. Firstly, it is assumed that bioconcentration is a thermodynamically driven partitioning process between water and the lipid phase of the exposed organism with no physiological barriers to impede accumulation (Barber, 2003). Secondly, it is implicitly assumed that only one route of exposure dominates an organisms total accumulation process. For example, it is assumed that fish accumulate virtually all their body burdens directly from water via the gill, or possibly, dermal exchange (Barber, 2008).

Deviations from the $logK_{ow}$-logBCF-relationship have been observed, in particular for extremely hydrophobic chemicals ($logK_{ow}>5$). Thomann and Connolly (1984) observed that the use of a lipid-based bioconcentration factor (BCF) underestimated PCB concentrations in the top-predator lake trout in Lake Michigan. They attributed this underestimation to a substantial contribution of dietary uptake to total chemical accumulation for chemicals with a $logK_{ow}>5$. Consequently, for carnivorous fish species uptake via ingestion of food should be incorporated in regression-models. In addition, for these extremely hydrophobic chemicals other non-thermodynamically driven elimination processes, like growth dilution, become increasingly important (Hendriks et al., 2001). Also for algae, for which exchange kinetics are fast, a non-linear $logK_{ow}$-logBCF relationship is found for $logK_{ow} > 6$ (Koelmans and Heugens, 1998). This is due to binding to extracellular carbon (Koelmans and Heugens, 1998).

At present, BAF-regression models for higher trophic level aquatic species are sparsely available (Arnot and Gobas, 2003). This is because bioaccumulation factors are subject to a large number of site-specific environmental variables and complex interactions between diverse uptake and elimination rate constants (Arnot and Gobas, 2003).

Metal bioaccumulation kinetics have not been linked to an intrinsic, metal-specific property, and similar regression-approaches are therefore absent for metals. BCFs of metals in aquatic organisms have, however, been linked to exposure concentrations in water (McGeer et al., 2003). However, this approach does not

recognize the complex dynamics of metal uptake, internal sequestration, storage, active elimination and nutrient essentiality or the potential for adverse effects (McGeer et al., 2003). Metal-specific BCFs are often highly variable between organisms and generally inversely related to exposure concentrations (McGeer et al., 2003; DeForest et al., 2007). These regressions are case-specific and cannot be extrapolated to other areas, conditions and contaminant levels.

1.5.2 Mass-balance bioaccumulation models

Mass-balance models originate from the 1980s and have traditionally been developed to predict accumulation of nonpolar organic pollutants in aquatic organisms (Neely, 1979; Spacie and Hamelink, 1982; Thomann and Connolly, 1984; Thomann, 1989; Gobas and Mackay, 1987). These early mass-balance models have undergone substantial development and evaluation in the last two decades, yet, the basic framework has remained similar. In these models, the organism is conceptualized as consisting of one-compartment that contains a number of homogeneously mixed phases, as lipid tissues, water and proteins. An organism can take up chemicals via absorption of water, ingestion of food and inhalation of air, and eliminate these chemicals via excretion with urine, egestion with feces, exhalation with air, growth dilution and possibly biotransformation. Each of these uptake and elimination processes is described by first-order kinetics i.e. uptake ($k_{x,j,in}$) and elimination rate constants ($k_{x,j,ex}$) are independent of time and independent of exposure concentrations. Chemical equilibration between the internal lipid-phase, aqueous-phase and protein phase is assumed to be rapid in comparison to exchange across the gills, the lungs and the gastro-intestinal tract. This assumption allows elimination rate constants to depend on chemical whole-body concentrations. A generic formula to describe the chemical concentration in an organism is:

$$\frac{\partial C_{x,i}}{\partial t} = k_{x,w,in} \cdot C_{x,w} + k_{x,n,in} \cdot C_{x,i-1} + k_{x,a,in} \cdot C_{x,a} - \left(k_{x,w,ex} + k_{x,n,ex} + k_{x,a,ex} + k_g + k_{x,m}\right) \cdot C_{x,i}$$

Equation 1.6

$C_{x,i}$	=	Internal chemical concentration	[$\mu g \cdot kg^{-1}_{wet\ weight}$]
$C_{x,w}$	=	Dissolved chemical concentration in water	[$\mu g \cdot L^{-1}$]
$C_{x,i-1}$	=	Chemical concentration in food	[$\mu g \cdot kg^{-1}_{wet\ weight}$]
$C_{x,a}$	=	Chemical concentration in air	[$\mu g \cdot dm^{-3}$]
$k_{x,w,in}$	=	Chemical absorption rate constant	[$L \cdot kg^{-1} \cdot d^{-1}$]

$k_{x,n,in}$	=	Chemical ingestion rate constant	[$kg \cdot kg^{-1} \cdot d^{-1}$]
$k_{x,a,in}$	=	Chemical inhalation rate constant	[$dm^3 \cdot kg^{-1} \cdot d^{-1}$]
$k_{x,w,ex}$	=	Chemical excretion rate constant	[d^{-1}]
$k_{x,n,ex}$	=	Chemical egestion rate constant	[d^{-1}]
$k_{x,a,ex}$	=	Chemical exhalation rate constant	[d^{-1}]
k_g	=	Growth dilution rate constant	[d^{-1}]
$k_{x,m}$	=	Chemical biotransformation rate	[d^{-1}]

Mass-balance models are often applied in steady-state, although they can provide a time-dependent concentration change as a function of organism growth or diet change. The steady-state chemical concentration in an organism can be estimated as:

$$C_{x,i} = \frac{k_{x,w,in} \cdot C_{x,w} + k_{x,n,in} \cdot C_{x,i-1} + k_{x,a,in} \cdot C_{x,a}}{k_{x,w,ex} + k_{x,n,ex} + k_{x,a,ex} + k_g + k_{x,m}} \qquad \text{Equation 1.7}$$

At present, models of various levels of complexity exist: from “simple” one-compartment models that are either organism-specific or apply to entire food chains (Hendriks et al., 2001; Kelly and Gobas, 2003), or even foodwebs of increasing complexity in which dietary interactions are described in matrix form (Campfens and Mackay, 2000; Arnot and Gobas, 2004).

The strength of the mass-balance model approach is that it applies to a wide range of species and various chemicals, including metals (Thomann et al., 1995; Hendriks and Heikens, 2001; Luoma and Rainbow, 2005). Another advantage of mass-balance bioaccumulation models is that uptake from the dissolved phase and from the diet are modeled as distinct processes, and the relative importance of these pathways can be assessed. Additionally, mass-balance models can explicitly address other important elimination pathways as growth dilution and biotransformation.
Problems remain, however, in parameterization of the uptake and elimination rate constants. The challenge is to predict uptake and elimination rate constants based on physiological characteristics of species and chemical specific properties, instead of using empirical rate constants that are measured for each species and each chemical. The advantage of this mechanistic approach is that the model can be generically applied to various chemicals and a wide range of species.

In the first mass-balance models (Neely, 1979; MacKay and Hughes, 1984) uptake and elimination rate constants of organochlorines in fish were empirically

calibrated and based solely on physico-chemical partitioning coefficients. These estimations of accumulation kinetics were gradually improved as a more mechanistic understanding of gill exchange (Gobas et al., 1986; Gobas and MacKay, 1987; McKim et al., 1985; Barber et al., 1988; Erickson and McKim, 1990; Hayton and Barron, 1990; Sijm and van der Linde, 1995) and dietary uptake was obtained (Gobas et al., 1993; Gobas et al., 1999). Nowadays, a commonly used approach to quantify chemical exchange for fish species, is to consider the uptake and elimination rate constants to be a function of the transport-efficiency through the two diffusion layers and a flow delay imposed by the ventilation rate or food ingestion rate (Gobas and Mackay, 1987; Gobas et al., 1993; Gobas et al., 1999; Arnot and Gobas, 2004; Hendriks et al., 2001). The transport efficiency is generally assumed to depend on the chemical K_{ow} (Gobas et al., 1986; Gobas and MacKay, 1987; McKim et al., 1985; Barber et al., 1988; Erickson and McKim, 1990; Hayton and Barron, 1990; Sijm and van der Linde, 1995). Values for the transfer coefficients are often obtained by calibration on species-specific experimental data (Mackay and Gobas, 1987; Gobas et al., 1993; Gobas et al., 1999). Some researchers have suggested an additional flow delay imposed by perfusion (Hayton and Barron, 1990; Erickson and McKim, 1990). Others assume that the role of blood flow in regulating the overall chemical uptake rate through gill ventilation is insignificant (Gobas and Morrison, 2000; Arnot and Gobas, 2004).

The elimination rate constant also depends on the chemical accumulation in different body compartments, as lipids, proteins and water (Gobas and MacKay, 1987; Sijm and van der Linde, 1995; Hendriks et al., 2001; Arnot and Gobas, 2004). The affinity for these body compartments is estimated as a function of the K_{ow}. Two additional elimination rate constants are generally included in mechanistic bioaccumulation models: growth dilution and biotransformation. Growth dilution is a pseudo-elimination pathway as internal chemical concentrations are lowered due to animal growth. There is however no nett loss of chemical mass. Growth rate constants can be based on approximations of the temperature-dependent growth rate of aquatic organisms (Arnot and Gobas, 2004) or on generic allometric and temperature relationships for growth (Hendriks et al., 2001). At present, generic methods to estimate biotransformation rates based on chemical properties and / or species characteristics are not available. As the model is often applied to non-metabolizable substances as PCBs, the biotransformation rate constant can assumed to be zero.

This approach has been successfully extended to estimate chemical accumulation in other aquatic organisms as crustaceans (Arnot and Gobas, 2004), to quantify dietary exchange of chemicals by mammals (Czub and MacLachlan, 2004; Czub and

MacLachlan, 2007; Armitage and Gobas, 2007) and inhalatory exchange of chemicals by air-breathing organisms (Cahill et al., 2003; Kelly and Gobas, 2003).

The major advantage of these mechanistic bioaccumulation models is their relative simplicity and the possibility to apply them consistently to a wide range of chemicals, as accumulation kinetics are related to the chemical's octanol-water partition coefficient. Yet, parameterization of most of these models is species-specific and therefore these models are not easily scaled from one animal species to another (but see exception in Section 1.6). Chemical risk assessment can benefit from a generic model that is applicable to a wide range of organisms and various chemicals without increasing model complexity or input-parameter requirements. Hence, the parameter values should only change as a result of physiological characteristics of the organism and physico-chemical properties of the substance.

Although mechanistic, mass-balance bioaccumulation models are common for organic chemicals, these models are largely absent for metals (Paquin et al., 2003). This is due to the complex environmental behaviour of metals. Metal bioaccumulation is highly variable depending on the species, metals and ecosystems studied (Luoma, 1983; van Straalen and van Wensem, 1986; Hendriks et al., 1995; Luoma and Rainbow, 2005). Geochemical conditions such as pH, organic matter and clay content determine the availability for uptake by organisms, whereas physiological processes, such as uptake and elimination kinetics, are highly species-specific and may be regulated by the organism (White and Rainbow, 1982; Bury and Wood, 1999; Lock and Janssen, 2001; Rainbow, 2002; Vijver et al., 2004; Schlekat et al., 2007). Additionally, while absorption and elimination rate constants of organic compounds have for long been linked to their octanol-water partition coefficient, no equivalent quantitative relationships exist for metals. It is, however, widely recognized that mass-balance models are a valuable tool in explaining differences in accumulation kinetics across metals, species and ecosystems (Thomann et al., 1995; Hendriks and Heikens, 2001; Luoma and Rainbow, 2005; Croteau and Luoma, 2008).

1.6 OMEGA (Optimal Modeling for Ecotoxicological Applications)

The mechanistic bioaccumulation model OMEGA (Optimal Modeling for Ecotoxicological Applications) has been developed as an additional tool for risk assessment purposes (Hendriks et al., 2001; Hendriks and Heikens, 2001). The main objective of the model is to explain and predict differences in accumumulation between substances (hydrophilic vs. hydrophobic and organic chemicals vs. metals) and species (aquatic vs. terrestrial species, low vs. high trophic level, warm-blooded vs. cold-blooded) (Hendriks et al., 2001). Here, a brief description of basic model

principles and assumptions is provided. A more detailed description of model equations can be found in the following chapters and corresponding appendices.

Similar to traditional mass-balance bioaccumulation models, the steady-state chemical concentration in an organism is estimated as the total uptake via water and food divided by the total elimination via excretion, egestion, growth dilution and biotransformation (Eqn. 1.7). All important contaminant fluxes are illustrated in Figure 1.2. Here, the term "excretion" is used to describe all elimination pathways via water fluxes, including excretion with urine, evapotranspiration via dermal surfaces and loss via gill ventilation.

The unique characteristics of the model are the combination of classical thermodynamic diffusion theory with biological allometry to predict chemical accumulation in biota. Internal chemical concentrations are estimated as a function of well-known properties of chemicals and species, such as the octanol-water partition coefficient (K_{ow}) of the chemical, and the adult mass (w), body composition and trophic level of the species (Hendriks et al., 2001; Hendriks and Heikens, 2001). The advantage of this approach is that data obtained from well-investigated substances and species can be applied to estimate the behaviour of new chemicals or bioaccumulation in (largely) unknown plants and animals, assuming that biochemical transport principles are universal.

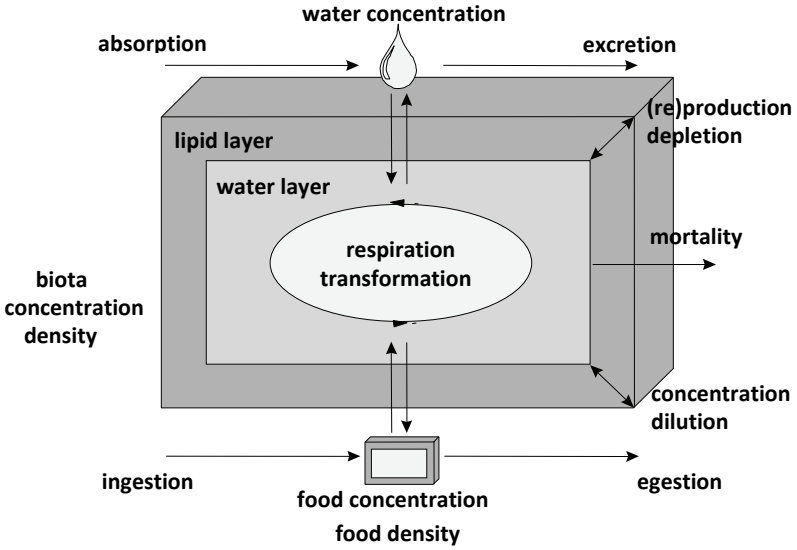

Figure 1.2. Contaminant fluxes are determined by metabolic flows at rate constants for absorption and excretion of water, ingestion and egestion of food / feces, (re)production, respiration and mortality of mass. Additionally, these contaminant fluxes are a function of

the lipid and water-layer resistance encountered during passage of absorbing epithelia (lung, gill, gastro-intestinal tract)

1.6.1 Quantifying accumulation kinetics of nonpolar, organic chemicals

Chemical exchange is limited by delays imposed by flows of water, food and biomass, and the resistance a chemical encounters in the two diffusion layers, the unstirred aqueous layer and the lipid layer. These flow delays and diffusion resistances are independent and act in series. The chemical uptake rate constant can thus be estimated as the reciprocal of the total transport resistance. Equation 1.8 provides an example for the uptake rate constant from water ($k_{x,w,in}$ in $L \cdot kg^{-1} \cdot d^{-1}$). Chemical uptake rate constants via dietary intake are predicted in a similar way, but these rate constants are additionally dependent on the food assimilation efficiency (AE), as specified in Section 1.3.2.

$$k_{x,w,in} = \frac{1}{\text{Aqueous layer resistance} + \text{Lipid layer resistance} + \text{Flow delay}}$$

Equation 1.8

Elimination via excretion ($k_{x,w,ex}$) and egestion ($k_{x,n,ex}$), is essentially the reverse process from uptake via water ($k_{x,w,in}$) and dietary ingestion ($k_{x,n,in}$), respectively. Elimination rate constants can thus be quantified analogous to uptake rate constants, except that chemicals accumulated in body tissues, such as lipids and proteins, are not easily eliminated. This is expressed by an accumulation ratio (K_{BW}), which reflects the chemical affinity for different components in organisms, such as neutral lipid, polar lipid, protein and lignin. The affinity for body components is a function of the substance K_{ow} and the composition of the species (Hendriks et al., 2005).

$$k_{x,w(n),ex} = \frac{1}{K_{BW}} \cdot k_{x,w(n),in}$$

Equation 1.9

Biotransformation is not explicitly considered in OMEGA, as, at present, these transformation rates can not be predicted based on physiological characteristics and chemical properties. When necessary, empirical biotransformation rates can easily be added to OMEGA.

The delays imposed by flows of water, food and biomass are related to species mass (w) to the power ¼ following allometric relationships (Hendriks et al., 2001; Hendriks, 2007). Additionally, these delays are related to a few, important species characteristics, such as trophic level, warm-blooded vs. cold-blooded, and water-ventilating vs. air-breathing. Trophic level is assumed to determine the species assimilation efficiency (AE) of food (Hendriks et al., 2001). The model generally considers five trophic levels: plants, herbivores, detritivores, primary carnivores and secondary carnivores, with default food assimilation efficiencies of 0%, 20%, 40%, 80% and 80%, respectively (Hendriks et al., 2001). Species can be further categorized in several functional groups: air-breathing vs. water-ventilating, and warm-blooded vs. cold-blooded. The first type determines the magnitude of the delay imposed by water flows. This flow delay is relatively small in water-breathing organisms, as large volumes of water are ventilated over the gills. For air-breathing organisms and plants the flow delay due to water exchange can, however, be substantial (Hendriks et al., 2001).

The second group, i.e. warm-blooded vs. cold-blooded, determines the magnitude of the delay imposed by food and biomass flows. Warm-blooded species need to spend extra energy on temperature regulation and consequently metabolic flows in warm-blooded species are higher compared to those in cold-blooded species (Hendriks et al., 2007). This is accounted for by a temperature correction factor ($q_{T:C}$).

The chemical transport resistance through the unstirred aqueous layer and the lipid layer is a function of both chemical properties and species characteristics. Both the aqueous layer resistance and the lipid layer resistance can be predicted as a function of adult mass, as exchange surfaces, i.e. gills, lungs and intestines, scale to species weight to the power ¼ (West et al., 1997). The thickness of the diffusion layers and the diffusivity in water and lipids is assumed to be independent of mass and relatively similar across various species. The total resistance in aqueous and lipid layers, therefore, scales to species weight to the power ¼. The lipid layer resistance is additionally assumed to be a function of the octanol-water partition coefficient of the chemical (Hendriks et al., 2001).

All coefficients and parameters required in OMEGA have been obtained by calibrating the equations on hundreds of rate constants from laboratory studies applying to species from various taxonomic groups, including algae, vascular plants, molluscs, fish, worms, arthropods, birds and mammals (Hendriks et al., 2001). The model was successfully validated with field data of organochlorine accumulation in species inhabiting the Rhine-Meuse delta, including aquatic and terrestrial

organisms (Hendriks et al., 2001). To evaluate the generic applicability of the model principles, the model should be validated with independent field data of other species, substances (non-organochlorines) and ecosystems (marine and estuarine). Additionally, contaminant exchange via air is not yet incorporated in OMEGA and the model is therefore likely to fail if exposure to air is dominant (Hendriks et al., 2001).

1.6.2. Quantifying accumulation kinetics of metals

Metal bioaccumulation is predicted based on the same model principles as for organic compounds, i.e. influx and efflux rate constants are a function of species characteristics as adult mass, trophic level and body composition and a generic metal-property, the tissue-water partition coefficient (Hendriks and Heikens, 2001). Metal exchange kinetics can be estimated analogously to exchange kinetics for organic pollutants, as a function of the resistance in water and lipid layers and a flow delay. In contrast to neutral organic compounds, metal uptake rate constants depend on the exposure concentration in water and food. Metals are transported through membranes by protein-carriers or protein channels (Bryan, 1984; Foulkes, 2000; Hendriks and Heikens, 2001). At high external concentrations, availability of these carriers may become more limited and uptake rate constants will decrease. Therefore, lipid-layer resistance for influx via absorption and ingestion is defined as a function of the exposure concentration, analogously to Michaelis-Menten kinetics for enzymes (Hendriks and Heikens, 2001).
The absorption rate constant is modeled according equation (Eqn. 1.10):

$$k_{x,w,in} = \frac{1}{\text{Aqueous layer resistance} + \text{Lipid layer resistance} \cdot C_{0w,x}^{kp} + \text{Flow delay}}$$

Equation 1.10

Elimination of metals may occur via three different pathways: excretion with water, egestion with feces, and dilution with biomass. Generally, metals are excreted slowly if they are bound to metal-binding proteins in soft tissues and / or incorporated in hard tissues, such as shells, feathers, and fur (Hendriks and Heikens, 2001). Binding to dry tissues is incorporated in OMEGA as a generic tissue-water distribution coefficient (K_{tw}). This coefficient describes the affinity of metals for dry tissue and is derived from calibration on thousands of metal accumulation ratios from laboratory and field studies (Hendriks and Heikens, 2001). For elimination of

metals, the lipid layer resistances are considered to be independent of the internal body concentration (Hendriks and Heikens, 2001). Additionally, uptake and elimination rate constants are assumed to be metal independent, as empirical rate constants show little metal-specific variation when averaged over different species (Hendriks and Heikens, 2001).

Substance parameters, i.e. the resistances for diffusion through aqueous and lipid-layers, were obtained by fitting rate constants on measured rate constants collected in a literature review. Data on all elements and species were collected but most applied to aquatic species, in particular molluscs and fish (Hendriks and Heikens, 2001). The results showed that measured and estimated rate constants were generally within an order of magnitude (Hendriks and Heikens, 2001). It was suggested that the fit could be improved by selecting relevant species- and element specific parameters (Hendriks and Heikens, 2001).
Additionally, model performance was evaluated with field accumulation data using average solid-water partitioning coefficients (K_{sw}) for Dutch soils. These solid-water partitioning coefficients are metal-specific, but have fixed values and consequently, do not incorporate important soil characteristics as pH and organic matter content that govern the bioavailability of metals. An additional improvement may be obtained by considering metal bioavailability in the abiotic environment.

1.7 Aim of this thesis

Summarizing the literature on bioaccumulation models, it is concluded that various mechanistic bioaccumulation models exist for nonpolar organic compounds. Accumulation kinetics is successfully related to substance properties as the octanol-water partition coefficient (K_{ow}) and to species-characteristics as the lipid content, the gill ventilation rate and the food ingestion rate. These models show good predictability of organochlorine bioaccumulation in aquatic ecosystems. Similar models for other ecosystems, i.e. terrestrial environments, and other substances, i.e. non-organochlorines including metals, are largely absent. Although most models estimate accumulation based on substance properties and species characteristics, key physiological parameters are often obtained by species-specific calibration or parameterization. These models are therefore not easily scaled from one species to another.

The unique characteristics of the bioaccumulation model OMEGA are the combination of the classical mass-balance approach with allometric relationships for metabolic rates, and species characteristics as trophic level, adult mass and body composition. The advantage of this approach is, that the model can be applied to a wide range of species, both aquatic and terrestrial, and various chemicals, including metals, without case-specific calibration. The model has been validated for PCBs and metal accumulation in various species of the Rhine–Meuse delta. To test the generic applicability of the model-concept, the model should be further validated in different ecosystems, such as marine environments, and for different substances, including non-organochlorines. Additionally, a further refinement of the model concept is necessary for metal accumulation kinetics. While uptake and elimination rate constants of organic compounds have for long been linked to their octanol-water partition coefficient, no equivalent relationships exist for metals. A mechanistic estimation of metal accumulation is therefore not yet possible. To advance science of metal bioaccumulation modeling, mechanistic estimation routines need to be developed that relate metal uptake and elimination kinetics to a metal specific property. Finally, chemical inhalation and exhalation rate constants have not been predicted based on allometric relationships and chemical properties. It is therefore useful to extend the model concept to exposure via air and develop quantitative relationships for chemical exchange via lung epithelia in mammals, analogous to the OMEGA-concept for chemical exchange via gill epithelia in aquatic organisms. The advantage for bioaccumulation modeling in general is the applicability of these relationships to a wide range of (terrestrial) organisms and various chemicals, without increasing model-complexity or input-

parameter requirements. Additionally, these relationships provide insight in species-specific differences in chemical uptake and elimination rate constants from air.

The aim of this thesis is to validate and improve the generic applicability of the combined allometry and mass-balance concept, as employed in OMEGA, for a wide range of species, various chemicals and other exposure routes.

This is done by:

1. Model validation
    - validation for non-chlorine organic compounds, such as organobromines and volatile organic compounds; metals and organotins
    - validation for various species, in particular mice, shrews, and estuarine and marine organisms

2. Model development
    - specification and calibration of metal accumulation kinetics via gill epithelia for aquatic organisms, using metal properties and species characteristics
    - specification of organic chemical accumulation kinetics via lung epithelia for various mammals, analogous to the OMEGA-concept for chemical exchange in gills in aquatic organisms

1.8 Outline of this thesis

In Chapter 2 and Chapter 3 the applicability of OMEGA to predict chemical accumulation in estuarine and marine food chains is explored. Chapter 2 focuses on the accumulation of neutral organic compounds, i.e. organochlorines and brominated flame retardants, in various marine species. In Chapter 3 the accumulation of tributyltin and triphenyltin in an estuarine food chain of the Western Scheldt is explored. In Chapter 4 and Chapter 5 the OMEGA model is applied to metal accumulation in terrestrial species inhabiting a polluted floodplain area.

Bioaccumulation kinetics of nonpolar, organic substances have long been linked to their octanol-water partition coefficient, however, metal accumulation has not been quantitatively linked to a metal-specific property, which is necessary for a more mechanistic estimation of uptake and elimination rate constants. In Chapter 6 it is evaluated whether metal absorption and elimination rate constants of aquatic species can be predicted based on species characteristics, such as ventilation rate and weight, and the metal-specific covalent index ($\chi^2_m r$), which is a measure of metal-affinity for sulphur-ligands. Finally, in Chapter 7 a generic modeling approach for contaminant exchange via lung epithelia in mammals is developed, based on chemical-specific properties and species-based allometric scaling, analogous to accumulation kinetics via the gill. The model validity is tested for nonpolar organic chemicals and various mammals, including rats, mice and humans.

Chapter 2.

Accumulation of organochlorines and brominated flame retardants in estuarine and marine food chains: field measurements and model calculations

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2.1 Abstract

Food chain accumulation of organochlorines and brominated flame retardants in estuarine and marine environments is compared to model estimations and fresh water field data. The food chain consists of herbivores, detritivores and primary and secondary carnivores i.e. fish, fish-eating birds and marine mammals. Accumulation of polychlorinated biphenyls is predicted well by OMEGA for herbi-detritivores and primary and secondary carnivorous fish. Ratios are similar to those found for fresh water species. Accumulation ratios for fish-eating birds and mammals are overestimated by the model, which is attributed partly to biotransformation of meta-para unsubstituted congeners. Additionally, birds may feed in other less polluted areas. For brominated diphenylethers (BDE) accumulation patterns are highly species and congener specific. Accumulation depends on both K_{ow} and metabolization capacities. BDE47 is the predominant congener in lower trophic levels. For marine birds and mammals accumulation ratios of BDE99 and 100 are similar to or higher than ratios of persistent PCBs.

2.2 Introduction

The last two decades, many chemicals have been identified as potentially hazardous because of their accumulation in food chains. Yet, most of the thousands of substances and species that are of interest for environmental management will not be monitored at all relevant locations and periods, because of financial, practical and ethical constraints. To allow risk assessment for many substances and species at different locations and periods, results from monitoring programs should be checked for consistency with data from other studies. With this goal in mind, we have developed the model OMEGA that estimates accumulation in food chains (e.g.; Hendriks et al., 2001; Hendriks and Heikens, 2001). OMEGA has been successfully applied to hazardous substances in fresh water and terrestrial communities. In the present investigation, its applicability to estuarine and marine systems is explored, with special emphasis on organochlorines and brominated flame retardants. A related investigation is carried out for organotins (Veltman et al., 2006).

The aim of this study is :

1. to determine the consistency of available estuarine and marine data sets with patterns of the same or related toxicants in other areas and food chains (diagnostic approach)
2. to test the OMEGA model with estuarine and marine data to allow predictions for other areas, periods, substances and species (prognostic approach).

For this purpose empirical data sets from monitoring surveys on estuarine and marine environments in the Netherlands from the last two decades were collected. These data comprise measured chemical concentrations in marine sediments and / or suspended solids as well as chemical residues in various species belonging to several trophic levels. Accumulation factors were calculated for a number of relevant food chains and results were compared to model estimations. Furthermore, the accumulation patterns of polychlorinated biphenyls (PCBs) and brominated diphenylethers (BDEs) were compared. Similar accumulation patterns were expected for these two groups as PCBs and BDEs are structurally related and have similar octanol-water partition coefficients. For polychlorinated biphenyls calculated accumulation factors were compared to results from fresh water studies. Brominated flame retardants have not been monitored extensively in Dutch fresh water environments, so a comparison could not be made for these compounds.

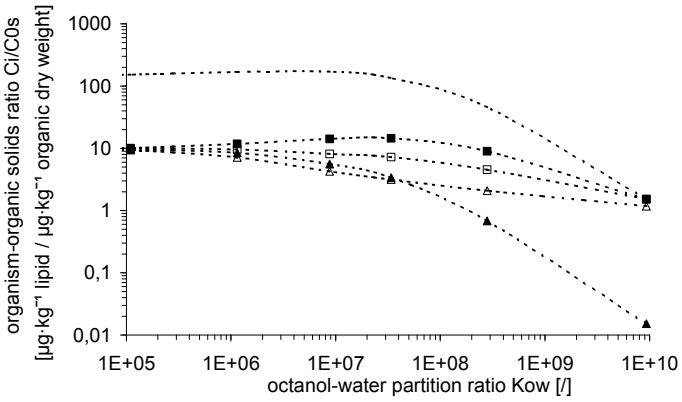
2.3 Methods

2.3.1 Chemical bioaccumulation model for marine food chains

The model OMEGA predicts chemical accumulation in food chains as a function of the chemical octanol-water partition coefficient, and the trophic level and adult mass of the species. A more detailed description of model equations is provided in the introduction and in Hendriks et al. (2001). The marine food chain in OMEGA consists of four species of different trophic levels. Predicted accumulation in the species occupying the trophic levels is presented in Figure 2.1. Figure 2.2 shows biomagnification of persistent organic compounds estimated by OMEGA for secondary carnivores with water ventilation and no water ventilation (air-breathing).

The first trophic level in marine systems are algae (*phycophyta*) and dead organic material. Accumulation in algae is determined by exchange with water. For hydrophobic substances ($10^2<K_{ow}<10^5$) influx from water increases and efflux via water decreases with increasing K_{ow}. For extremely hydrophobic substances

(K_{ow}>10^5), exchange levels off. Resistance in aqueous layers restricts uptake, while growth dilution determines release. As a result, the organism-water concentration ratio (BCF) increases (10^2<K_{ow}<10^5) and levels off as well (K_{ow}>10^5). Biota-suspended solids-accumulation ratios (BSAF) however, decline because sorption to dead organic matter is assumed to be proportional to the dissolved concentration, also for extremely hydrophobic compounds (Hendriks et al., 2001).



▲ herbivores, Δ detritivores, □ 1.carnivorous fish, ■ 2. carnivorous fish,
"dashed curve" 2. carnivorous mammal

Figure 2.1: Model predictions of organism-organic matter C_i/C_{0s} [µg·kg^{-1} lipid weight / µg·kg^{-1} dry weight] bioaccumulation ratios for herbivores, detritivores, primary and secondary carnivores versus octanol-water partition ratios K_{ow} [/].

The second trophic level consists of herbivores and detritivores that feed on algae and dead organic material respectively. In addition to exchange with water, these species are also exposed to contaminated food. For hydrophobic substances (10^2<K_{ow}<10^5) and organisms that live on aqueous oxygen, uptake from water is dominant. The organism-water concentration ratio is similar to that of plants. For extremely hydrophobic substances (K_{ow}>10^5) however, intake from food becomes more important. For these compounds accumulation of detritivores is larger than that of herbivores, as the first species feed on dead organic material. Unlike algae, dead organic material can not eliminate toxicants by growth dilution, which is the major elimination route for extremely hydrophobic chemicals.

The third and fourth trophic level exists of fish as well as marine mammals and birds. Uptake from food is very important for these species. Accumulation factors for carnivores are clearly larger than those for detritivores and herbivores. Differences between primary and secondary carnivorous fish are small, as these

fish are assumed to have similar assimilation efficiencies. Magnification ratios for secondary carnivorous fish are very similar to ratios for primary carnivorous fish. If the secondary carnivore is a bird or marine mammal and taking up oxygen from air, differences in accumulation and magnification ratios are larger (Figure 2.2). These animals accumulate substances mainly from food (Hendriks et al., 2001) and are, unlike fish, not able to eliminate contaminants via water ventilation. Moreover, their assimilation efficiency may be higher than those for fishes. In the present study, a default assimilation efficiency of 80% is used for carnivores. Occasionally, the implication of an assimilation efficiency of 90% is explored for birds and marine mammals. As levels for mammals and birds are equal, only accumulation in mammals is presented (Figure 2.2).

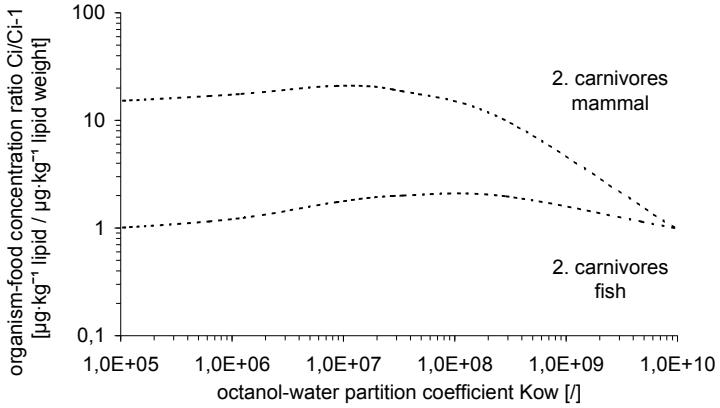


Figure 2.2: Biomagnification ratios C_i/C_{i-1} [µg·kg^{-1} lipid weight / µg·kg^{-1} lipid weight] of secondary carnivorous fish and mammals versus octanol-water partition ratios K_{ow} [/].

2.3.2 Organic solids–water partitioning

Residues of substances in biota are calculated from the concentration in water. However, chemical concentrations are mainly measured in sediment and/or suspended solids. The dissolved chemical concentration in water is predicted from the chemical concentration in organic solids, assuming equilibrium partitioning between the organic carbon-phase and the water-phase (Eqn. 2.1). Neutral, organic chemicals have a strong affinity for the organic carbon (oc)-fraction in solids. Therefore, sorption to solids can be described by the organic-carbon partition coefficient (K_{oc}) and the oc-fraction in solids (Eqn. 2.2). The oc-fraction in marine sediments is approximately half of the organic matter fraction (EC, 2004). The K_{oc} is related to the octanol-water partition coefficient (K_{ow}), as chemicals are assumed to

bind to an apparent (octanol-equivalent) lipid fraction present in organic carbon (Eqn. 2.3) (Hendriks et al., 2001).

$$C_{0w,x} = \frac{C_{0,x} \cdot f_{s\_oa}}{K_{sw}}$$ Equation 2.1

$$K_{sw} = f_{oc} \cdot K_{oc}$$ Equation 2.2

$$K_{oc} = f_{lso} \cdot K_{ow}$$ Equation 2.3

K_{sw}	=	organic solids water partition coefficient [L·kg^{-1}]
K_{oc}	=	organic carbon partition coefficient [L·kg^{-1}]
$C_{ow,x}$	=	dissolved concentration in water [µg·L^{-1}]
$C_{0,x}$	=	concentration in sediment or suspended solids [µg·kg^{-1} organic dry weight]
f_{oc}	=	organic carbon fraction in marine solids (10%) (van der Kooij et al., 1991)
f_{s_oa}	=	organic matter fraction of suspended particles (20%) (van der Kooij et al., 1991)
f_{lso}	=	octanol equivalent lipid of organic weight fraction (18%) (Hendriks et al., 2001)

2.4 Data collection

Data were obtained from monitoring programs carried out by RIKZ and supplemented with other studies to cover various substances, species and areas (Table 2.1). To this end, a literature search was carried out using the Scirus and Picarta publication databases. Additional papers were collected from journals of Elsevier ScienceDirect (Aquatic Toxicology, Chemosphere, Environmental Pollution, Water Research), the American Chemical Society (Environmental Science and Technology, Journal of Agricultural and Food Chemistry), the Society of Environmental Toxicology and Chemistry (Environmental Toxicology and Chemistry) and of Springer Verlag (Archives of Environmental Contamination and Toxicology, Reviews of Environmental Contamination and Toxicology). In addition, several marine journals were consulted (Marine Pollution Bulletin, Journal of Marine Research, Marine Ecology Progress Series, Marine Environmental Research, Journal of Experimental Marine Biology and Ecology, Marine Biology).

The search was carried out with combinations of various keywords. The subject was defined by “accumulation”, “magnification”, “absorption”, “assimilation”, “elimination”, “biotransformation” and / or “food chain”. Organobromines were characterized as “bromo”, “brominated”, “(poly)brominated diphenyl ethers”,

"(P)BDE", "hexabromocyclododecane", "HBCD", "tetrabromobisphenol-A", "TBBP-A". In addition, information on biotransformation of organobromines was searched using "metabolism" and "debromination" as keywords. For organochlorines "PCB" and "(poly)chlorinated biphenyls" were used as keywords. Additional literature on congener specific transformation of PCBs was looked up using "recalcitrant" and "congener specific" in combination with "PCB" and/or "(poly)chlorinated biphenyls" and "biotransformation". Species were searched by the Latin names, "*Nereis diversicolor*", "*Haematopus ostralegus*", "*Buccinum undatum*", "*Sterna hirundo*", "*Phoca vitulina*". For regional data, "North Sea", "Wadden Sea", "Scheldt" were used as locations.

Table 2.1 Data sets (food chains based on available data)

Area	**Organochlorines: PCB, HCB, PCP**	**Brominated flame retardants: BDE, HBCD, TBBP-A**
trophic level		
Zeehavenkanaal, Delfzijl		
0	sediment[Eg04] (PCB<d.l.)	
1		
2	*N. diversicolor*[Eg04] (PCB<d.l.)	
3	*H. ostrealegus*[Eg04] (PCB<d.l.)	
Dutch Wadden Sea		
0		sediment[De03], suspended solids[De03]
1		
2	*N. diversicolor*[Bo89], M. balthica[Bo89]	*M. edulis*[De03]
3-3.5	*S. solea*[Bo89], *P. platessa*[Bo89], *H. ostrealegus*[Bo89,]	*P. flesus*[De03]
4	*P. vitulina*[Bo89]	
Western Scheldt		
0	sediment[St88], suspended solids[St88, vdZ04]	sediment[Va03,De03], suspended solids[Va03,De03,Le04]
1		
2	*A. marina*[St88,vdZ04], *N. diversicolor*[St88], *C. volutator*[St88], *C. edule*[vdZ04]	*M. edulis*[Va03]
3-3.5	*C. crangon*[St88, vdZ04], *Clupeidae*[St88], *C. harengus*[vdZ04], *P. platessa*[vdZ04], *C. gulosus*[vdZ04], *E. gurnardus*[vdZ04], *Ammodytes*[vdZ04], *P. flesus*[St88, vdZ04], *T. luscus*[vdZ04], *M. merlangus*[vdZ04], *S. solea*[vdZ04]	*C. crangon*[Le04], *Clupeidae*[Va03], *C. harengus*[Le04], *P. platessa*[Le04], *C. gulosus*[Le04], *E. gurnardus*[Le04], *Ammodytes*[Le04], P. flesus[De03, Le04], *T. luscus*[Le04], *M. merlangus*[Le04], *S. solea*[Le04]
4	*S. hirundo*[St88, vdZ04]	*S. hirundo*[Va03, Le04]

Table 2.1 Data sets (food chains based on available data) (*continued*)

Area	**Organochlorines: PCB, HCB, PCP**	**Brominated flame retardants: BDE, HBCD, TBBP-A**
trophic level		
Eastern Scheldt		
0		suspended solids[De03]
1		
2	*M. edulis*[De93], *O. edulis*[De93]	*M. edulis*[De03]
3-3.5	*P. flesus*[De01]	*P. flesus*[De03]
4		
central North Sea and Dutch coast		
0	sediment[De01]	sediment[De03]
1		
2		*M. edulis*[De03]
3-3.5	*P. flesus*[De01]	*C. crangon*[Bo02], *B. undatum*[Bo02], *C. harengus*[Bo02], *G. morhua*[Bo02], *P. flesus*[De03], *M. merlangus*[Bo02]
4		*P. phocoena*[Bo02], *P. vitulina*[Bo02]

2.4.1 Species specific data

In order to compare measured accumulation and magnification ratios with model predictions, a food chain based on feeding preferences of the organisms was set up (Table 2.2). A distinction was made between the benthic, heterotrophic and estuarine food chain on the one hand and the pelagic, autotrophic and marine food chain on the other hand (Hamerlynck, 1993). The first trophic level, consisting of phytobenthos and phytoplankton was not measured in the monitoring programs included here. The second trophic level consists of herbi-detritivores that feed non-selective on living algae and dead and suspended organic material. The diet of blue mussel (*Mytilus edulis*) is generally of a planktonic origin, whereas the clam *Macoma balthica* feeds about equally on pelagic and benthic sources in the Western Scheldt (Herman et al., 2000). The annelid lugworm Arenicola marina and the ragworm Nereis diversicolor obtain their food largely (>80%) from the benthic layer (Herman et al., 2000). Both species may feed as well on animal particles, including small blue mussels (Hiddink, 2002).

The next trophic level exists of true primary carnivores. This includes molluscs as the common whelk (*Buccinum undatum*), crustaceans as the brown shrimp (*Crangon crangon*), fish species as plaice (*Pleuronectes platessa*), sandeel

(*Ammodytidae*), herring (*Clupea harengus*) and grey gurnard (*Eutriglia gurnardus*). The bird species, oystercatcher (*Haematopus ostralegus*), belongs to the primary carnivores as well. These primary carnivores usually feed opportunistically on the different invertebrate herbi-detritivores present. *C. crangon* mainly feeds on Myisidacea and Amphipoda in the Irish Sea (Oh et al., 2001). *H. ostralegus* prefers *M. balthica* but switches to, e.g., *M. edulis* and *N. diversicolor* in their absence (Kater pers. comm.).

Flounder (*Platichthys flesus*), sole (*Solea solea*), cod (*Gadus morhua*), whiting (*Merlangius merlangus*) and pout (*Trisopterus luscus*) are reported to feed on both herbi-detritivores and primary carnivores. These fish species are therefore classified in the intermediate level of 3.5. Finally, several top-predators were sampled. The diet of common tern (*Sterna hirundo*) in the Scheldt estuary largely consists of small fish, in particular herring (*C. harengus*), sprat (*Sprattus sprattus*) and sandeels (Ammodytidae) (Jongbloed et al., 1995). Harbour seals (*Phoca vitulina*) in the Wadden Sea mainly consume flounder (*P. flesus*) and herring (*C. harengus*), supplemented by other species. In addition to these preferences, consumption by opportunistic predators also depends on the food chain that is dominant in the system.

Table 2.2. Trophic levels in different suggested food chains with representative species

Trophic level		**Benthic**	**Intermediate hyper-benthic**	**Pelagic**
1				
2	herbi-detritivores	*A. marina*, *C. volutator*, *N. diversicolor*	*M. balthica*	*M. edulis*
3	pr. carnivores	*P. platessa*, *H. ostralegus*	*C. crangon*, *Ammodytidae*, *E. gurnardus*	*C. harengus*, *Clupeidae*
3.5	pr.-sec. carnivores	*S. solea*	*P. flesus*, *G. morhua*, *M. merlangus*, *T. luscus*	
4	sec. carnivore		*P. vitulina*, *P. phocoena*	*S. hirundo*

Stronkhorst 1987, Jongbloed et al. 1995, Mensink et al. 1997, Oh et al. 2001, Herman et al. 2000, http://www.fishbase.org, primary carnivores feed on molluscs, annelids and/ or crustaceans, secondary carnivores feed on fish.

Accumulation in biota is estimated by the model on a lipid weight basis for organic compounds. Measured concentrations were converted to this unit when necessary using dry and lipid weight fractions of organisms determined in the respective study. Where possible, 95%-confidence intervals were obtained using samples from different locations, periods or species, if belonging to the same trophic level.

2.4.2 Substance specific data

The organochlorines studied include PCBs (polychlorinated biphenyls), hexachlorobenzene (HCB) and pentachlorophenol (PCP). PCBs are persistent organic compounds that are known to accumulate in food chains. These compounds have been the focus of environmental research for years. The molecular structure consists of two coupled benzene rings (biphenyls) with a maximum of ten chlorine substituents. In total 209 different congeners exist, which are classified according to the number of chlorine atoms and the number of ortho-substituents. Increasing chlorination generally increases the octanol-water partition coefficient and reduces biotransformation rates (Gobas and Morrison, 2000; Mackay et al., 2000). BFRs (brominated flame retardants) include brominated diphenyl ethers (BDEs), hexabromo-cyclododecane (HBCD) and tetrabromobisphenol A (TBBP-A). BDEs show structural resemblence to PCBs and are classified analogue to PCBs on the number of bromine substituents.

Octanol-water partition coefficients were compiled from different databases (Appendix 2.1) (Terrabase, Epiwin). Coefficients obtained by slow stirring were used where possible (Braekevelt et al., 2003), if not available, estimated ratios based on structure-fragment distributions were taken.

Residues of chemicals in organic solids were reported on a total sediment basis, dry weight or (total) organic carbon basis. OMEGA requires input concentrations in $kg \cdot kg^{-1}$ organic dry weight for neutral organic contaminants. Data were converted to this unit, using organic carbon or organic matter contents of sediments and suspended solids determined in the same study. If these fractions were not mentioned, values from samples taken at nearby locations or in other years were used.

2.5 Results

2.5.1 Organochlorines

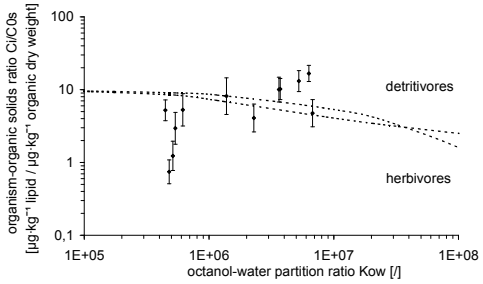
Organism-solid concentration ratios (Biota-Sediment-Accumulation-Factors)

Patterns for the herbi-detritivores, *A. marina*, *N. diversicolor* and *C. volutator* in the Western Scheldt, are largely similar and separate species are therefore not shown (Figure 2.3a). Accumulation ratios of most chlorobiphenyls are at the level estimated by the model from suspended solids concentrations. Some less ($K_{ow}<5\cdot10^5$) and more ($K_{ow}>3\cdot10^6$) hydrophobic substances are below and above this level, respectively. Based on a few substances detected in sediments in the Western Scheldt however, accumulation in *N. diversicolor* is below the level expected for both lower and higher chlorinated biphenyls (Figure 2.3b). Hexachlorobenzene levels in *N. diversicolor* in Zeehavenkanaal do not deviate significantly from the model, although somewhat lower (Figure 2.3b). Residues of pentachlorophenol in *N. diversicolor* are somewhat above model predictions (Figure 2.3b). PCB levels in the detritivore *C. edule* in the Western Scheldt are in good agreement with model predictions (Figure 2.3c).

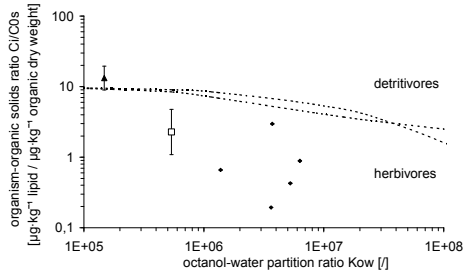
For the primary carnivorous crustacean, *C. crangon*, (Figure 2.3d) ratios are approximately three times higher than model predictions. The accumulation level of 2,2',4,5,5'-pentachlorobiphenyl is somewhat below model lines. Residues of PCB and HCB in the fish species Clupeidae are largely at the increased level expected for primary carnivores (Figure 2.3e). Accumulation ratios in primary carnivorous fish are similar for the separate species and presented in one figure (Figure 2.3f). Residues are somewhat higher (1.5 - 2.5 times) than model predictions, but follow the pattern predicted by the model.

Accumulation factors for various, secondary carnivorous fish species are in good agreement with model predictions, although 2.5 – 3 times higher (Figure 2.4a). Accumulation in *P. flesus*, based on sediment concentrations, is lower than expected (Figure 2.4b). Ratios for the fish-eating bird *S. hirundo*, are somewhat lower than model predictions, although confidence intervals still cover model curves (Figure 2.4c). Accumulation is significantly lower than predicted for the lower chlorinated congeners (2,4,4'-trichloro-, 2,2',4,5'-tetra-, 2,2',5,5'-tetrachlorobiphenyl).

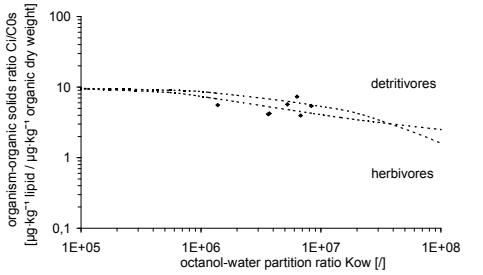
a. Herbi-detritivores :
A. marina, *N. diversicolor* and *C. volutator*[St88] , Western Scheldt, suspended solids



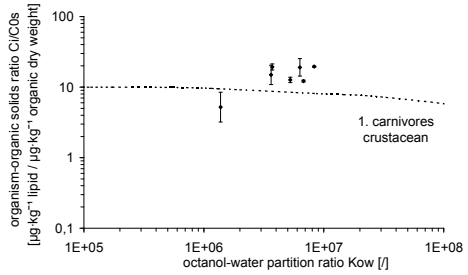
b. Herbi-detritivores:
N. diversicolor, Western Scheldt[St88] and Zeehavenkanaal[Eg03], (▲ PCP ;□ HCB), sediment



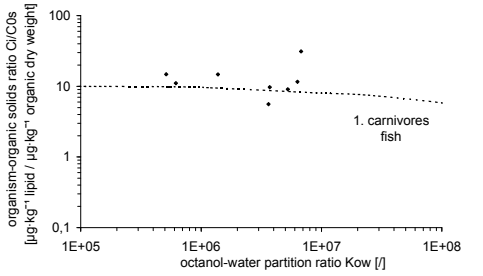
c. Herbivores: *C. edule*[vdZ04]
Western Scheldt, suspended solids



d. Primary carnivores: *C. crangon*[vdZ04]
Western Scheldt, suspended solids



e. Primary carnivores: *Clupeidae*[St88], Western Scheldt, suspended solids



f. Primary carnivores: *C. harengus*, *P. platessa*, *C. gulosus*, *E. gurnardus*, *Ammodytes*[vdZ04], Western Scheldt, suspended solids

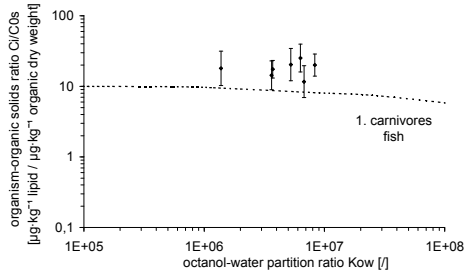
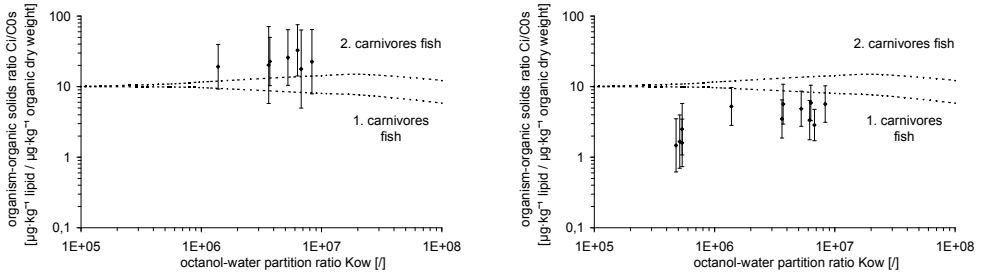


Figure 2.3: Organism-organic matter C_i/C_{0s} [µg·kg^{-1} lipid weight / µg·kg^{-1} dry weight] bioaccumulation ratios for organochlorines versus octanol-water partition ratios K_{ow} [/]. Model estimations (dashed curves) and field surveys on different trophic levels (closed circles ●, geometric mean±95%-CI). Sources: van der Zande, 2004; Stronkhorst, 1988

a. Primary-secondary carnivores: *M. merlangus*, *S. solea*, *P. flesus* (juvenile and adult)[vdZ04], Western Scheldt, suspended solids

b. Primary-secondary carnivores: *P. flesus*[De01], North Sea, sediment



c. Secondary carnivores: *S. Hirundo*[vdZ04, St88], Western Scheldt, suspended solids

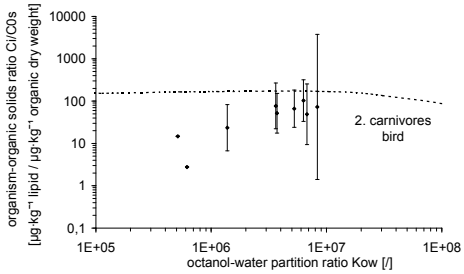


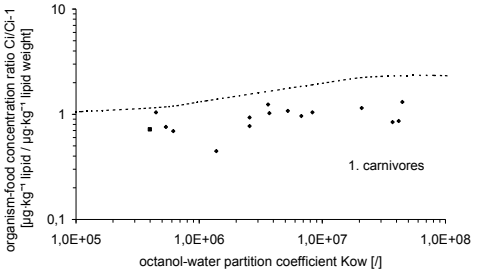
Figure 2.4: Organism-organic matter C_i/C_{0s} [µg·kg^{-1} lipid weight / µg·kg^{-1} dry weight] bioaccumulation ratios for organochlorines versus octanol-water partition ratios K_{ow} [/]. Model estimations (dashed curves) and field surveys on different trophic levels (closed circles ●, geometric mean±95%-CI). Sources: van der Zande, 2004; Stronkhorst, 1988; De Boer et al., 2001

Biomagnification factors

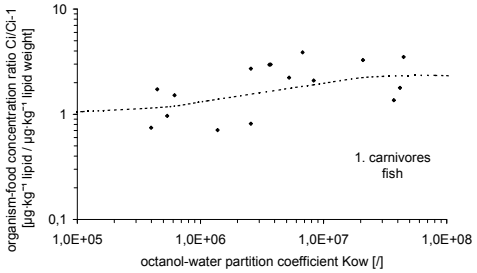
In the Wadden Sea, taking *Macoma balthica* as a representative animal at the basis of the carnivorous food chain, ratios for *N. diversicolor* are lower than model predictions for primary carnivores (Figure 2.5a). Biomagnification ratios for *P. platessa* and *S. solea* are within the range expected for benthivorous and piscivorous fish (Figure 2.5b and c). Values for the seabird *H. ostralegus* are below that in their food (Figure 2.5d). Biomagnification ratios for the harbour seal *P. vitulina*, feeding on *P. platessa*, are in the range of 3 to 4 for the higher chlorinated PCBs and between 0.8 and 1 for lower chlorinated congeners (2,4',5-tri-, 2,4,4'-tri-, 2,2',5-tri-, 2,2',5,5'-tetra-, 2,2',4,5'-tetra-, 2,2',3,5'-tetra-, 2,2',4,5,5'-pentachlorobiphenyl) (Figure 2.5e). For both lower and higher chlorinated

congeners these values are significantly below model expectations for secondary carnivorous mammals.

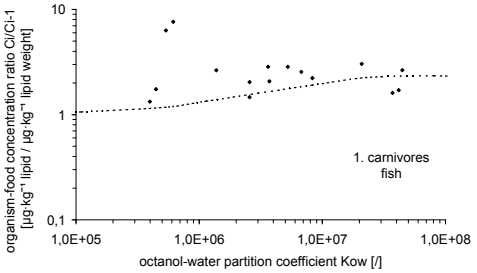
a. *N. diversicolor* (n=14) vs. *M. balthica* (n= 17)



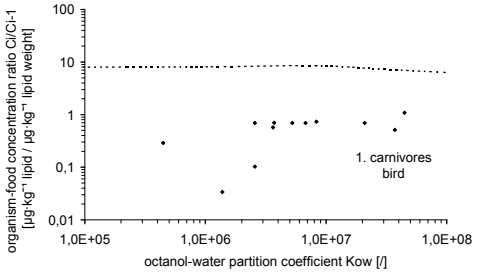
b. *P. platessa* (n=7) vs. *M. balthica* (n =17)



c. *S. solea* (n=8) vs. *M. balthica* (n=17)



d. *H. ostralegus* (brain, n =14) v. *M. balthica* (n=17)



e. *P. vitulina* (blood, n=18) vs. *P. platessa* (n =7)

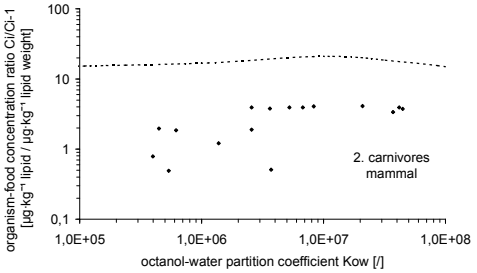
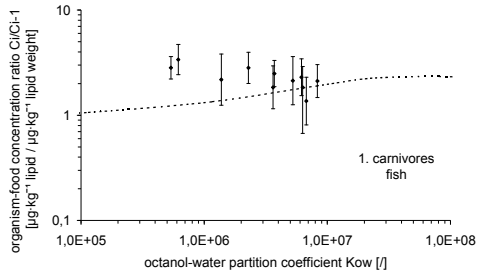
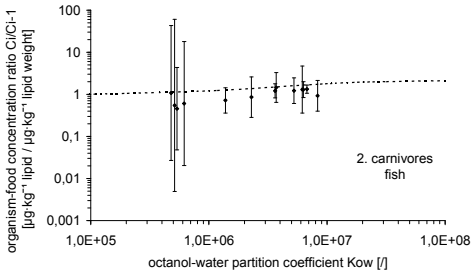


Figure 2.5. Biomagnification ratios C_i/C_{i-1} [µg·kg^{-1} lipid weight / µg·kg^{-1} lipid weight] of organochlorines in Wadden Sea food chain. Model estimations (dashed curves) and field surveys (closed circles ●, geometric mean±95%-CI closed circles). Source: Boon et al., 1989

a. Different primary carnivorous fish species vs. *A. marina*[vdZ04] (Western Scheldt, 2004)

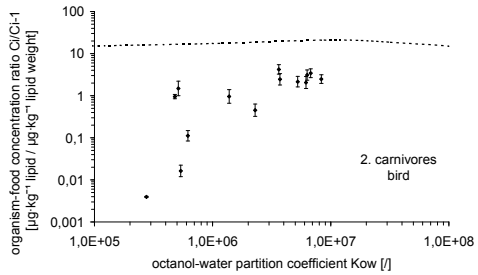
b. *M. merlangus* vs. *Ammodytes* and *C. harengus*[vdZ04] (Western Scheldt, 2004)





c. *S. Hirundo* vs. different fish species[vdZ04] (Western Scheldt, 2004)

d. *S. Hirundo* vs. *Clupeidae*[St88] (Western Scheldt, 1988)



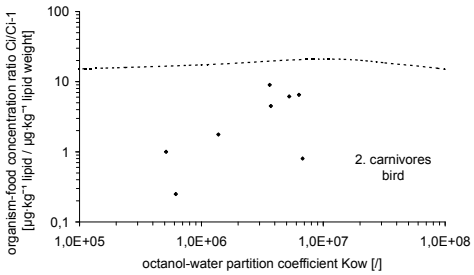


Figure 2.6. Biomagnification ratios C_i/C_{i-1} [µg·kg^{-1} lipid weight / µg·kg^{-1} lipid weight] of organochlorines in Western Scheldt food chain. Model estimations (dashed curves) and field surveys (closed circles ●, geometric mean±95%-CI). Source: van der Zande, 2004; Stronkhorst, 1988

Residues in various primary carnivorous fish versus *A. marina* are in good agreement with model predictions for higher chlorinated biphenyls. However, biomagnification ratios for the lower chlorinated congeners are higher than expected (Figure 2.6a). Values for the secondary carnivore *M. merlangus* versus the fish species *Ammodytes* and *C. harengus*, are in good agreement with model predictions (Figure 2.6b). Large confidence intervals exist for the lower chlorinated compounds. Biomagnification in the secondary carnivore *S. hirundo* is significantly lower than expected for all monitored PCBs (Figure 2.6c). Ratios are similar to those obtained for *P. vitulina* in the Wadden Sea. Biomagnification ratios of *S. hirundo*, feeding on Clupeidae, are below model estimations (Figure 2.6d). Lowest ratios are observed for 2,4,4'-trichlorobiphenyl, 2,2',5,5'-tetrachlorobiphenyl, 2,2',4,5,5'-pentachloro-biphenyl and 2,2',3,3',4,4'-hexachlorobiphenyl.

2.5.2 Brominated flame retardants

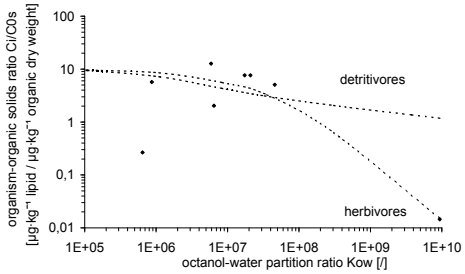
Organism-solid concentration ratios (Biota-Sediment-Accumulation-Factors)

In the Western Scheldt two herbi-detritivores were monitored, namely *A. marina* and *C. edule* (Figure 2.7a,b). For both organisms accumulation ratios of brominated diphenylethers are in reasonable agreement with model predictions. However, for both species the residues of HBCD are significantly, that is 30 – 50 times, overestimated.

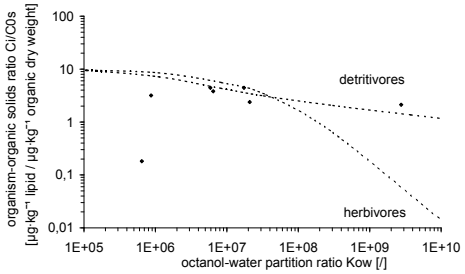
Accumulation ratios for the primary carnivore *C. crangon* are generally similar to model estimations. Highest accumulation ratios are observed for BDE47 and BDE49 (Figure 2.7c). Again, HBCD residues are significantly, that is three orders of magnitude, lower than model predictions. Accumulation ratios for several primary carnivorous fish (*Ammodytes*, *C. harengus*, *E. gurnardus* and *P. platessa*), are generally in line with model predictions, although large confidence intervals exist (Figure 2.7d). Levels of BDE99 and BDE100 are somewhat lower than model predictions, but confidence intervals still cover model curves. The predominant congener in most fish species is BDE47, similar to observations for C. crangon. HBCD residues are a factor of 30 lower than model estimations.

The secondary carnivorous fish monitored in the Western Scheldt could not be plotted in the same figure as large variations occur between the different fish species (Figure 2.7e,f; Figure 2.8a,b,c). However, HBCD residues are similar in most secondary carnivorous fish and significantly, that is 1 – 2 orders of magnitude, below model predictions. In several fish species, *P. flesus* (juvenile and adult) and *S. solea*, accumulation ratios of BDE99 and BDE100 are lower than predicted. As observed for primary carnivorous fish, BDE47 is the predominant congener in secondary carnivorous fish. In P. *flesus*, residues of BDE47 and BDE71 are significantly higher than expected. In the secondary carnivorous fish *M. merlangus*, only two of the analysed brominated flame retardants (BDE154 and HBCD) are above detection limits. The accumulation ratio of BDE154 is in good agreement with model estimations, whereas accumulation of HBCD is significantly below model curves. For *T. luscus* most of the accumulation ratios are in reasonable agreement with model predictions. For the secondary carnivore *P. flesus*, monitored by De Boer et al. (2003) accumulation ratios of BDE99 and BDE47 are below model curves (Figure 2.8d). Accumulation ratios are lowest for BDE 99 (0.4 – 1.0) and highest for BDE47 (1.1 – 1.7).

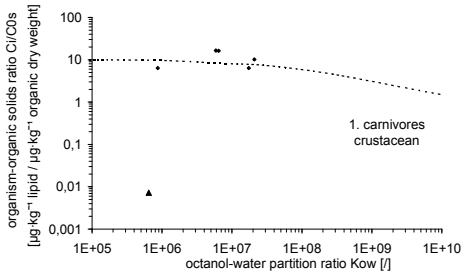
a. Herbi-detritivores: *A. marina*[Le04], Western Scheldt, suspended solids



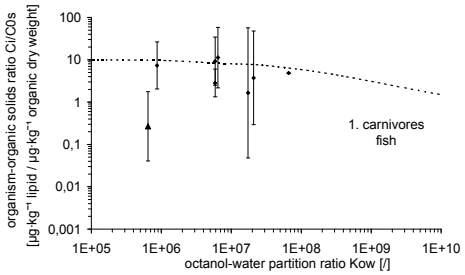
b. Herbivores: *C. edule*[Le04], Western Scheldt, suspended solids



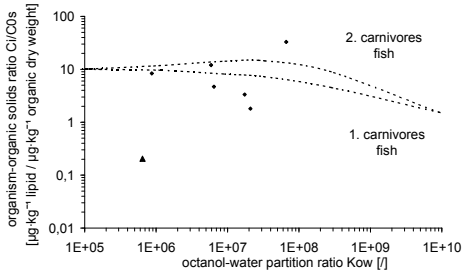
c. Primary carnivores: *C. crangon*[Le04], Western Scheldt,suspended solids



d. Primary carnivores: different fish species (*C. harengus*, *E. gurnardus*, *P. platessa*, *Ammodytes*)[Le04], Western Scheldt, suspended solids



e. Primary–secondary carnivores: *S. solea*[Le04], Western Scheldt, suspended solids



f. Primary–secondary carnivores: *T. luscus*[Le04], Western Scheldt, suspended solids

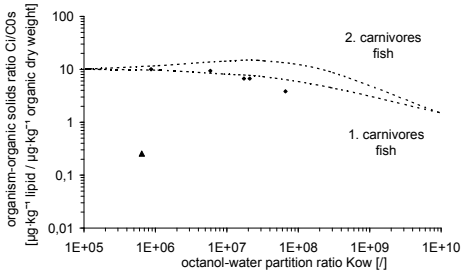
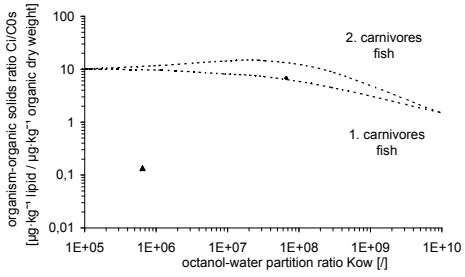


Figure 2.7: Organism-organic matter C_i/C_{0s} [µg·kg^{-1} lipid weight / µg·kg^{-1} dry weight] bioaccumulation ratios for brominated flame retardants versus octanol-water partition ratios K_{ow} [/]. Model estimations (dashed curves) and field surveys on different trophic levels (closed circles ●, geometric mean±95%-CI). Sources: Leonards et al., 2004

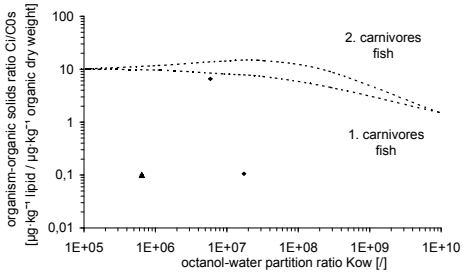
Levels of brominated flame retardants in the secondary carnivorous bird, *S. hirundo*, are all below model estimations (Figure 2.8e). According their

accumulation ratios the brominated diphenylethers and HBCD can be divided in two groups. BDE71, BDE85 and HBCD have accumulation ratios between 0.8 and 1.5. Higher residues are observed for BDE28, BDE47, BDE99, BDE100 and BDE154. Ratios of these congeners are between 8 and 40.

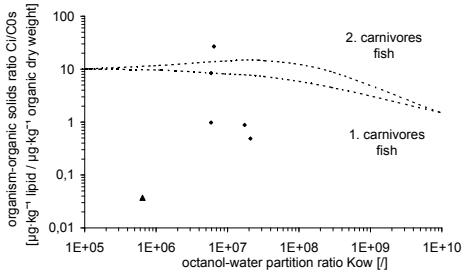
a. Primary–secondary carnivores: *M. merlangus*[Le04], Western Scheldt, suspended solids



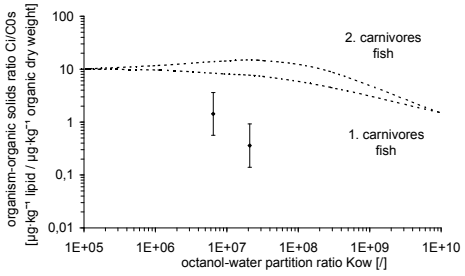
b. Primary–secondary carnivores: *P. flesus* (juvenile)[Le04], Western Scheldt, suspended solids



c. Primary–secondary carnivores: *P. flesus* (adult)[Le04], Western Scheldt, suspended solids



d. Primary–secondary carnivores: *P. flesus*[De03], different locations, sediment



e. Primary–secondary carnivores: *S. Hirundo*[Le04], Western Scheldt, suspended solids

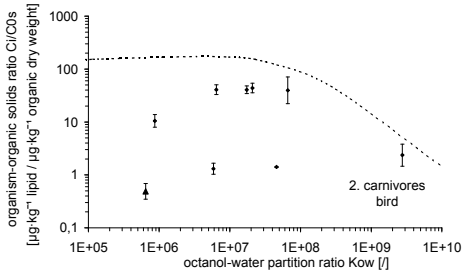


Figure 2.8: Organism-organic matter C_i/C_{0s} [µg·kg^{-1} lipid weight / µg·kg^{-1} dry weight] bioaccumulation ratios for brominated flame retardants versus octanol-water partition ratios K_{ow} [/]. Model estimations (dashed curves) and field surveys on different trophic levels (closed circles ●, geometric mean±95%-CI). Sources: Leonards, 2004; De Boer et al., 2003

Organism-food concentration ratios (Bio-Magnification-Factors)

Biomagnification ratios of brominated flame retardants in *G. morhua* and *M. merlangus* are in good agreement with model estimations (Figure 2.9a). Ratios for BDE99 and BDE153 are somewhat lower than model predictions, although confidence intervals of the latter congener still cover model curves. Residues of brominated flame retardants in *P. flesus* compared to *M. edulis*, are up to a factor of four lower than model predictions (Figure 2.9b). Deviations are not significant. For *P. flesus* versus *C. harengus* magnification of BDE154 is approximately four times higher than expected. Levels of BDE47 and BDE71 are in good agreement with model predictions. Magnification factors of HBCD, BDE49, BDE99 and BDE100 are up to two orders of magnitude lower than model estimations (Figure 2.9c). Residues in *P. phocoena*, feeding on *C. harengus* as well, are in good agreement with model estimations for three congeners, BDE17, BDE99 and BDE100 (Figure 2.9d). The ratios for BDE153 and BDE154 are somewhat higher than expected, whereas the level of BDE47 is lower than predicted. For the secondary carnivorous bird *S. hirundo*, magnification factors are lower than model predictions for most congeners, including BDE47 (Figure 2.9e). However, the magnification ratio of BDE100 is higher than expected. Levels of the other penta-brominated congener BDE99 and the hexa-brominated congener BDE154 are somewhat lower than model predictions.

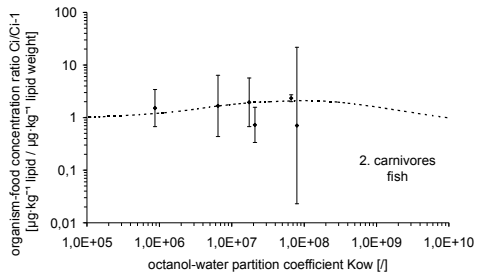
2.6 Discussion

2.6.1 Organochlorines

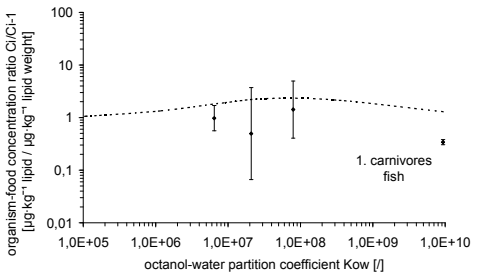
Accumulation and magnification ratios for polychlorinated biphenyls are predicted well by OMEGA for species in the lower end of the food chain i.e. for herbi-detritivores, crustaceans and primary and secondary carnivorous fish. For the herbi-detritivores, *A. marina*, *N. diversicolor* and *C. volutator*, PCB accumulation patterns are similar. Accumulation ratios for *C. volutator* are somewhat larger than ratios observed for *A. marina* and *N. diversicolor*, which can be attributed to the very low fat-fraction of C. volutator. Large uncertainties in the analysis of this fraction are likely (Stronkhorst, 1988) and accumulation in non-fat tissues may interfere. Consequently, presenting ratios for *C. crangon* on a lipid weight basis may overestimate the true accumulation.

A few deviations from model predictions are observed. These deviations can largely be attributed to three factors, namely reduced bioavailability of PCBs, biotransformation of specific congeners by marine birds and mammals and additional feeding in other less polluted areas.

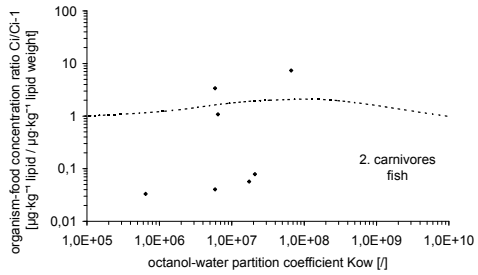
a. *G. morhua* or *M. merlangus* vs. *Clupea harengus*[Bo02]



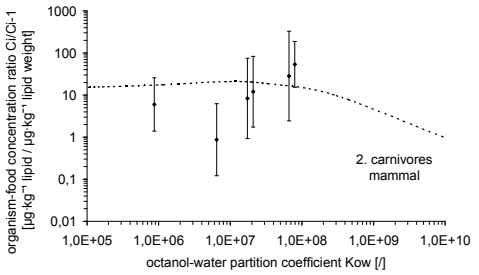
b. *P. flesus* vs. *M. edulis*[De03]



c. *P. flesus* vs. *C. harengus*[Le04]



d. *P. phocoena* or *P. vitulina* (liver/blubber) vs. *C. harengus*[Bo02]



e. *S. hirundo* vs. different fish species[Le04]

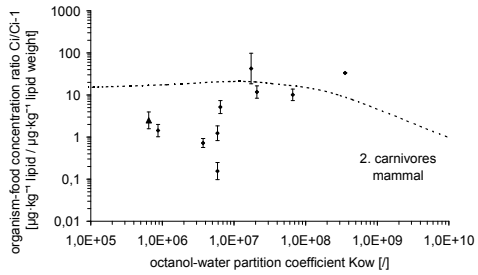


Figure 2.9: Biomagnification ratios C_i/C_{i-1} [µg·kg^{-1} lipid weight / µg·kg^{-1} lipid weight] of brominated flame retardants in secondary carnivorous fish, birds and marine mammals versus primary carnivorous fish. Model estimations (dashed curves) and field surveys (closed circles●, geometric mean±95%-CI). Source: Leonards et al., 2004

Accumulation ratios based on sediment concentrations are generally lower than model predictions. This is due to higher chemical residues in sediment layers compared to suspended solids because of historically higher emissions. These residues may be less bioavailable. Concentrations in the overlaying water phase are lower than estimated based on sediment concentrations resulting in lower accumulation ratios than model predictions.

For fish-eating birds, *S. hirundo* and *H. ostralegus*, and marine mammals, *P. vitulina*, the model generally overestimates accumulation and magnification ratios. This can partly be attributed to metabolization of specific PCB congeners. It is reported that seabirds and marine mammals possess efficient biotransformation mechanisms for these PCBs (Borgå et al., 2004). In general, biotransformation of PCBs depends on the number and the place of chlorine substitution on the phenylring. Both seabirds and marine mammals are capable of metabolizing PCBs with any number of chlorine substituents, if at least one of the phenylrings is meta-para unsubstituted (Boon et al., 1994; Boon et al., 1997) In *S. hirundo* accumulation ratios of 2,2',4,5'-tetra, and 2,2',5,5'- tetrachlorobiphenyl are lower than predicted. Each of these congeners has vicinal hydrogen atoms at the meta- and para-position on at least one of the phenylrings. Consequently, these congeners can be metabolized by *S. hirundo* resulting in low accumulation ratios. The other congeners have chlorine substituents on at least one of these positions and are consequently not metabolized. An exception to this rule is 2,4,4'-trichlorobiphenyl, which is para-substituted at both phenylrings, implying persistency. However, low ratios are observed for these congener. Results on magnification of PCBs in *S. hirundo* in the Western Scheldt food chain are consistent with these observations. Magnification ratios of meta-para unsubstituted congeners (2,2',3,5-tetra, 2,2'4,5,5'-penta, 2,2',4,5-tetra, 2,2',5,5'-tetra-, 2,2',5-, 2,4,5'-trichlorobiphenyl) are lower than ratios for other congeners with chlorine atoms at these positions. Biotransformation also explains observed low ratios of three congeners (2,3'5-trichloro, 2,2',4,5,5'-penta and 2,2',3,4',5',6-heptachlorobiphenyl) in the bird *H. ostralegus* feeding on *M. balthica*.

Seals are also capable of metabolizing meta-para unsubstituted PCBs The metabolic capacity of seals is considered to be higher than that of birds (Boon et al., 1994; Boon et al., 1997). Seals are capable of metabolizing PCBs with adjacent H-atoms at the ortho-meta position and a maximum of one ortho-chlorine as well, whereas birds are not. (Boon et al., 1994; Boon et al., 1997). Applying this biotransformation rules on the data for *P. vitulina*, the congeners that show low magnification posses adjacent H-atoms in the m,p-position or vicinal H-atoms in the o,m-position with a maximum of one ortho-chlorine atom. The other congeners for which higher magnification ratios are observed, all have either no vicinal H-atoms or vicinal H-atoms at the ortho,meta positions, but more than one ortho-chlorine substitution, implying persistency.

However, accumulation and magnification ratios of congeners considered to be persistent in seals and birds are below model predictions as well. For the bird species *S. hirundo* and *H. ostralegus* feeding in other less polluted areas than the

Western Scheldt and the Wadden Sea, respectively, may explain this observation. For seals different feeding behaviour is unlikely as all the seals studied were held in captivity and fed with fish, *P. platessa*, from the Dutch Wadden Sea. PCB concentrations in fish were determined in the same study. A possible explanation for the observed reduced accumulation ratios is that PCB residues were determined in seal blood. PCB patterns in blood on a lipid basis is assumed to represent patterns of all other organs and tissues of seals (Boon, 1987). However, blood concentrations on a lipid basis may not reflect blubber concentrations i.e. PCB residues in blubber may be higher. Consequently the true accumulation in seals may be higher than predicted based on measurements. Different feeding behavior may explain comparable PCB concentrations in *N. diversicolor* versus its prey *M. balthica* as well. According to Hiddink (2002), *N. diversicolor* feeds on *M. balthica* only occassionaly and mainly consumes living algae and dead suspended organic material.

The two other chlorinated compounds studied, hexachlorobenzene and pentachlorophenol, were only monitored in *N. diversicolor* in the Zeehavenkanaal. HCB concentrations were below detection limits in suspended solids in other studies. Based on this study, PCP seems to accumulate in *N. diversicolor* whereas residues of HCB are somewhat lower than model predictions. However, PCP is a metabolite of HCB and biotransformation of HCB may attribute to PCP residues in *N. diversicolor*.

Organism-organic solid concentration ratios can be compared with field survey measurements for fresh water species in the Rhine-Meuse delta (Hendriks et al., 2001). In Hendriks et al. (2001) accumulation ratios are presented for the total group of persistent organic compounds: PCBs, chlorobenzenes and chlorobiocides (including HCH, drins, DDD and DDE). For mollusks and arthropods (herbi-detritivores), freshwater accumulation ratios between 3.3 and 4.5 are observed. These ratios are comparable to values for *C. edule*, but are somewhat lower than accumulation factors for marine herbi-detritivores.

For freshwater fish (*Rutilus rutilus, Anguilla anguilla*, *Stizostedion lucioperca*) organism-suspended solids concentration ratios are in the range of 7.1 – 11.1 (Hendriks et al., 2001). This is in good agreement with the obtained accumulation ratios of PCBs for marine and estuarine fishes. No organism-suspended solid concentrations ratios are available for freshwater birds.

Magnification ratios in marine food chains can be compared with magnification ratios for fresh water species of the Rhine-Meuse delta (Hendriks et al., 2001). Organism-food concentration ratios for “fresh water” bird species (*Aythya fuligula*, *Recurvirostra avosetta*, *Sterna hirundo*, *Phalacrocorax carbo*) are between 1.7 and

12, which is comparable to ratios for PCBs in *S. hirundo* (egg). However, these values are higher than ratios observed for *H. ostralegus*. Supporting the assumption that *H. ostralegus* is not fully exposed to PCBs in the food chain of this area i.e. preying on species from other locations as well. Residues in mammalia (*Crocidura russula*, *Sorex araneus*, *Mustela nivalis*, *Mustela erminea*, *Mustela putorius*, *Lutra lutra*) from the Rhine-Meuse delta vary between 4 and 20, with exceptional values in the 46 – 146 range. Levels for *P. vitulina* are in the range of 3 to 4 for persistent PCBs and thus in agreement with the lower values for the Rhine-Meuse delta. Magnification ratios are much lower for labile PCBs and vary between 0.4 and 1.0. This may indicate that at least *P. vitulina* has higher metabolic capacities than the observed freshwater species. In Norwegian marine waters magnification ratios of PCBs in the harbor and grey seal feeding on cod are approximately two times higher (BMF 8.7 – 10.0) than the values obtained in this study (Ruus et al., 1999). De Swart et al. (1996) found a magnification factor of 3.7 (∑PCB) for seals fed on herring from the Baltic Sea. In the same study magnification factors of seals fed with Atlantic ocean herring were determined and appeared to be more in line with the higher reported values. (BMF 7.9 – 8.1) Although these magnification ratios are higher than those observed for *P. vitulina* in the Wadden Sea, they confirm the observation that PCB accumulation in seals is significantly lower than expected. Moreover, these ratios are significantly lower than magnification ratios observed for freshwater species as well.

2.6.2 Brominated flame retardants

Accumulation of brominated diphenyl ethers is highly congener and species specific. Differences in accumulation and magnification ratios can largely be attributed to species specific metabolization capacities. Accumulation is generally predicted well by OMEGA for herbi-detritivores and crustaceans. Preferential accumulation of tetra- (BDE47 and BDE49) and penta-brominated diphenylethers (BDE85, BDE99 and BDE100) is observed. These observations are consistent with Gustafsson et al. (1999), who found that accumulation ratios of BDE47, BDE99 and BDE153 were similar to or higher than ratios of PCBs in the herbi-detritivore *M. edulis*.

In primary and secondary carnivorous fish accumulation and magnification ratios of BDE99 and BDE100 are generally lower than predicted. Recent studies have illustrated that various fish species are capable of metabolizing several BDE congeners (Stapleton et al., 2004[a,b,c]; Tomy et al., 2004) This biotransformation occurs via oxidative and debromination pathways (Hakk and Letcher, 2003). Stapleton et al. (2004[a,b,c]) reported that the common carp (*Cyprinus carpio*) is

capable of debrominating several BDE-congeners, one of which is BDE99. Debromination of BDE99 probably results in formation of BDE47 (Stapleton et al., 2004[b]). This may explain the observed lower residues of BDE99 in primary carnivorous fish and the predominance of BDE47. To provide a quantitative explanation of the observed differences between accumulation ratios in fish species, elimination rate constants as estimated by the model for fish are compared to literature values for juvenile lake trout (Table 2.3) The estimated elimination rate constants are higher than measured values, suggesting no substantial biotransformation. Tomy et al. (2004) stated that their elimination rate constants may be underestimated as fish were fed with a group of selected BDEs and bioformation of the lower brominated compounds may occur. No distinction can be made between BDEs resulting from uptake of the compound by fish or those resulting from bioformation.

Table 2.3: Independent empirical elimination rates (Tomy et al., 2004) compared to model predictions of physical-chemical minimum elimination (Hendriks et al., 2001)

		Model	**Empirical**	**Ratio**
Compound -bromodiphenylether	Abbrevation	Elimination rate $k_{X,ex}$ [d^{-1}]	Elimination rate $k_{X,ex}$ [d^{-1}]	Emp. / Mod.
2,2',4,4'-tetra-	BDE47	$2.2 \cdot 10^{-2}$	$1.2 \cdot 10^{-2}$	$5.4 \cdot 10^{-1}$
2,3',4,4'-tetra	BDE66	$2.5 \cdot 10^{-2}$	$4.0 \cdot 10^{-3}$	$1.6 \cdot 10^{-1}$
2,2',4,4',5'-penta	BDE99	$1.9 \cdot 10^{-2}$	$8.0 \cdot 10^{-3}$	$4.1 \cdot 10^{-1}$
2,2',4,4',6'-penta	BDE100	$2.0 \cdot 10^{-2}$	$2.0 \cdot 10^{-3}$	$1.0 \cdot 10^{-1}$
2,2',4,4',5,5'-hexa	BDE153	$1.8 \cdot 10^{-2}$	$6.0 \cdot 10^{-3}$	$3.3 \cdot 10^{-1}$
2,2',3,3',4,4',5,5',6,6'-deca	BDE209	$9.4 \cdot 10^{-3}$	$2.7 \cdot 10^{-2}$	2.9

In contrast to observations for fish, high accumulation and magnification ratios of BDE99 and BDE100 are observed for the secondary carnivorous bird *S. hirundo*. This may imply that these congeners are not metabolized by *S. hirundo*, which is consistent with the biotransformation rules that exist for PCBs. The two congeners are both meta-para substituted and consequently not metabolized. However, this is not true for the two other congeners, BDE85 and BDE190. These compounds posses bromine substituents at the meta-para positions, implying persistency. However, accumulation ratios for both congeners are lower than predicted. Possibly the PCB-biotransformation rules do not apply in the same manner to BDEs. In the marine mammal *P. phocoena* accumulation ratios for BDE99 and BDE100 are in good agreement with model predictions, which is consistent with observations for the secondary carnivorous bird *S. hirundo*. Levels of BDE153 and BDE154 are higher than model predictions. BDE47 does not biomagnify in *P. phocoena* in

contrary to observations for other studied species. The results on secondary carnivorous birds and marine mammals indicate that the pentabrominated congeners, BDE99 and BDE100, acummulate preferentially, reaching levels similar to or higher than model predictions.

At present little is known about biotransformation of BDEs. Metabolization of BDEs is reported to occur via two pathways, by oxidation and debromination. Debromination is only reported for fish species at the moment and it is not known if other species are capable of debromination as well. Furthermore, the pathway by which BDE congeners are debrominated and the rate at which this occurs is unknown. At the moment no clear structurally related rules for biotransformation exist as are available for PCBs. Consequently, a quantitative explanation of differences between model estimation and field data can not be given. The results indicate that accumulation of brominated diphenylethers is highly congener and species specific. In fish species the pentabrominated compounds BDE99 and BDE100 seem to be metabolized, whereas high accumulation of these compounds is observed in birds and marine mammals.

The results also indicate that hexabromocyclododecane does not accumulate in food chains in spite of high organic solid concentrations. Recent literature (Morris et al., 2004) has shown that HBCD exists of three diastereomers, namely α-, β- and γ-HBCD. Generally, γ-HBCD is the dominant stereomer in solids, accounting for approximately 80% of the total HBCD concentration. α-HBCD is present at <10% of the total concentration only. However, in biota the α-stereomer is dominant (>80%) and γ-HBCD is accounting for approximately 20% of the total concentration only. Consequently, conclusions on accumulation potential of HBCD can not be drawn based on total HBCD concentrations as the α-stereomer may accumulate in food chains (Morris et al., 2004).

2.7 Conclusion

PCBs generally behave as expected based on their K_{ow} and accumulation is predicted well by the model. PCB accumulation ratios are comparable among different species of the same trophic level. Magnification ratios were similar in the two studied food chains, the Western Scheldt and the Wadden sea food chain. Biota-organic solids accumulation ratios of PCBs in fresh water herbi-detritivores and fish are comparable to ratios obtained for marine water species.

However, in the upper end of the food chain accumulation is generally overestimated by the model. Residues in the secondary carnivorous bird *S. hirundo* and mammal *P. vitulina* are significantly lower than model predictions. This is

attributed to biotransformation of the meta-para unsubstituted congeners for both *S. hirundo* and *P. vitulina*. Additionally, *S. hirundo* may feed in other less polluted areas.

Magnification ratios of PCBs in several fresh water bird species are comparable to ratios calculated for *S. hirundo*. On the contrary, magnification ratios for *P. vitulina* are significantly lower than ratios found for fresh water mammals. This is confirmed by reported magnification ratios for seals in (arctic) marine waters. The results indicate that magnification of PCBs in at least seals is significantly lower than magnification in fresh water mammals.

Accumulation of brominated diphenylethers is highly congener and species specific. In lower trophic level species, as herbi-detritivores and crustaceans, accumulation of BDEs is predicted reasonably well by the model and behavior is similar to PCBs. In higher trophic level species, biotransformation occurs and accumulation ratios depend on metabolizing capacities as well. Fish may be capable of debrominating BDE99 and BDE100, resulting in low accumulation ratios of these congeners. However, some BDE congeners, in particular BDE47, reach similar levels as persistent PCBs in fish. In piscivorous birds and marine mammals, accumulation ratios of BDE99 and BDE100 are similar to or higher than ratios for persistent PCBs, in contrast to observations for fish.

No conclusions can be drawn on accumulation of HBCD as concentrations are presented for total HBCD. Recent studies have shown that the diastereomer α-HBCD in particular may accumulate in food chains.

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Chapter 3.

Organotin accumulation in an estuarine food chain. Comparing field measurements with model estimations

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3.1 Abstract

The bioaccumulation model OMEGA is used to explore accumulation of organotins in the Western Scheldt food chain, consisting of herbi-detritivores, primary and secondary carnivorous fish and a piscivorous bird. Organotins studied are tributyltin (TBT) and triphenyltin (TPT) and the respective di- and mono-organotin metabolites. Empirical elimination rate constants are compared to model predictions for organic substances and metals. It is found that field bioaccumulation ratios are higher than predicted based on elimination kinetics relevant for organic compounds. The results indicate that uptake of organotins mainly occurs via hydrophobic mechanisms, whereas elimination may occur via metal-like kinetics. This results in very low elimination rates, which are comparable to model predictions for metals.

3.2 Introduction

Tributyltin (TBT) and triphenyltin (TPT) have been extensively used as antifouling boat paints since the early 1970s. As a result, large quantities have been introduced in the aquatic environment (Berg et al., 2001; Hoch, 2001). Both triphenyltin and tributyltin were shown to be hazardous to aquatic life (Hoch, 2001). Even at low nanomolar aqueous concentrations (1-2 ng·l-1), TBT causes chronic and acute toxic effects in most sensitive aquatic organisms, such as algae, zooplankton, molluscs and the larval stage of some fishes (Gibbs and Bryan, 1996). Since the awareness of these effects, use of TBT as antifouling paint on ships has been prohibited in EU countries for crafts smaller than 25 meter (EU directive 1989). However, at present larger ships still use TBT and emission in the environment is continuing (Ten Hallers-Tjabbes et al., 2003), prolonging the concern over this contaminant.

Tributyltin and triphenyltin are ionizable, organometallic compounds that form complexes with various ligands present in the abiotic and biotic environment (Arnold et al., 1997; Buck et al., 2003). Speciation of organotin compounds strongly determines their environmental fate and accumulation kinetics, which therefore differ from neutral organic substances. In water, triorganotins (TOT: tributyltin (TBT) and triphenyltin (TPT)) exist as various neutral, non-dissociated molecules (triorganotin hydroxide (TOT-OH), triorganotin chloride (TOT-Cl)) and / or as organotin cations (TOT+). Each of these compounds exhibits different sediment-water partitioning behaviour (Arnold et al., 1997, Burton et al., 2005). Generally, speciation depends on pH and salinity of the water. The pH of seawater is usually well buffered slightly above pH 8. At this pH the predominant compounds of both TBT and TPT are the neutral hydroxy-complexes (>93%) (Arnold et al., 1997). Under

more acidic conditions (pH 6), the TBT-cation exists besides the neutral TBT-Cl and TBT-OH complexes (Burton et al., 2005).

At the conditions of the Western Scheldt estuary, i.e. a minimum pH of 7.1 at the brackish or fresh water interface (Schaar van Ouden Doel) and a value of 8 near the North Sea (Waterstat, 2005), TBT and TPT exist as neutral compounds. Hydrophobic partitioning to organic matter is likely the dominant sorption process (Burton et al., 2004) and partitioning behaviour may be similar to those of organic contaminants.

Speciation of TBT and TPT also influences uptake and elimination kinetics. In a laboratory study Fent and Looser (1995) found a significantly higher accumulation of TBT in *Daphnia magna* at pH 8 than at pH 6, which they attributed to the occurrence of TBT as a neutral hydroxide at pH 8. These neutral complexes may cross biomembranes more easily than the hydrophilic cations (Fent and Looser, 1995), resulting in higher accumulation levels.

However, Tanabe (1999) reported that the distribution of organotins in body tissues is similar to that of mercury, and unlike that of organochlorines, i.e. organotins accumulate in liver or kidney instead of blubber. This preferential accumulation has been reported for various fish (Tanabe, 1999), bird species (Stäb et al., 1996; Guruge et al., 1996), and marine mammals (Kim et al., 1996; Tanabe, 1999). This is suggested to result from selective protein binding (Kannan et al., 1996; Kim et al., 1996). Organotins form complexes with various kinds of biotic ligands as sulfide groups in glutathione, cysteine and histidine (Elliott et al., 1979; Nishikimi et al., 2001; Hunziker et al., 2001). This complexation may result in lower elimination rates compared to neutral organic substances. Moreover, elimination rates of organotins may be more comparable to rate constants of metals than of organic substances, as metals are known to bind to proteins as well (Hendriks and Heikens, 2001).

In the present investigation, the model OMEGA is used to explore accumulation of organotins in the estuarine food chain of the Western Scheldt. Data from monitoring programs were collected, comprising measured chemical concentrations in marine suspended solids as well as chemical residues in various species belonging to several trophic levels (all field data are provided in the Supporting Information). Field-based biota-suspended solids accumulation ratios (BSAF) were calculated for the different species and compared to model predictions. Additionally, empirical elimination rate constants for organotins were compared to model estimations. Elimination was modeled following two approaches, respectively: modeling elimination as an organic compound and as a metal.

3.3 Methods

3.3.1 Bioaccumulation factors

In order to compare model predictions with field data, a food chain based on feeding preferences of the monitored organisms is set up (Table 3.1). This food chain is identical to the Western Scheldt food chain used in Chapter 2 to estimate accumulation of organochlorines and brominated flame retardants.

Table 3.1: Trophic levels in suggested Western Scheldt food chain with representative species

Trophic level		**Species**
1	suspended matter	
2	herbi-detritivores	*Arenicola marina* (lugworm), *Cerastoderma edule* (common cockle)
3	primary carnivores	*Crangon crangon* (common shrimp), *Ammodytidae* (sand lances), *Eutrigla gurnardus* (grey gurnard), *Pleuronectes platessa* (plaice), *Clupea harengus* (herring), *Clupeidae* (herring, sardines, shads, sprats, pilchards), *Gobius niger* (black goby)
3.5	primary-secondary carnivores	*Platichthys flesus* (flounder), *Gadus morhua* (Atlantic cod), *Merlangius merlangus* (whiting), *Trisopterus luscus* (pout), *Solea solea* (sole)
4	secondary carnivore	*Sterna hirundo* (tern)

Stronkhorst 1988; Jongbloed et al., 1995; Mensink et al., 1997; Oh et al. 2001; Herman et al. 2000, http://www.fishbase.org, primary carnivores feed on molluscs, annelids, crustaceans, secondary carnivores feed on fish.

3.3.2 Uptake and elimination kinetics

Uptake of organotin compounds is modeled similar to neutral organic compounds as the uncharged hydroxo-complex of organotins, i.e. TBT-OH and TPT-OH, crosses membranes by hydrophobic mechanisms (Fent and Looser, 1995).

Elimination of organotins is modeled following two approaches (equations are provided in Appendix 3):

- similar to a neutral organic compound (Hendriks et al., 2001)
- similar to a metal (Hendriks and Heikens, 2001)

In the first approach it is assumed that the neutral hydroxo-complex of organotins does not dissociate in biota. In this case elimination rates should be similar to rate constants estimated for organic compounds. In the second approach, it is assumed that dissociation or ligand exchange does occur in biota and that elimination of the

organotin cation is similar to metal elimination kinetics as metals bind to proteins as well. An important metal-binding protein in animals is metallothionein (Ikemoto et al., 2004). This binding to proteins is incorporated in OMEGA as a generic dry tissue – water distribution coefficient (K_{tw}), which results in a lower elimination rate compared to hydrophobic organic substances. The K_{tw} describes the affinity of a metal for proteins, and is derived from calibration on hundreds of accumulation ratios from laboratory and field studies (Hendriks and Heikens, 2001). To test the validity of the two approaches, elimination and uptake rate constants as estimated by OMEGA are compared to empirical elimination rate constants from literature.

3.3.3 Suspended solids-water partitioning

OMEGA requires dissolved water concentrations as input to estimate chemical residues in biota. As the monitoring data from the Western Scheldt do not include water concentrations of organotins, these concentrations were calculated from the concentration in suspended matter according to Equation 3.1. The suspended solids-water partition coefficient (K_{sw}) can be estimated from the organic carbon-based partition coefficient (K_{oc}) and the organic carbon fraction (f_{oc}) in suspended matter according Equation 3.2 (Karickhoff et al., 1979). As the K_{oc} is normalized to the organic carbon content in suspended solids, its value is independent of the actual organic carbon fraction.

$$C_{0w,x} = \frac{C_{0,x} \cdot f_{s\_oa}}{K_{sw}} \qquad \text{Equation 3.1}$$

$$K_{sw} = f_{oc} \cdot K_{oc} \qquad \text{Equation 3.2}$$

K_{sw}	=	solids–water partition coefficient	[L·kg^{-1} $_{\text{wet weight}}$]
K_{oc}	=	organic carbon normalized partition coefficient	[L·kg^{-1} $_{\text{organic carbon}}$]
$C_{0w,x}$	=	dissolved concentration in water	[µg·L^{-1}]
$C_{0,x}$	=	concentration in suspended matter	[µg·kg^{-1} $_{\text{organic dry weight}}$]
f_{oc}	=	percentage of organic carbon in suspended solids	(9.15%)
f_{om}	=	percentage of organic matter in suspended solids	(20%)

3.4 Data collection and treatment

Data were obtained from a monitoring program in the Western Scheldt estuary in spring 2003, carried out by the Dutch National Institute for Coastal and Marine Management (RIKZ) (all field data are provided in the supporting information). Organotins in suspended solids and biota were analysed according a standard RIKZ procedure as described in de Boer et al. (2001) and Verslycke et al. (2005), respectively. All analytical methods used, are accredited by the Dutch council for accreditation (RvA) according ISO 17025 .

Organotin concentrations in biota and organic solids are presented on a dry weight basis. All dry weight concentrations in suspended solids were converted to concentrations on an organic matter dry weight basis by using the organic carbon content of suspended solids (9%), determined in the same study. In marine sediments, organic matter generally contains 50% of organic carbon (EC, 2004).

Octanol-water partition coefficients of TBT-OH and TPT-OH were taken from Arnold et al. (1997). K_{ow}`s for the di- and mono- compounds were compiled from EPIWIN (Estimations Program Interface for Windows, U.S. Environmental Protection Agency) database (USEPA, 2001). EPIWIN is an estimation program of USEPA that predicts, among other parameters, octanol-water partition coefficients based on structure-fragment distributions. Additionally, empirically obtained K_{ow}`s are given in this database. Empirical $K_{ow}$`s were used where possible, if not available, estimated K_{ow}`s were taken.

While relationships for estimating the organic carbon distribution coefficient (K_{oc}) as a function of the K_{ow} are available for a range of nonpolar compounds, no such relationship has previously been developed for organotin species (Burton et al., 2005). Therefore, we chose to use empirically obtained K_{oc}`s from literature. As sorption and desorption behaviour of organotins, and consequently $K_{oc}$, is strongly dependent on pH and salinity, empirical $K_{oc}$`s were used only if they were derived under estuarine / marine conditions (pH > 7.2). A range of K_{oc} values exist (Table 3.2) and the geometric mean was used in the OMEGA calculations (Table 3.3).

Table 3.2: Empirical organic carbon normalized partition coefficients (K_{oc})

Compound	**Koc [L·kg$_{oc}^{-1}$]**	**Location**	**pH**	**Reference**
tributyltin	$3.2 \cdot 10^4$	Laboratory		Meador, 2000
tributyltin	$1.2 \cdot 10^3$ - $9.6 \cdot 10^4$	Sediment of mangrove forest, mud flat, sand bank and commercial marina. Southeast Queensland, Australia	8	Burton et al., 2005
tributyltin	$1.1 \cdot 10^4$ - $4.9 \cdot 10^5$	harbor and marine sediment	7.35	Berg et al., 2001
tributyltin	$7.6 \cdot 10^3$ – $4.1 \cdot 10^5$	In-situ, marina sediment Southeast Queensland, Australia	7.6 – 8.1	Burton et al., 2004
tributyltin	$1.9 \cdot 10^4$ - $2.8 \cdot 10^5$	Estuarine sediment, UK		Langston and Pope, 1995
tributyltin	$5.0 \cdot 10^5$	Marine sediment, Rhine estuary	7.7	Stronkhorst et al., 1999
dibutyltin	$8.5 \cdot 10^3$ - $6.0 \cdot 10^5$	Harbor and marine sediment	7.35	Berg et al., 2001
dibutyltin	$3.1 \cdot 10^2$	In-situ, marina sediment Australia	7.6 – 8.2	Burton et al., 2004
monobutyltin	$4.8 \cdot 10^3$ - $1.3 \cdot 10^5$	Harbor sediment , Lake Zürich	7.35	Berg et al., 2001
triphenyltin	$5.4 \cdot 10^4$ - $2.3 \cdot 10^5$	Harbor and marine sediment	7.35	Berg et al., 2001
diphenyltin	$3.0 \cdot 10^4$ - $3.0 \cdot 10^5$	Harbor and marine sediment	7.35	Berg et al., 2001
monophenyltin	$1.1 \cdot 10^4$ - $4.8 \cdot 10^4$	Harbor and marine sediment	7.35	Berg et al., 2001

Table 3.3: Empirical organic carbon normalized partition coefficients (K_{oc}, geometric mean), estimated suspended solids – water partition coefficient (K_{sw}), measured organotin concentrations in Western Scheldt suspended solids($C_{0,x}$) and estimated dissolved water concentrations ($C_{0w,x}$) of organotins studied

Compound	**K_{ow}** [-]	**K_{oc}** [$L \cdot kg_{oc}^{-1}$]	**K_{sw}** [$L \cdot kg^{-1}$]	**$C_{0,x}$** [$\mu g \cdot kg^{-1}$ organic dry weight]	**$C_{0w,x}$** [$\mu g \cdot L^{-1}$]
Tributyltin-hydroxide	$1.3 \cdot 10^4$	$8.5 \cdot 10^4$	$8.5 \cdot 10^3$ *	$4.1 \cdot 10^2$	$9.6 \cdot 10^{-3}$
Triphenyltin-hydroxide	$3.1 \cdot 10^3$	$1.1 \cdot 10^5$	$1.1 \cdot 10^4$	$4.5 \cdot 10^1$	$8.4 \cdot 10^{-4}$
Dibutyltin	$3.1 \cdot 10^1$	$1.2 \cdot 10^5$	$1.2 \cdot 10^4$	$7.4 \cdot 10^1$	$1.3 \cdot 10^{-3}$
Diphenyltin	$7.9 \cdot 10^1$	$9.0 \cdot 10^4$	$9.0 \cdot 10^3$	$1.5 \cdot 10^1$	$3.4 \cdot 10^{-4}$
Monobutyltin	$2.0 \cdot 10^3$	$3.9 \cdot 10^4$	$3.9 \cdot 10^3$	$7.4 \cdot 10^1$	$3.7 \cdot 10^{-3}$
Monophenyltin	$1.1 \cdot 10^3$	$2.3 \cdot 10^4$	$2.3 \cdot 10^3$	< d.l.	<d.l.

The suspended solids-water partition coefficient was derived from the K_{oc} according equation 3.2. Subsequently, the water concentration of TBT and TPT can be calculated (Eqn. 3.1). In Table 3.3 K_{ow}, K_{oc}, K_{sw} and water concentrations are presented.

3.5 Results

3.5.1 Biota-suspended solids-accumulation ratios (BSAFs)

Field and model BSAFs for the different species of the Western Scheldt food chain are presented in figure 1. Generally, field bioaccumulation ratios are relatively low (< 1). Only in *C. crangon*, ratios are higher than one. Accumulation ratios of tributyltin (TBT) decline in the food chain, whereas ratios of triphenyltin (TPT) are higher in primary and secondary carnivorous fish compared to herbi-detritivores.

In the two herbi-detritivores monitored, *A. marina* and *C. edule*, empirical accumulation ratios of TBT are comparable, that is 0.6 and 0.8, respectively. TPT accumulation ratios are lower, i.e. 0.3 for *A. marina* and 0.5 for *C. edule*. The metabolites of TPT are below detection limits in both species. In contrast, accumulation ratios of the metabolites of TBT, i.e. dibutyltin (DBT) and monobutyltin (MBT), are relatively high in *A. marina* (approximately 2 for both DBT and MBT). In the mollusc, *C. edule*, ratios of MBT are below detection limits and accumulation ratios of DBT are 0.6.

Accumulation ratios of TBT and TPT for the crustacean *C. crangon*, are approximately twice as high as ratios of herbi-detritivores, which is 1.4 for TBT and 1.1 for TPT. Metabolites of TPT are not detected in *C. crangon*. DBT and MBT ratios are lower than ratios of TBT. BSAFs of TBT in primary carnivorous fish are twice as high as values for secondary carnivorous fish. In contrast, accumulation ratios of

TPT are comparable among primary and secondary carnivorous fish, with typical values of 0.9 and 0.7, respectively. The metabolite diphenyltin (DPT) is detected in both trophic levels.
The lowest accumulation ratios for TBT and TPT are determined for eggs of the secondary carnivorous bird, *S. hirundo*. Typical values of TBT and TPT are 0.01 and 0.18, respectively.

Figure 3.1 shows that field accumulation ratios of TBT and TPT in all species, except in eggs of *S. hirundo*, are 1 to 2 orders of magnitude higher than expected by OMEGA estimations for organic compounds. However, accumulation of TPT is in reasonable agreement with model predictions using metal elimination ($BSAFs_{metal}$) in the lower part of the food chain, i.e for herbi-detritivores and *C. crangon*. Deviations from model predictions are within a factor 8 (Figure 3.1a,b,c). For primary and secondary carnivorous fish deviations are larger, up to a factor 30 (Figure 3.1d,e). For TBT, modeled accumulation ratios with metal elimination are comparable to field BSAFs in the lower part of the food chain. In contrast, tributyltin accumulation in primary and secondary carnivorous fish is significantly overestimated when elimination is modeled as a metal. Accumulation of TBT in eggs of *S. hirundo* is substantially overestimated following both approaches. For TPT, $BSAFs_{metal}$ are 2 orders of magnitude higher than field ratios.
For all species accumulation ratios of DBT are comparable to model estimations using metal elimination. Accumulation of monobutyltin in *A. marina* and *C. edule* is in good agreement with BSAFsmetal. However, for higher trophic level species accumulation is substantially overestimated following this approach (up to 4 orders of magnitude for *S. hirundo*).

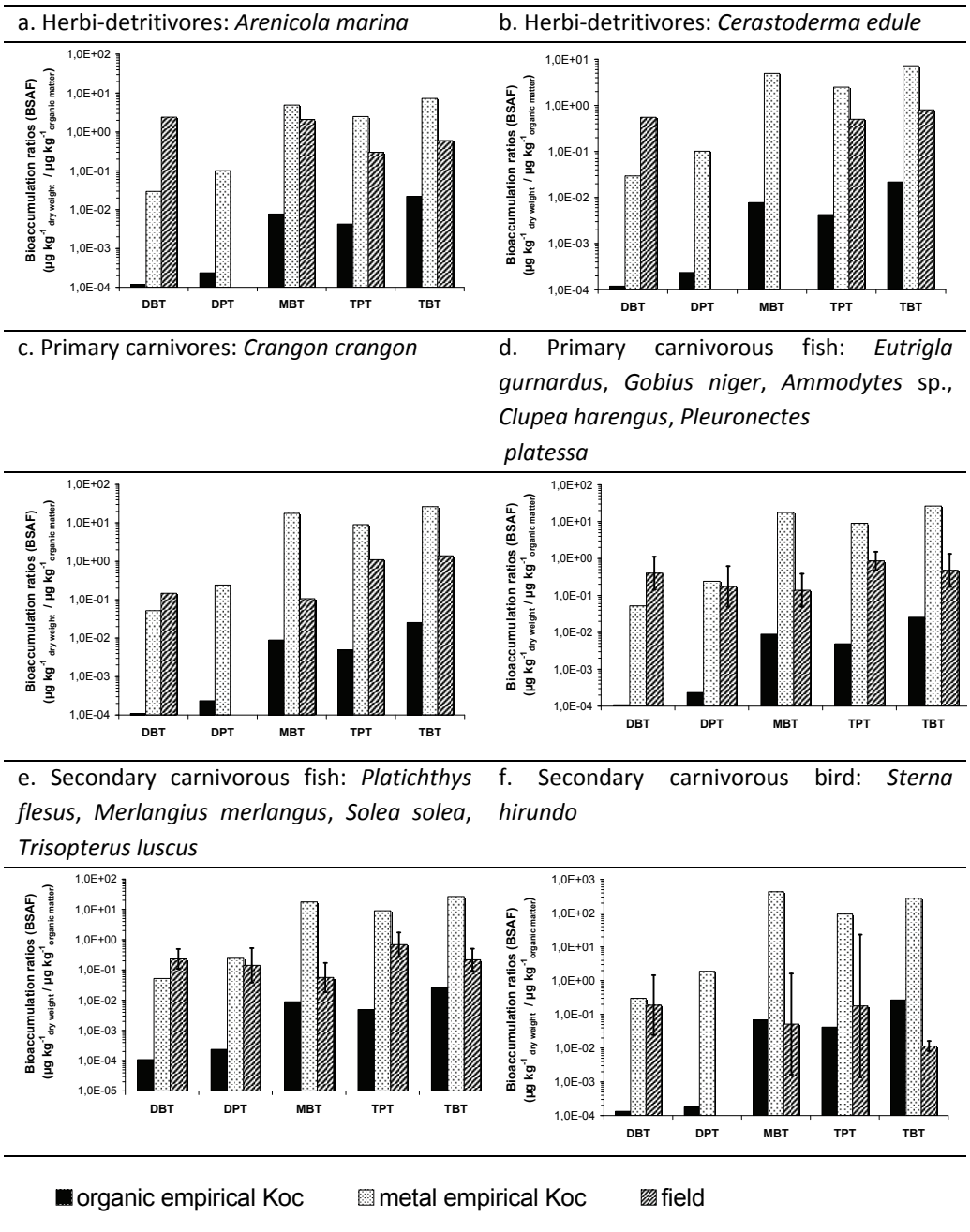
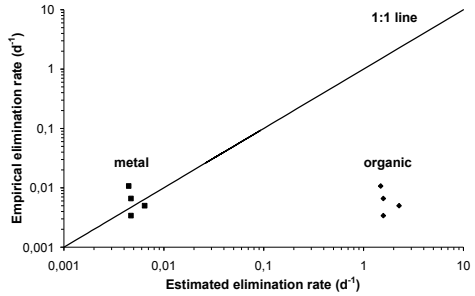


Figure 3.1. Empirical biota-suspended solids-accumulation ratios (BSAF) ($\mu g \cdot kg^{-1}_{\text{dry weight}}$ / $\mu g \cdot kg^{-1}_{\text{organic matter}}$) compared to modeled BSAFs following two approaches: 1) modeling elimination similar to organics and 2) modeling elimination similar to metals. Accumulation ratios are calculated for five organotin compounds: dibutyltin (DBT), diphenyltin (DPT), monobutyltin (MBT), triphenyltin (TPT) and tributyltin (TBT)

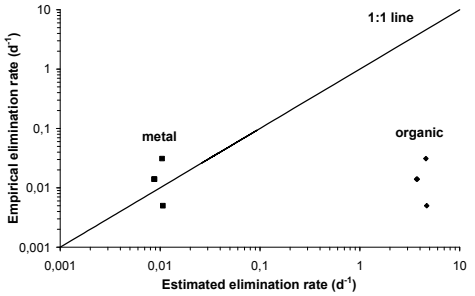
3.5.2 Elimination rate constants

In Figure 3.2 empirical elimination rates of TBT and TPT from literature are compared to model predictions. For herbi-detritivores, i.e. molluscs, (Figure 3.2a) (Shiraishi and Soma, 1992; Stäb et al. 1995, Suzuki et al., 1998) and fish (Figure 3.2b) (Tas, 1993) empirical elimination rates of TPT are in good agreement with model predictions for metals, deviations are within a factor 2. Estimations for organic compounds overestimate the empirical elimination rate by two orders of magnitude.

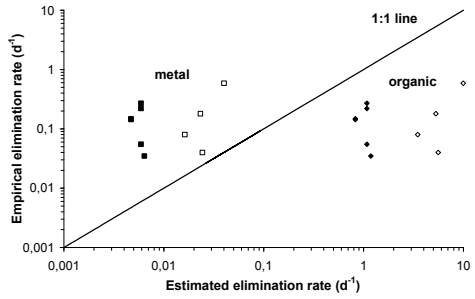
a. Elimination rate constants triphenyltin: detritivores (molluscs)



b. Elimination rate constants triphenyltin: fish



c. Elimination rate constants tributyltin: herbi-detritivores



d. Elimination rate constants tributyltin: fish

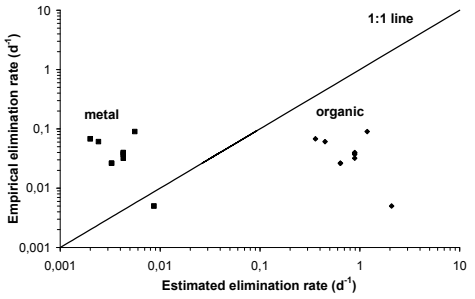
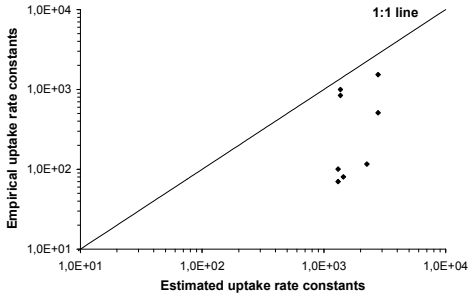


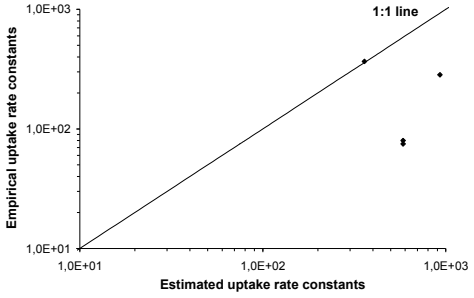
Figure 3.2: Empirical elimination rate constants versus model estimations for metals (■) and neutral organic compounds (◆). Panel c: ■ - elimination metal molluscs, ◆ - elimination organic molluscs, □ - elimination metal polychaeta and amphipods, ◇ - elimination organic polychaeta and amphipods. Line represents theoretical 1:1 relationship (Tas, 1993; Meador, 1997; Krone and Stein, 1999; Yamamoto et al., 1997; Ikeda and Yamada 2003; GomezAriza et al. 1999; Laughlin et al., 1986; Suzuki et al., 1998; Shiraishi and Soma 1992; Stäb et al., 1995; Fent and Looser, 1995)

For molluscs (Figure 3.2c), empirical elimination rates of TBT (Gomez-Ariza et al., 2001; Laughlin et al. 1986, Suzuki et al. 1998) are between a factor of 5 and 34 lower than model estimations for organic compounds. In contrast, elimination is substantially, that is 1 to 2 orders of magnitude, overestimated using metal elimination. For amphipods and polychaeta empirical elimination rates (Meador, 1997, Fent and Looser, 1995) vary with an order of magnitude for the different species (Figure 3.2c). For some species, i.e. *Armandia brevis* and *Eohausterius washingtonianius*, rate constants are comparable to predictions for metals, for other species empirical values are in the middle of model estimations for metals and organics. TBT elimination rate constants for various fish species (Tas, 1993; Meador, 1997; Krone and Stein, 1999; Yamamoto et al., 1997; Ikeda and Yamada, 2003) are in between estimated values for metals and organics (Figure 3.2d). An exception is the elimination of TBT by *Poecilia reticulata* (Tas, 1993), for this species empirical values are more comparable to predictions for metals.

a. Uptake rate constants of tributyltin: polychaeta



b. Uptake rate constants of tributyltin: fish



c. Uptake rate constants triphenyltin: fish

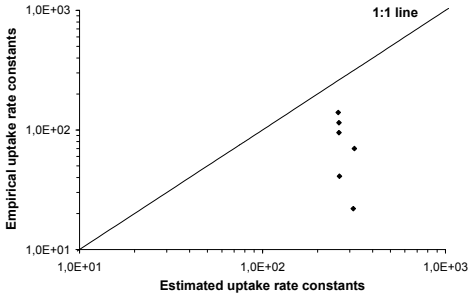


Figure 3.3: Empirical uptake rate constants (µg·kg^{-1} / µg·l^{-1} d^{-1}) (Tas, 1993; Meador, 1997; Fent and Looser ,1995) compared to model predictions for neutral organic compounds

3.5.2 Uptake rate constants

In Figure 3.3, empirical absorption rate constants from literature (Tas, 1993; Fent and Looser, 1995; Meador, 1997) are compared to model estimations for organic substances. TBT uptake by polychaeta is in good agreement with model predictions for most species (Figure 3.3a). However, for the amphipod *Rhepoxynius abronius* (Meador, 1997), uptake is overestimated by a factor 15. Uptake of TBT and TPT by fish (Tas, 1993; Meador, 1997) is overestimated by OMEGA with a maximum factor of 8 and 14, respectively (Figure 3.3b,c).

3.6 Discussion

3.6.1 Accumulation

The observed low accumulation ratios (BSAF) can mainly be attributed to relatively strong binding of organotins to suspended matter (Burton et al., 2004). Additionally, TBT may be present in minute paint particles in suspended matter (Coelho et al., 2002). This TBT fraction is less available for partitioning to the water phase and accumulation is lower than expected.

The highest accumulation ratios of both TBT and TPT are observed in the crustacean *C. crangon*. This is consistent with Takahashi et al. (1999), who found a limited metabolization capacity of TBT in crustaceans. Metabolization may explain lower BSAFs in *C. edule* compared to *C. crangon*. For molluscs' metabolism of TBT may be dependent on season i.e. enhanced biotransformation is suggested in summer (Mensink et al., 1997). As organotin levels in *C. edule* were monitored in spring, metabolization of TBT may be important. No literature evidence on metabolization of TBT by the herbi-detritivore *A. marina* is available. However, comparable TBT levels in *A. marina* with *C. edule* and high concentrations of metabolites DBT and MBT, suggest TBT metabolization capacity of *A. marina*.

TBT is easily metabolized in the liver of fish into DBT (Reader et al., 1996; Stäb et al., 1996), which explains lower accumulation ratios in primary and secondary carnivorous fish compared to *C. crangon*. As a consequence, DBT residues result from both uptake from water and food and metabolization of TBT. TPT is not as easily metabolized as TBT (Stäb et al., 1996). This is consistent with our results as higher accumulation ratios are determined for TPT in fish than for TBT.

Extremely low accumulation ratios are found for eggs of *S. hirundo*. This finding may be explained by the fact that birds are capable of metabolizing TBT as well as TPT (Evers et al., 1995; Stäb et al., 1996). Secondly, seasonal moulting may be an efficient elimination route of organotins. Feathers of cormorants may contain 20-30% of the total body-burden of butyltins (Guruge et al., 1996). Apparently,

feathers contain proteins that have a high affinity for tin. Finally, residues in eggs may not be representative for concentrations in the adult birds as organotins mainly accumulate in liver and kidney of birds (Stäb et al., 1996).

TPT levels in primary carnivorous fish are higher compared to residues in its diet *A. marina*, suggesting biomagnification of TPT. Biomagnification factors (BMF = $[C_i]_{predator}/[C_{i-1}]_{prey}$, concentrations in $\mu g \cdot kg^{-1}$ wet weight) for both TPT and TBT can be calculated for two suggested predator – prey relationships: firstly, for primary carnivorous fish feeding on *A. marina* and secondly for *M. merlangus* and *P. flesus* (adult) feeding on *C. harengus* and *Ammodytes* sp. (Table 3.4). Biomagnification ratios of TPT are substantially higher than those of TBT. A low biomagnification potential of TBT has been shown in various predator-prey relationships (Stäb et al., 1996; Takahashi et al., 1999; Kim et al., 1996). Few studies on biomagnification of TPT are available. However, Mensink et al. (1997) observed biomagnification of TPT in *B. undatum* feeding on *M. edulis* in the Eastern Scheldt estuary. Stäb et al. (1996) also showed magnification of TPT in piscivorous fish in a freshwater lake. In the Western Scheldt food chain biomagnification ratios of TPT for primary and secondary carnivorous fish, approach those of PCBs (Table 3.4). However, accumulation mechanisms of TPT are likely to be different as those for PCBs as discussed in this paper.

Table 3.4: Biomagnification ratios (BMFs) of tributyltin (TBT), triphenyltin (TPT) and polychlorinated biphenyls (PCBs) for two suggested predator-prey relationships in Western Scheldt food chain

Predator - prey	**Biomagnification ratios[1] ($kg_{wet\ weight} \cdot kg^{-1}_{wet\ weight}$) (geometric mean)**		
	TBT	TPT	PCBs[2]
Primary carnivorous fish[3] vs. *Arenicola marina*	0.8	3.4	4.9 - 12
Merlangius merlangus and *Platichthys flesus* vs *Ammodytes* sp. and *Clupea harengus*	0.2	0.4	0.1 – 0.7[4]

1 To allow comparison between organotins and PCBs all magnification ratios are expressed on a wet weight basis

2 PCBs comprise different congeners, therefore a range in BMFs is provided (Veltman et al., 2005).

3 Primary carnivorous fish include *Ammodytidae*, *Eutriglia gurnardus*, *Pleuronectes platessa*, *Clupea harengus*, *Clupeidae* and *Gobius niger*

4 Note that predator fish species are not true secondary carnivores i.e. they feed on herbi-detritivores as well. BMFs of PCBs are therefore lower than one.

3.6.2 Empirical bioaccumulation ratios compared to BSAFs and BCFs from literature

To determine the consistency of accumulation ratios of Western Scheldt species with similar species from other areas, BSAFs are compared to accumulation ratios obtained from literature (Table 3.5). However, as few field BSAFs are available for marine species, laboratory and freshwater studies, were included as well. Additionally, field bioaccumulation factors (C_i/C_{0w}) were estimated for Western Scheldt species, to enable a comparison with empirical BCFs (Table 3.6). Calculated field BAFs are based on empirical internal concentrations and estimated dissolved water concentrations. These field BAFs do include exposure via contaminated food.
Generally, BAFs and BSAFs of TBT are in good agreement with literature values. For fish, only BSAFs for freshwater species were found in literature. These freshwater accumulation ratios were an order of magnitude larger than accumulation ratios for Western Scheldt fish. However, freshwater-BSAFs may not be comparable to marine-BSAFs as speciation of organotins does differ in freshwater i.e. the cationic compounds are more prevalent in freshwater compared to marine water.
In contrary to results for TBT, our TPT bioaccumulation factors for fish are substantially higher than literature BCF values. This is expected, as BCFs obtained from literature are solely from laboratory studies. In these studies, fish are exposed to TPT via the water phase only, i.e. food exposure is excluded. However, determined biomagnification ratios (Table 3.4) provide evidence that intake via food is important for triphenyltin. Consequently, field BAFs of TPT are higher than those determined in a laboratory study, in which exposure via food is not included.

3.6.3 Model estimations

In low trophic level species, both TBT and TPT accumulate more than expected based on their respective K_{ow}, arising from their low elimination. Particularly, for TPT elimination rates for fish and molluscs are 1 to 2 orders of magnitude lower than expected based on the K_{ow}. Moreover, empirical elimination rate constants are comparable to OMEGA estimations for elimination of metals (Figure 3.2a,b). This is consistent with Laughlin et al. (1986), who found a 10-fold excess in TBT-accumulation in *M.edulis* compared to predictions based on the K_{ow}.
Metal elimination rate constants underestimate the true elimination rate of TBT for fishes as well as for various herbi-detritivores. For both fishes and molluscs, elimination rate constants are expected to be higher than model predictions, as TBT is eliminated via biotransformation (Stäb et al., 1996; Mensink et al. 1997), which is not quantified in the model.

Accumulation of both TBT and TPT is overestimated when elimination is modeled as a metal. An explanation, additional to metabolization capacity, is that predicted absorption rate constants are larger than empirical uptake rate constants (Figure 3.3). For low trophic level species overestimation of uptake from water largely explains the overestimation of accumulation ratios. For fishes, overestimation of absorption of TPT explains approximately half of the deviation from field ratios. In the modeling approach it is assumed that uptake of TBT and TPT is related to the K_{ow}. However, membrane-water partitioning of organotins may not be fully related to their partitioning behaviour between a bulk solvent (1-octanol) and water, due to complex formation of organotins with ligands present in membrane constituents (Hunziker et al., 2001), which may result in lower absorption rates.

3.7 Conclusion

Low accumulation ratios (BSAF<1) are observed for all species, except *C. crangon*, as organotins are strongly bound to suspended matter. No magnification of tributyltin (TBT) in the food chain is observed, probably due to substantial biotransformation. In contrast, triphenyltin shows biomagnification potential in fish species, with BMFs approaching those of PCBs. However, accumulation kinetics is different from organochlorines. Our modeling suggests that uptake of organotins occurs mainly via hydrophobic mechanisms, while elimination rates of organotins are more comparable to elimination rates for metals.

3.8 Acknowledgements

The authors thank the following persons: Jan Boon (NIOZ), Joost Stronkhorst (DGW), Martin Eggens (RIKZ), Joop Bakker (RIKZ), Gert-Jan Zwolsman (KIWA), Belinda Kater (RIKZ) and Ton van der Zande (RIKZ) for providing monitoring data and Theo Traas (RIVM) for comments on the manuscript

Table 3.5:Predicted biota-organic solids- accumulation factors (BSAF in kg organic dry weight / kg dry weight) of tributyltin (TBT) in Western Scheldt species compared to BSAFs from literature

Western Scheldt species	**BSAF predicted** ($kg_{dw} \cdot kg^{-1}_{dw}$)	**BSAF literature** ($kg_{dw}\ kg^{-1}_{dw}$)	**Species**	**Location**	**References**
Arenicola marina	0.6	0.03 – 0.4	*Armandia brevis* (polychaete)	Laboratory	Meador and Rice, 2001
Cerastoderma edule	0.8	0.9 – 1.5	*Potamocorbula amurensis* (Asian clam)	Mare Island, San Fransisco Bay	Pereira et al.,1999
		4.6 – 6.9	*Nuculana pernula* (northern nutclam)	Subtidal zone of waters between Denmark and Sweden	Strand et al., 2003
Primary and secondary carnivorous fish	0.2 – 0.5	4.8	*Perca fluviatilis* (perch), *Esox lucius* (pike), *Stizostedion lucioperca* (pike perch)	Lake Westeinder, the Netherlands (freshwater)	Stäb et al., 1996

Table 3.6: Empirical bioconcentration factors (BCF in $L \cdot kg^{-1}_{wet\ weight}$ and $L \cdot kg^{-1}_{dry\ weight}$) compared to predicted BAFs for Western Scheldt species.

Western Scheldt species	**Compound**	**BAF** predicted ($L \cdot kg^{-1}_{dw}$)	**BCF** empirical ($L \cdot kg^{-1}_{dw}$)	**BAF** predicted ($L \cdot kg^{-1}_{ww}$)	**BCF** empirical ($L \cdot kg^{-1}_{ww}$)	**Species**	**Location**	**References**
A. marina	tributyltin	$2.6 \cdot 10^4$	$1.3 \cdot 10^3$ – $2.2 \cdot 10^3$	$6.1 \cdot 10^3$		*Rhepoxynius abronius* (amphipod) *Armandia brevis* (polychaete) *Eohaustorius washingtonianus* (amphipod)	Laboratory	Meador, 1997
C. edule	tributyltin	$3.4 \cdot 10^4$	$1.7 \cdot 10^4$- $>4.8 \cdot 10^5$	$3.4 \cdot 10^3$		*Crassostrea angulata* (Portuguese oyster), *Cerastoderma edule* (common cockle), *Venerupis decussate* (carpet shell clam), *Venerupis semidecussata* (carpet shell clam), *Chamelea gallina* (striped venus), *Ruditapes filipinaris* (clam), *Mytilus galloprovincialis* (Mediterranean mussel), *Patella vulgata* (common limpet)	Southwest of Spain	Gomez-Ariza et al., 2001
					$5.1 \cdot 10^3$	*Mytilus edulis* (blue mussel)	North sea	Shawky and Emons, 1998
					$1.0 \cdot 10^4$	*Mytilus graynus* (mussel), *Mytilus edulis* (blue mussel)	Laboratory	Suzuki et al., 1998
					$7.7 \cdot 10^3$ – $1.1 \cdot 10^4$	*Mytilus edulis* (blue mussel)		Guolon and Yong, 1995

Crangon crangon	tributyltin	$6.0 \cdot 10^4$		$1.5 \cdot 10^4$	$1.2 \cdot 10^3$ - $7.7 \cdot 10^3$	*Caprella* spp. (skeleton shrimp)	Otsuchi bay, Japan	Takahashi et al., 1999
Primary carnivorous fish	tributyltin	$2.7 \cdot 10^4$		$6.7 \cdot 10^3$	$4.0 \cdot 10^3$ – $1.4 \cdot 10^4$	*Lateolabrax japonicus* (Japanese perch), *Pennehia argentatus* (white croaker), *Seriola quinqueradiata* (yellowtail), *Trichiurus japonicus* (bandfish), *Sillago japonica* (silver whiting), *Psenopsis anomala* (butter fish), *Stephanolepis cirrhifer* (filefish), *Takifugu xanthopterus* (triped puffer) (muscle)	Osaka Bay, Japan	Harino et al., 2000
Secondary carnivorous fish	tributyltin	$1.1 \cdot 10^4$		$2.1 \cdot 10^3$	$3.2 \cdot 10^3$- $1.1 \cdot 10^4$	*Pagrus major* (red sea bream) (whole-body) *Mugil cephalus* (mullet) *Rudarius ercodes* (filefish)	Laboratory	Yamada and Takayanagi, 1992
					$1.1 \cdot 10^3$ - $6.0 \cdot 10^4$	*Synodus foetens* (lizard fish) (muscle and liver) *Leiognathus elongates* (ponyfish) (whole-body)	coastal waters of Taiwan	Dong et al., 2004
			$1.0 \cdot 10^4$			*Pagrus major* (red sea bream) (liver)	Laboratory	Yamamoto et al., 1997
					$1.9 \cdot 10^3$ – $3.1 \cdot 10^3$	*Dicentrachus labrax* (seabass) (liver)	Laboratory	El Hassani et al., 2005
			$2.1 \cdot 10^4$			*Platichthys stellatus* (starry flounder)	Laboratory	Meador, 1997

Cerastoderma edule	triphenyltin	$2.4 \cdot 10^4$	$2.4 \cdot 10^3$	$3.6 \cdot 10^4$ – $4.3 \cdot 10^4$	*Mytilus graynus* *Mytilus edulis* (blue mussel)	Laboratory	Suzuki et al., 1998
Primary carnivorous fish	triphenyltin	$5.1 \cdot 10^4$	$1.2 \cdot 10^4$	$3.1 \cdot 10^3$ – $3.3 \cdot 10^3$	*Pagrus major* (red sea bream) (whole-body) *Rudarius ercodes* (filefish)(whole-body)	Laboratory	Yamada and Takayanagi, 1992
Secondary carnivorous fish		$4.6 \cdot 10^4$	$9.3 \cdot 10^3$	$6.6 \cdot 10^2$ – $9.0 \cdot 10^2$	*Dicentrachus labrax* (seabass) (liver)	Laboratory	El Hassani et al., 2005

Chapter 4.

Metal accumulation in the earthworm *Lumbricus rubellus*. Model predictions compared to field data

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4.1 Abstract

The mechanistic bioaccumulation model OMEGA (Optimal Modeling for Ecotoxicological Applications) is used to estimate accumulation of zinc (Zn), copper (Cu), cadmium (Cd) and lead (Pb) in the earthworm *Lumbricus rubellus*. Our validation to field accumulation data shows that the model accurately predicts internal cadmium concentrations. In addition, our results show that internal metal concentrations in the earthworm are less than linearly (slope < 1) related to the total concentration in soil, while risk assessment procedures often assume the biota-soil accumulation factor (BSAF) to be constant. Although predicted internal concentrations of all metals are generally within a factor of five compared to field data, incorporation of regulation in the model is necessary to improve predictability of the essential metals zinc and copper.

4.2 Introduction

Ecological risk assessment of metal-polluted ecosystems is commonly based on total soil metal concentrations (Crommentuijn et al., 2000; Lock and Janssen, 2001). However, total concentrations do not necessarily indicate bioaccumulation and toxicity of metals to biota. In the first place, soil specific parameters as pH and organic matter content can strongly determine the chemical availability of metals in soils (Sauvé et al., 2000). Secondly, the metal fraction available for uptake by biota is dependent on the exposure route. Soft-bodied, soil-dwelling organisms are exposed to metals either through direct dermal contact with metals in soil solution or by ingestion of bulk soil or specific soil fractions (Lanno et al., 2004). Finally, some species may limit bioaccumulation of some metals by active excretion and / or reducing uptake, thereby maintaining low metal body burdens even at high exposure concentrations (Rainbow, 2002). Most species, however, cannot regulate bioaccumulation of non-essential metals (Rainbow, 2002; Cain et al., 2004). These organisms may prevent toxicity by effectively storing metals in non-toxic forms, i.e. bound to metal-binding proteins like metallothionein (MT) or incorporated in non-soluble granules (Vijver et al., 2004). Species relying on sequestration as detoxification mechanism may excessively accumulate metals with increasing exposure concentrations without suffering toxic effects. Adverse effects may occur, however, when the capacity of the detoxification mechanism is exceeded (Lock and Janssen, 2001; Rainbow, 2002).

Consequently, information of metal accumulation studies may differ widely, depending on the ecosystems, species and conditions (lab, field) investigated. Bioaccumulation models provide insight in metal- and species-specific differences

in accumulation kinetics and facilitate interpretation of field data. Additionally, models allow prediction for other cases. Various bioaccumulation models have been developed for aquatic (Luoma and Rainbow, 2005; Steen Redeker et al., 2004) and terrestrial species (Saxe et al., 2001; Heikens et al., 2001). Here, we use the OMEGA (Optimal Modeling for Ecotoxicological Applications) model developed for studying accumulation of metals and neutral organic substances in terrestrial and aquatic food chains. OMEGA has been successfully applied to estimate accumulation of hazardous substances in freshwater, marine and terrestrial communities (Hendriks et al., 2001; Hendriks and Heikens, 2001; Veltman et al., 2005; Veltman et al., 2006). The added value of OMEGA in comparison to most other bioaccumulation models is that estimations of uptake and elimination rate constants are based on allometric and biochemical transport principles. This facilitates extrapolation to other soils, contaminant levels, and species and even other metals, without data intensive and case specific calibration.

The aim of the present investigation is two-fold. On the one hand, we want to validate OMEGA for accumulation of zinc, copper, cadmium and lead in the earthworm *Lumbricus rubellus*, thriving in floodplains. On the other hand, we want to check whether field data from a large monitoring program are consistent with data sets on which the model was calibrated. Elimination kinetics were modeled following two approaches. Firstly, a maximum elimination rate was obtained by adding the rate constants of excretion, egestion and "biomass dilution". Secondly, earthworms may sequester metals in an irreversible form, yielding a minimum elimination rate, assuming release via biomass dilution only. Pore water-mediated dermal uptake of metals is assumed to be the dominant exposure route for earthworms, based on research of Vijver et al. (2003) and Saxe et al. (2001). Vijver et al. (2003) showed that earthworms with sealed mouths accumulated almost equal amounts of metals as "normal" earthworms, suggesting that uptake via the skin is the main exposure route. We did not model dietary uptake of metals due to lack of information on the metal fraction in the organic matter phase of solids, for most metals, and the (metal-specific) assimilation efficiency from the gut of this fraction. In our mechanistic model approach, this information is required, as it is known that earthworms selectively feed on specific soil fractions that are rich in organic matter (Bolton and Phillipson, 1976). Internal concentrations predicted by OMEGA were compared to field accumulation data in earthworms from different floodplain soils in the Netherlands. To justify future application of the model for different soil types or other areas, monitoring data from various locations in the Netherlands were included too (n = 9 – 15 locations, see Supporting Information). We studied the essential metals, copper and zinc, and the non-essential metals,

cadmium and lead. Regression analysis was used to determine the relationship between earthworm metal concentrations and external concentrations. The latter comprises both total soil levels and pore water concentrations.

4.3 Methods

4.3.1 Model predictions of internal concentrations

Earthworms predominantly accumulate metals via pore water–mediated dermal uptake (Vijver et al., 2003; Saxe et al., 2001). Ingestion of soil is therefore excluded as a route of metal uptake for earthworms and steady-state internal concentrations are calculated as the influx via water (absorption), divided by total elimination rate (sum of excretion with water, egestion with feces and growth dilution) (Eqn. 4.1).

$$C_{i,x} = \frac{k_{x,w,in} \cdot C_{0w,x}}{k_{x,w,ex} + k_{x,n,ex} + k_9}$$ Equation 4.1

$C_{i,x}$	=	Metal concentration in *Lumbricus rubellus*	[kg·kg^{-1} $_{\text{wet weight}}$]
$C_{0w,x}$	=	Metal concentration in pore water	[kg·L^{-1}]
$k_{x,w,in}$	=	Metal absorption rate constant	[kg·kg^{-1} $_{\text{wet wt}}$·d^{-1}/ kg·L^{-1}]
$k_{x,w,ex}$	=	Metal excretion rate constant	[d^{-1}]
$k_{x,n,ex}$	=	Metal egestion rate constant	[d^{-1}]
k_g	=	Growth dilution rate constant	[d^{-1}]

4.3.2 Uptake and elimination kinetics

In contrast to neutral organic substances, predicted uptake rate constants of metals depend on the exposure concentration (Lock and Janssen, 2001). Metals are transported through membranes by protein-carriers or protein-channels (Bryan, 1984; Foulkes, 2000; Hendriks and Heikens, 2001). At high external concentrations, availability of these carriers may become limited and uptake rates will decrease. Therefore, lipid layer resistance for influx via absorption is defined as a function of the exposure concentration (Eqn. 4.2), analogous to Michaelis-Menten kinetics for enzymes or Langmuir kinetics for sorption.

By movement through moist soils earthworms efficiently exchange substances with water via their skin. Hence, the total delay imposed by the water flux in earthworms is less than that in hard-bodied organisms where exchange with water is limited by drinking and excretion. This is accounted for by using a value of 200 for

γ_0 (similar to aquatic species), instead of the typical value of 0.2 for terrestrial species (Hendriks and Heikens, 2001).

$$k_{x,w,in} = \frac{1}{\left(\rho_{H2O,0} + \rho_{CH2,w,in} \cdot C_{0w,x}^{\kappa_p} + \frac{1}{\gamma_0}\right) \cdot w^{\kappa}}$$ Equation 4.2

$\rho_{H2O,0}$	=	Water layer diffusion resistance ($2.8 \cdot 10^{-3}$)	[$d \cdot kg^{-\kappa}$]
$\rho_{CH2,w,in}$	=	Lipid layer resistance for influx of metals from water ($1.0 \cdot 10^{-3}$)	[$d \cdot kg^{-\kappa}$]
γ_0	=	Water absorption – excretion coefficient (200)	[$kg^{\kappa} \cdot d^{-1}$]
κ	=	Rate exponent (0.25)	[-]
κ_p	=	Lipid layer resistance exponent (0.41)	[-]
w	=	Species wet weight ($2.6 \cdot 10^{-3}$)	[kg]

Elimination of metals may occur via three different pathways: excretion with water (Eqn. 4.3), egestion with feces (Eqn 4.4) and dilution with biomass (Eqn 4.5). Egestion with feces is included in the model, as metals may be egested with feces, irrespective of dietary uptake. Biomass dilution includes individual growth as well as reproduction and replacement of tissues. Generally, metals are excreted slowly if they are bound to metal-binding proteins in soft tissues and / or incorporated in hard tissues, such as shells, feathers, and fur (Hendriks and Heikens, 2001). Binding to dry tissues is incorporated in OMEGA as a generic tissue - water distribution coefficient (K_{tw}). This coefficient describes the affinity of metals for dry tissue and is derived from calibration on thousands of metal accumulation ratios from laboratory and field studies (Hendriks and Heikens, 2001).

$$k_{x,w,ex} = \frac{1}{K_{tw} \cdot p_{s,i}} \cdot \frac{1}{\left(\rho_{H2O,0} + \rho_{CH2,ex} + \frac{1}{\gamma_0}\right) \cdot w^{\kappa}}$$ Equation 4.3

$$k_{x,n,ex} = \frac{1}{K_{tw} \cdot p_{s,i}} \cdot \frac{1}{\left(\rho_{H2O,faeces} + \rho_{CH2,ex} + \frac{1}{K_{tw} \cdot p_{s,i-1} \cdot (1 - f_{as}) \cdot \frac{(\gamma_2 + \gamma_{resp} \cdot q_{ap})}{f_{as}}}\right) \cdot w^{\kappa}}$$ Equation 4.4

$$k_g = \gamma_2 \cdot w^{-\kappa}$$ Equation 4.5

K_{tw}	=	Dry-tissue water partition coefficient ($8.0 \cdot 10^3$)	[$kg \cdot kg^{-1}$ $_{dry\ wt}$ / $kg \cdot L^{-1}$]
f_{as}	=	Fraction of food assimilated (40%)	[-]
$p_{s,i}$	=	Dry weight fraction of *Lumbricus rubellus* (i) (15%)	[$kg_{\ dry\ wt}$ / $kg_{\ wet\ wt}$]
$p_{s,i-1}$	=	Dry weight fraction of diet (i-1) (10%)	[$kg_{\ dry\ wt}$ / $kg_{\ wet\ wt}$]
$\rho_{CH2,ex}$	=	Lipid layer resistance for efflux of metals (0.3)	[$d \cdot kg^{-\kappa}$]
$\rho_{H2O,feces}$	=	Water layer resistance to feces ($1.1 \cdot 10^{-5}$)	[$d \cdot kg^{-\kappa}$]
γ_2	=	Biomass (re)production coefficient ($7.5 \cdot 10^{-4}$)	[$kg^{\kappa} \cdot d^{-1}$]
γ_{resp}	=	Average respiration rate coefficient ($7.5 \cdot 10^{-4}$)	[$kg^{\kappa} \cdot d^{-1}$]
q_{ap}	=	Animal to plant respiration coefficient (6.0)	[-]

Uptake rate constants are assumed to be metal independent because empirical absorption rates show little metal–specific variation when averaged over different species (Hendriks and Heikens, 2001). Earthworms rely on sequestration to prevent damage of metals (Vijver et al., 2006). This affects metal elimination kinetics because strongly bound substances are likely hardly eliminated via excretion or egestion. Therefore, elimination is modeled in two ways: Firstly, a maximum elimination rate is calculated as the sum of excretion, egestion and dilution with biomass. Secondly, a minimum elimination rate is obtained by assuming that metals are only eliminated by "dilution with biomass". In other words, tight binding to proteins or storage in detoxified forms is incorporated in the model, by increasing the value of K_{tw} (Eqn. 4.3 and 4.4). As a result, excretion and egestion rate constants (Eqn. 4.3 and 4.4) approximate zero, and the total modeled elimination rate equals the rate for biomass dilution. These maximum and minimum elimination rates are assumed to be uniform constants for all metals. The approach results in two estimated internal concentrations for each soil concentration.

OMEGA requires dissolved pore water concentrations to predict internal metal concentrations. Pore water concentrations are estimated using a semi-mechanistic adsorption model that accounts for pH, total soil metal concentrations and soil organic matter content (Sauvé et al., 2000). This adsorption model was derived from a large variety of soils, with a pH range that includes the values noted in our soils (Sauvé et al., 2000). The pore water concentrations predicted by the Sauvé model are comparable to estimated levels using solid-solution partitioning regressions specific for floodplain soils (data not shown) (Schröder et al., 2005). The model of Sauvé et al. (2000) was used instead of the Schröder-regressions, as we

included accumulation data of non-floodplain soils (Appendix 4) and this generic model allows extrapolation to other soil-types.

4.3.3 Data collection and treatment

Field data on the accumulation of metals in the earthworm species *L. rubellus* and total soil concentrations were collected from five studies: Hendriks et al. (1995), Hobbelen et al. (2004), van Vliet et al. (2005), Koolhaas (unpublished data) and Peijnenburg (unpublished data). The first four studies comprise accumulation data of different Dutch floodplain soils, namely two locations of the river Rhine delta (Gelderse Poort and Ochten) (Hendriks et al., 1995), the Biesbosch (Hobbelen et al., 2004), and the Afferdensche and Deestsche Waarden (van Vliet et al., 2005). The data of Peijnenburg represent different locations in the Netherlands, including various soil types.

95%-Confidence intervals were obtained using samples from the same location. For Biesbosch data these confidence intervals represent the "individual" variability in metal concentrations in L. rubellus. For data of Peijnenburg the 95%-confidence intervals represent the seasonal variability in earthworm metal body concentrations. Information on variation in total soil concentrations, pH and organic matter fraction is provided in the supporting information.

Analytical procedures were carried out as described in previous papers (Hendriks et al., 1995; Hobbelen et al., 2004; van Vliet et al., 2005). Analytical methods used by Koolhaas are described in Hobbelen et al. (2004). For data of Peijnenburg, soils were characterised in terms of pH(pw) and loss-on-ignition (indicated as: LOI, in units of: %). LOI is considered representative of the organic matter content of the solid phase and was determined from the weight loss of approximately 5 g of dried soil (105 °C), heated at 550 °C for 3 hours. pH(pw) was determined directly in the pore water, with a pH glass electrode Sentix® stored in a buffer solution, employing an electronic voltmeter (pH meter) against a saturated solution of KCl.

For metal analysis in soils by Peijnenburg, about 1.0 g of ground air-dry soil was weighed into a microwave digestion bomb and 4 ml concentrated nitric acid and 12 ml concentrated hydrochloric acid were added to each bomb. The soil samples were digested in a microwave oven (CEM corporation-MDS 2000) for 1 hour at 180 psi. Following cooling of the samples, the solution was quantitatively transferred into a volumetric flask and diluted to a final volume of 100 mL with milli-Q water. This solution was passed through a 0.45-μm filter. For reference purposes, seven blanks and seven standard soils were digested simultaneously. Metal cation concentrations in the sieved solution were determined by ICP-AES (Spectro Analytical Instruments, Kleve Germany). Adult, clittelate, earthworms were

collected by means of hand sorting from the top 10 cm layer of the soil. They were allowed to void their guts on wetted filter paper during 48 hours in the dark at 15 °C. Subsequently, the worms were euthanized at –18 °C and stored at this temperature until they were thawed and dried on a paper tissue. Earthworm tissues were digested overnight in concentrated HNO3 at 100 °C, after which the remaining acid was removed by boiling. The remaining material was dissolved in 0.1 M HNO3 and metal concentrations in the digest were determined by means of ICP-AES (Spectro Analytical Instruments, Kleve, Germany). Average dry weight of the organisms (15%) was determined by means of freeze-drying 10 species during 48 hours.

In the Afferdensche and Deestsche Waarden and some locations in the Biesbosch, soil metal contents were determined at different depths. As *L. rubellus* is an epigeic, i.e. surface-dwelling earthworm (Bouché, 1977), total metal concentrations in the top-soil layer (0–10 cm) were included from these studies.

The adsorption model requires organic carbon content, soil solution pH and total metal concentration in soils, to estimate the metal pore water concentration. In all studies, total metal concentrations in soil were measured in aqua regia extracts. Measured organic matter (OM) fractions were converted to organic carbon fractions assuming 17% of the OM-fraction in soils exists of organic carbon (EC, 2004). pH-$CaCl_2$ was measured in each study except for Hendriks et al. (1995). Pore water pH was calculated from these pH-$CaCl_2$ according equation 4.6 (Peijnenburg et al., 2001). For data from Hendriks et al. (1995) a pore water pH–value of 7 was used, which is the average pH for floodplain soils (Schröder et al., 2005).

$$pH_{pw} = \frac{pH_{CaCl_2}}{1.13} + 1.02$$

Equation 4.6

pH_{pw}	=	pore water pH	[-]
$pHCaCl_2$	=	$CaCl_2$ pH	[-]

Linear regression analysis was performed to relate earthworm metal concentrations to both total soil levels and estimated pore water concentrations. The regression equations were optimized using a linear least squares fit to find appropriate values for the slope (a) and intercept (b) of the regressions. Apart from the regression parameters a and b, the correlation coefficient (r^2) and the residual standard error (SE) were derived. All data were log-transformed in order to normalize their distribution. To compare empirical regression equations with

predictions of OMEGA, model equations were rewritten in a form similar to the regression equations.

4.4 Results

4.4.1 Empirical data

Figure 4.1 and 4.2 show that earthworm metal concentrations are not linearly related (slope<1) to total soil concentrations and estimated pore water concentrations. Compared to the non-essential metal cadmium, internal concentrations of zinc and copper increase slightly with increasing total soil concentrations (Figure 4.1a and b). The slope of the regression line is approximately 0.27 for both metals. A relatively steep slope (> 0.43) is observed for cadmium and lead (Figure 4.1c and d). Zinc concentrations in *L. rubellus* range from 500 to 1906 $mg \cdot kg^{-1}$ dry body weight, except of one high concentration of 3653 $mg \cdot kg^{-1}$ dry weight. Substantially lower internal levels are observed for copper, cadmium and lead (Figure 4.1b, c and d). Copper concentrations range between 6 and 72 $mg \cdot kg^{-1}$ dry body weight. Cadmium concentrations in *L. rubellus* vary between 2 and 154 $mg \cdot kg^{-1}$ dry body weight and lead concentrations are within 3 and 132 $mg \cdot kg^{-1}$ dry body weight.

A significant correlation is found between earthworm metal concentrations and total soil concentrations ($p<0.001$) (Table 4.1). Particularly, for copper and cadmium the explained variance is relatively high (r^2 of 0.59 and 0.65, respectively). In addition, there is a statistically significant relationship between internal concentrations of the metals Zn, Cd, Cu and Pb, and estimated pore water concentrations ($p<0.05$) (Table 4.2), with an explained variance between 11 and 47%. The explained variance found for lead is relatively low ($r^2 = 0.11$) compared to the other metals.

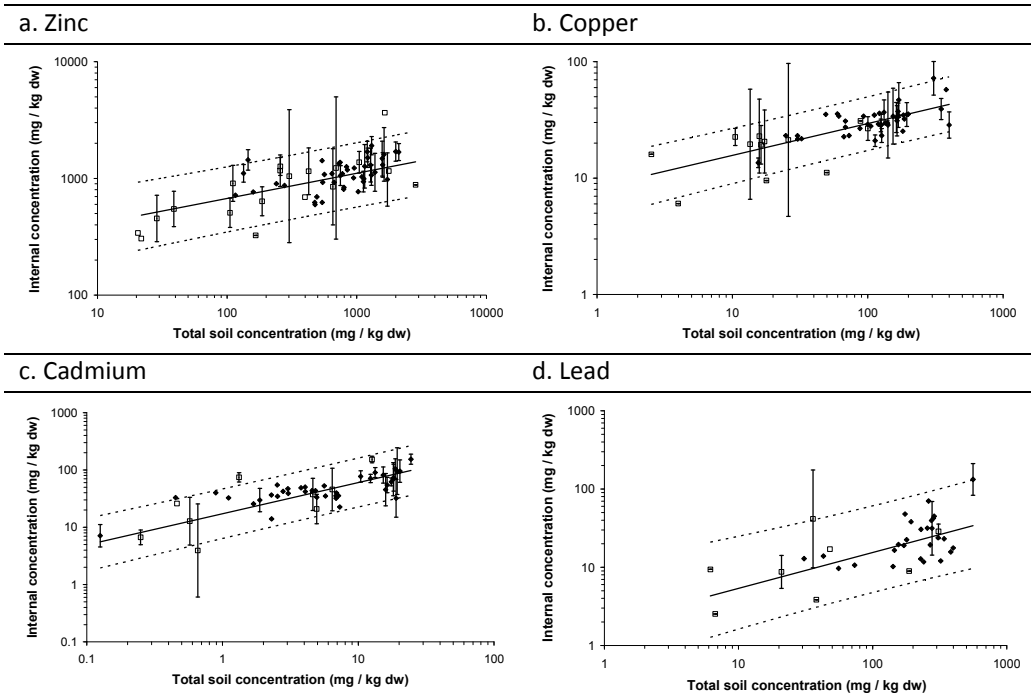

Figure 4.1. Measured internal metal concentrations in *Lumbricus rubellus* (C_i, $mg \cdot kg^{-1}$ dry body weight) plotted against measured total soil concentrations (C_{soil}, $mg \cdot kg^{-1}$ dry soil). ◆ data from Hendriks et al. (1995), Hobbelen et al. (2004) and van Vliet et al. (2005) (Rhine, Biesbosch, Afferdensche and Deestsche Waarden), □ unpublished data from Peijnenburg (various locations in the Netherlands), full line represents regression. Dashed lines represent 97.5th and 2.5th percentile of the field data. 95% confidence intervals are plotted when possible. For three data points confidence intervals were too large to plot in the figure. These data points (C_{soil}, C_i) are: cadmium (0.46, 25.9), lead (48.1, 17.1), copper (380, 57.6).

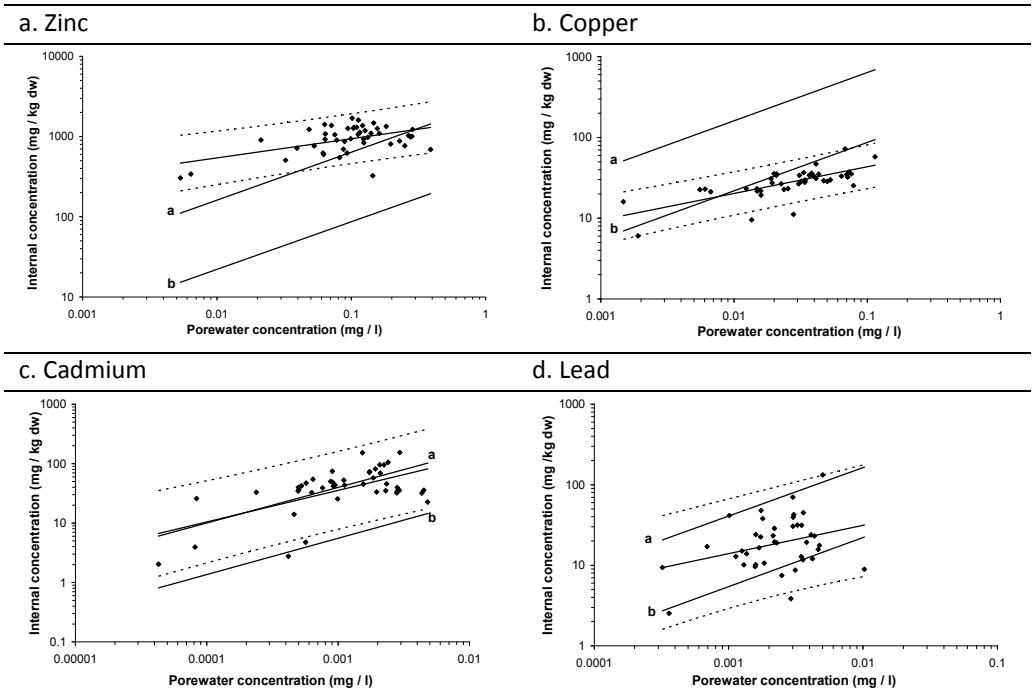

Figure 4.2. Measured internal concentrations in *Lumbricus rubellus* (C_i, $mg \cdot kg^{-1}$ dry body weight) plotted against estimated pore water concentrations (C_{pw}, $mg \cdot L^{-1}$), compared to OMEGA model predictions. Full line represents regression for field data. The full lines a and b represent, respectively, (a) minimum elimination (growth dilution only) and (b) maximum elimination (sum of egestion, excretion and growth dilution). Dashed lines represent 97.5th and 2.5th percentile of the field data. See text for further explanation.

Table 4.1: Correlation analysis for internal metal concentrations in *Lumbricus rubellus* (C_i, $mg \cdot kg^{-1}$ dry body weight) with total soil concentrations (C_s, $mg \cdot kg^{-1}$ dry soil), using data from Hendriks et al. (1995); Hobbelen et al. (2004); van Vliet et al. (2005) and Peijnenburg (unpublished data)

Metal	**Log (C_i)**	r^2	***SE***	***p***
Zn[a]	$0.27 \cdot Log(C_s) + 2.3$	0.49	0.19	< 0.001
Cd	$0.54 \cdot Log(C_s) + 1.23$	0.65	0.24	< 0.001
Cu	$0.28 \cdot Log(C_s) + 0.91$	0.59	0.11	< 0.001
Pb	$0.43 \cdot Log(C_s) + 0.38$	0.41	0.25	< 0.001

[a]One data point (C_s = 2100 $mg \cdot kg^{-1}$ dry soil, C_i =155 $mg \cdot kg^{-1}$ dry body weight) was identified as an outlier, and therefore excluded from the regression analysis.

Table 4.2: Correlation analysis for measured internal metal concentrations in *Lumbricus rubellus* (C_i, $mg \cdot kg^{-1}$ dry body weight) with estimated pore water concentrations (C_{pw}, $mg \cdot L^{-1}$). Also included are regression equations estimated with the OMEGA model, using minimum and maximum elimination. See text for further explanation.

Metal		Log (C_i)	r^2	*SE*	*p*
Zn[a]	Empirical	$0.23 \cdot Log(C_{pw}) + 3.19$	0.22	0.15	<0.001
Cd	Empirical	$0.53 \cdot Log(C_{pw}) + 3.14$	0.37	0.32	<0.001
Cu	Empirical	$0.33 \cdot Log(C_{pw}) + 2.0$	0.47	0.13	<0.001
Pb	Empirical	$0.37 \cdot Log(C_{pw}) + 2.24$	0.11	0.31	0.03
[Me]	OMEGA (min. elimination)	$0.60 \cdot Log(C_{pw}) + 3.4$			
	OMEGA (max. elimination)	$0.60 \cdot Log(C_{pw}) + 2.6$			

[a]One data-point (C_{pw} = 0.45 $mg \cdot L^{-1}$, C_s = 2100 $mg \cdot kg^{-1}$ dry soil, C_i =155 $mg \cdot kg^{-1}$ dry body weight) was identified as an outlier, and therefore excluded from the regression analysis.

4.4.2 Model predictions

Accumulation of cadmium in *L. rubellus* is accurately predicted by OMEGA using a minimum elimination rate. The deviations between model estimations and field data are within a factor 3 (with exception of two data points, which are within a factor 8) (Figure 4.2c). The slope of the empirical regression line is comparable to the slope of the model line, i.e. 0.53 versus 0.60, respectively. A large variation in internal lead concentrations is observed (Figure 4.2d) and model predictions with both maximum and minimum elimination rate are within the 97.5th and 2.5th percentile of the field data. For both essential metals, the slope of the empirical regression line is lower than expected by OMEGA, i.e. 0.23 for Zn and 0.33 for Cu compared to a value of 0.60 predicted by the model. Earthworm zinc concentrations are underestimated by OMEGA at low pore water concentrations, with a maximum factor of 3 (Figure 4.2a). Internal copper concentrations in *L. rubellus* are best predicted using a maximum elimination rate (Figure 4.2b).

4.5 Discussion

4.5.1 Empirical regressions

Our results show that internal metal concentrations are statistically significantly correlated to total metal concentrations in soil, as observed in several other studies (Hobbelen et al., 2004; Marinussen et al., 1997; Becquer et al, 2005; Dai et al., 2004). The field data, especially of cadmium and lead, show a relatively large scattering, which partly results from including accumulation data from various soil types, with significant differences in pH and organic matter content (data of

Peijnenburg). This results in differences in bioavailability of metals and consequently, variance in accumulation.

The significant relationship between internal concentrations in earthworms and total soil levels, is not necessarily contradicting the assumption that uptake via the skin is the major exposure route. Total soil concentrations are relatively stable in time and may therefore be a good predictor of metal accumulation in chronically exposed earthworms via pore water. Additionally, a statistically significant relationship between internal concentrations and estimated pore water concentrations is observed for all metals. Coefficients of determination (r^2) are somewhat lower for the relation between internal metal concentration in *L. rubellus* and estimated pore water concentrations than for the relationship with total soil levels. This probably arises from additional uncertainty introduced by estimating pore water concentrations with the adsorption model. Furthermore, bioavailability of metals is influenced by speciation and various metal species may have different contributions to the total uptake (Chuang and Wang, 2006; Vink, 2002). Regressions may therefore be improved considering different metal species instead of total dissolved pore water concentrations.

4.5.2 Model predictions

Figure 4.2 shows that OMEGA accurately predicts Cd accumulation using a minimum elimination rate and that internal Cu concentrations are in reasonable agreement with model predictions. However, for Zn and Pb the model has less predictability. Deviations between model estimations and field data may result from the assumption that total dissolved metal concentrations are bioavailable for uptake by earthworms, whereas Chuang and Wang (2006) showed that various metal species have different contributions to the total uptake. Also predicting pore water concentrations by the Sauvé model may explain differences between model estimations and field data. We compared pore water concentrations estimated with the Sauvé model with those predicted by K_p-regressions developed for floodplain soils (Schröder et al., 2005) (data not shown). The predicted pore water concentrations are comparable for cadmium and copper, i.e. within a factor 2 (excluding one exception for Cd which is within a factor 5). Model predictions, and conclusions, are not influenced using Schröder regressions instead of the Sauvé model, for cadmium, copper and zinc. However, for lead, pore water concentrations estimated with Schröder regressions tend to be lower (factor 5-12) than predictions with the Sauvé model. A low coefficient of determination (r^2 = 0.10) is found for the relation between earthworm lead levels and estimated pore water concentrations. This may partly be attributed to the lower predictability of

lead pore water concentrations compared to other metals (Sauvé et al., 2000). Due to the large variability in internal concentrations and the lower predictability of pore water concentrations conclusions on lead accumulation cannot be drawn.
For the essential metals zinc and copper, the slope of the field regression line is lower than expected by OMEGA, i.e. approximately 0.30 versus 0.60. This can be explained by regulation of internal concentrations of these metals. Regulation of zinc has been observed in different earthworm species, *Eisenia fetida* (Spurgeon and Hopkin, 1999; Lock and Janssen, 2001), E. andrei (Peijnenburg et al, 1999; Van Gestel et al., 1993) and *L. rubellus* (Ireland et al., 1979). Copper is one of the more toxic essential metals (Finney and O'Halloran, 2003). Therefore, it is of utmost importance for species to effectively detoxify and / or regulate internal copper levels above metabolic requirements. Morgan and Morgan (1999) concluded that *L. rubellus* is not able to sequester Cu by metal binding ligands in some tissues. To compensate this lack of a storage mechanism the earthworm probably invests in regulating uptake or elimination rates copper, which may explain the slight increase in earthworm copper concentrations with increasing total soil concentrations and increasing pore water levels.
Even at low exposure concentrations *L. rubellus* contains relatively high zinc levels compared to levels of other metals. At these low external concentrations, internal zinc concentrations are underestimated by OMEGA. This may result from either underestimation of the uptake rate or overestimation of the elimination rate. The latter explanation is unlikely as the minimum modeled elimination rate is already rather low, i.e. $3.3 \cdot 10^{-3}$ d^{-1}. Vijver et al. (2003) and Saxe et al. (2001) showed that 20 - 30% of internal zinc concentrations resulted from ingestion of soil, whereas for the other metals, Cd, Cu and Pb, uptake could be completely attributed to absorption via the skin. Therefore, excluding ingestion of soil may not be valid for zinc, and total uptake rate constants, i.e. absorption plus ingestion, may be underestimated.

4.5.3 Elimination rates

Internal concentrations of cadmium are best predicted assuming that this metal is only eliminated by growth dilution, although a relatively high variation in internal Cd concentrations is observed. From immunohistochemical observations it is known that a significant proportion (> 70%) of the earthworm Cd concentration is sequestered by cystein-rich metal-binding proteins, such as metallothionein (MT) (Vijver et al., 2006; Morgan et al., 2004; Stürzenbaum et al., 2004; Stürzenbaum et al., 2001). This detoxification mechanism is highly efficient (Stürzenbaum et al., 2004). Cadmium is tightly bound by MT-2 (isoform 2 metallothionein) and very low

elimination rates have been reported ($4.6 \cdot 10^{-3}$ d^{-1} for *L. terrestris*) (Spurgeon and Hopkin, 1999; Sheppard et al., 1997). These empirical rate constants of loss are in good agreement with the predicted minimum elimination rate of $3.3 \cdot 10^{-3}$ d^{-1}.

Comparing elimination rate constants estimated using OMEGA with values reported in literature (Table 4.3), contradicting results are observed as empirical clearance rates vary as much as two orders of magnitude. For example elimination rate constants reported for copper range between 0.02 and 1.5 d^{-1} (Spurgeon and Hopkin, 1999). Empirical elimination rates are consistent for cadmium only, with low elimination rates reported for most species. Modeled minimum elimination rates are comparable to the lowest values in the range.

For zinc, copper and lead, empirical elimination rate constants may only be used in a relative manner. Apparently, copper and lead are more easily eliminated than cadmium, which is in agreement with our observation that internal concentrations of these metals are best modeled using a maximum elimination rate.

Table 4.3: Empirical constants for the elimination of zinc, copper, cadmium and lead from different earthworm species reported in the literature. Also included are minimum and maximum elimination rate constants of metals calculated for *Lumbricus rubellus* using the OMEGA model. See text for further explanation.

Metal	**Species**	**Elimination rate (d^{-1})**	**References**
Cadmium	*Eisenia andrei*	0.078	Honeycutt et al., 1995
Cadmium	*Allolobophora tuberculata*	0.018	Neuhauser et al., 1996
Cadmium	*Eisenia andrei*	0.032	Peijnenburg et al., 1999
Cadmium	*Eisenia fetida*	0 – 0.081	Spurgeon and Hopkin, 1999
Cadmium	*Lumbricus terrestris*	0.0046	Sheppard et al., 1997
Cadmium	geometric mean[a]	0.03	
Zinc	*Allolobophora tuberculata*	0.034	Neuhauser et al., 1996
Zinc	*Eisenia fetida*	0 – 1.84	Spurgeon and Hopkin, 1999
Zinc	*Lumbricus terrestris*	0.01	Sheppard et al., 1997
Zinc	geometric mean	0.25	
Copper	*Eisenia fetida*	0 – 1.63	Spurgeon and Hopkin, 1999
Copper	*Lumbricus rubellus*	0.04 – 0.95	Marinussen et al., 1997
Copper	geometric mean	0.37	
Lead	*Eisenia andrei*	1.2	Peijnenburg et al., 1999
Lead	*Eisenia fetida*	0.024 – 0.47	Spurgeon and Hopkin, 1999
Lead	geometric mean	0.26	
OMEGA	Max. elimination rate constant	0.02	
OMEGA	Min. elimination rate constant	0.003	

[a] Elimination rates with a value of '0' are not included in the geometric mean

4.5.4 Relevance for environmental risk assessment

Bioaccumulation is often used as a criterion for prioritization and risk assessment of both organic substances and metals (McGeer et al., 2003). Our validation to field data shows that OMEGA is capable of accurately predicting bioaccumulation of the non-essential metal cadmium in the earthworm *L. rubellus*. In addition, our results show that internal metal concentrations in the earthworm are less than linearly (slope <1) related to the total concentration in soil, while risk assessment procedures often assume the biota-soil accumulation factor (BSAF) to be constant (Crommentuijn et al., 1997; Lock and Janssen, 2001; McGeer et al., 2003). Obviously, the regressions collected in the present and previous studies can be used to obtain a concentration-dependent accumulation factor. However, the advantage of using a model like the one proposed here is that it facilitates extrapolation to soils of various origins, with different physico-chemical properties, and to other species, with similar physiology and metal detoxification mechanisms as earthworms. An additional application for risk assessment purposes is that model predictions underpin the correctness of field measurements, as field accumulation data can show large variability as well. OMEGA may also be used for other non-essential metals in addition to cadmium. However, the model needs incorporation of regulation to improve predictability for essential metals as zinc and copper. Additionally, the model may be improved by considering metal speciation in pore water, to account for differences in bioavailability between various metal species.

4.6 Conclusion

Internal metal concentrations in *L. rubellus* are significantly related to total soil concentrations and estimated pore water concentrations. These internal concentrations show a less than linear relationship (slope <1) with external concentrations. The model accurately predicts accumulation of the non-essential metal cadmium. However, insight in accumulation kinetics of metals, especially regulation of uptake and elimination of essential metals, is necessary to improve the predictability of copper and zinc.

Chapter 5.

Cadmium accumulation in herbivorous and carnivorous small mammals. Meta-analysis of field data and validation of the bioaccumulation model OMEGA

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5.1 Abstract

Environmental risk assessment procedures often use bioaccumulation as a criterion for hazard identification of a polluted location. Field studies on metal concentrations in food chains have, however, provided widely different information, as accumulation is shown to vary between the extremes of bioreduction and biomagnification. Bioaccumulation models provide insight in species-specific uptake and elimination kinetics of metals and assist in the interpretation of field data. Here we use the bioaccumulation model OMEGA to estimate cadmium accumulation in herbivorous voles and carnivorous shrews. In addition to model validation a meta-analysis of cadmium accumulation data is performed, as earlier studies generally focused on relationships between cadmium concentrations in either specific tissues (kidney, liver) or whole-body concentrations and total soil levels. We additionally included the food – small mammal relationship. Our results show that cadmium whole body concentrations are significantly related to Cd levels in food items as earthworms and plants. In addition, a significant relationship is found between cadmium accumulation in the liver and kidney of small mammals and total soil levels. Cadmium concentrations in shrews are typically an order of magnitude higher than metal levels in voles, as a result of higher metal accumulation in earthworms compared to plants. Model predictions for both voles and shrews are in good agreement with field observations, deviations are generally within a factor of five. Small mammals prevent cadmium toxicity by binding this metal to metallothionein, which likely results in low elimination rates. Comparison with empirical elimination rates shows that rate constants of loss are accurately predicted assuming that cadmium is only released via growth dilution.

5.2 Introduction

Floodplain soils in The Netherlands are historically polluted with metals as cadmium, copper, zinc, nickel, and lead. In other areas, elevated levels of heavy metals are due to extensive mining in the past, occurrence of metal smelters or other industrial sources, and application of metal rich sewage sludge. Soil dwelling organisms inhabiting these areas are known to accumulate metals (Hobbelen et al., 2004; Hunter et al., 1987[a]) and dietary transfer to predators as shrews and moles has been observed (Ma, 1987; Ma et al., 1991). Bioaccumulation studies demonstrated that metal accumulation in small mammals can vary between the extremes of bioreduction and biomagnification (Ma, 1987; Ma et al., 1991; Hunter et al., 1989; Shore, 1995; Andrews et al., 1989; Torres and Johnson, 2001; Rogival

et al., 2006), which complicates interpretation of field data and environmental risk assessment of metals.

Several interacting factors affect bioaccumulation, e.g., environmental conditions, metal specificity (essential versus non-essential) and species-specific characteristics (Luoma and Rainbow, 2005; Wijnhoven et al., 2006; Wijnhoven et al., 2007). First, environmental conditions as pH and organic matter content of the soil determine the availability for metal uptake by earthworms and plants (Sauvé et al., 2000), which are important diet items for small mammals. Second, internal concentrations of essential metals as copper and zinc are efficiently regulated by some species, including small mammals, by actively increasing excretion and / or reducing the uptake (Rainbow, 2002). This results in stable whole-body concentrations even at high exposure concentrations (Hunter et al., 1989). Additionally, internal concentrations of these metals can be regulated in invertebrates and plants and food chain transfer of essential metals is generally limited (Rogival et al., 2006). Homeostatic regulation is probably disrupted at high exposure concentrations and biomagnification may occur in these specific cases. Most species cannot regulate bioaccumulation of non-essential metals as cadmium and lead (Rainbow, 2002). These organisms may prevent toxicity by effectively storing metals in non-toxic forms, i.e. bound to metal binding proteins as metallothionein, or incorporated in non-soluble granules. Species relying on sequestration as detoxification mechanism may excessively accumulate metals with increasing exposure concentrations due to very low elimination of tightly bound metals. Carnivorous small mammals feeding on accumulators as earthworms are therefore exposed to relatively high metal concentrations compared to herbivorous species (Hunter et al., 1989; Hamers et al., 2006).

The aim of the present study is two-fold: First, a meta-analysis of field accumulation data of cadmium in herbivorous voles and carnivorous shrews was performed to examine if cadmium concentrations are consistently related to exposure concentrations, which is necessary for model validation. Second, field data are used to validate the mechanistic bioaccumulation model Optimal Modeling for Ecotoxicological Applications (OMEGA). The bioaccumulation of cadmium in small mammals in relation to environmental contamination has been the focus of many studies. However, these studies generally present empirical data and few models have been developed. We are aware of only two comparable studies of Shore (1995) and Sample et al. (1998) (www.hsrd.ornl.gov/ecorisk/tm219.pdf), in which cadmium accumulation in tissues (Shore, 1995) or whole-body (Sample et al., 1998) of small mammals is quantitatively related to total soil concentrations. In this study, we also included

the food – small mammal relationship, in addition to both tissue- and whole-body relationships with soil levels.

Bioaccumulation models serve as an additional tool for risk assessment purposes and facilitate the interpretation of field data. Regression models as developed by Shore (1995) and Sample et al. (1998) generally accurately predict metal accumulation for specific species and locations. The added value of the bioaccumulation model proposed here is that uptake and elimination rate constants are predicted based on allometric and physiological transport principles. This facilitates extrapolation to other contaminant levels and species, without data intensive and case specific calibration. Additionally, the model provides quantitative information on specific parts of the bioaccumulation process, i.e., uptake and elimination, allowing for a mechanistic explanation of species-specific differences in metal accumulation.

In a previous study it was shown that the model correctly predicts accumulation of cadmium in the earthworm *Lumbricus rubellus* (Veltman et al., 2007a). Species included in the present study are the carnivorous shrew, *Sorex araneus*, and herbivorous voles, *Clethrionomys glareolus* and *Microtus agrestis*. Metal uptake kinetics are modeled using an empirically derived metal assimilation-efficiency and a predicted food ingestion rate constant based on allometric relationships. Small mammals prevent cadmium toxicity by binding this metal to the protein metallothionein, which likely results in low elimination rates. Therefore, elimination of cadmium is modeled as growth dilution only, yielding a minimum elimination rate. In addition to the validation with field accumulation data, modeled Cd elimination rate constants and food ingestion rate constants are compared to empirical values.

5.3 Materials and methods

5.3.1 Bioaccumulation model

Small mammals predominantly accumulate metals via ingestion of food (Hunter et al., 1989). Therefore, uptake via absorption of water and inhalation of air is excluded in the current modeling approach. Steady-state whole-body cadmium concentrations are predicted as the total uptake via food divided by the elimination rate constant (Eqn 5.1).

$$C_{i,x} = \frac{k_{x,n,in} \cdot C_{i-1,x}}{k_g} \qquad \text{Equation 5.1}$$

$C_{i,x}$	=	Internal metal (x) concentration in small mammals (i) [$kg_x \cdot kg^{-1}$ body wet weight]	
$C_{i-1,x}$	=	Metal concentration in food (i -1)	[$kg_x \cdot kg^{-1}$ food wet weight]
$k_{x,n,in}$	=	Metal uptake rate constant from food (n) [$kg_x \cdot kg^{-1}$body wet weight d^{-1} / $kg_x \cdot kg^{-1}$ food wet weight]	
k_g	=	Rate constant for growth dilution	[d^{-1}]

Binding of cadmium to the metal-binding protein metallothionein (MT) is the predominant detoxification strategy for most mammals (Klaassen et al., 1999). This influences elimination kinetics, as strongly bound substances are poorly eliminated by excretion and / or egestion. Therefore it is assumed that cadmium is eliminated via growth dilution only, yielding a minimum elimination rate (k_g) (Eqn. 5.2). The temperature correction factor ($q_{T:c}$) accounts for the fact that metabolic flows in warm-blooded species are higher compared to cold-blooded species. Species weights (w) are obtained from field data of Wijnhoven et al. (2006, 2007) and Hamers et al. (2006) and a geometric mean of adult weight is used.

$$k_g = q_{T:c} \cdot \gamma_2 \cdot w^{-\kappa}$$ Equation 5.2

γ_2	=	Biomass (re)production coefficient ($6 \cdot 10^{-4}$)	[$kg^{\kappa} \cdot d^{-1}$]
w	=	Weight of species ($1.0 \cdot 10^{-2}$ for *S. araneus*, $3.0 \cdot 10^{-2}$ for *M. agrestis*, $2.1 \cdot 10^{-2}$ for *C. glareolus*)	[kg]
κ	=	Rate exponent (0.25)	[/]
$q_{T:c}$	=	Temperature correction factor (3.5)	[/]

The uptake rate constant of metals from food ($k_{x,n,in}$) depends on the food ingestion rate constant (k_n) and the metal assimilation efficiency from the food matrix ($p_{x,a}$) (Eqn. 5.3). The assimilation efficiency of cadmium in small mammals is generally low and depends on external conditions as pH in the gastrointestinal tract, available biological ligands in food, and stability of these metal-complexes in the food matrix (Andersen et al., 2004). At present, the mechanisms of metal uptake via ingestion are not fully understood (Klaassen et al., 1999, Zalups and Ahmad, 2003) and therefore metal assimilation efficiency cannot be modeled mechanistically. Instead an empirical cadmium assimilation efficiency ($p_{x,a}$) of 0.51% (Appendix 5) was used to predict the uptake rate constant of cadmium from food ($k_{x,n,in}$) (Eqn. 5.3). This empirical cadmium assimilation efficiency is a geometric mean value of data from 10 long-term experimental studies, including various experimental designs.

$$k_{x,n,in} = k_n \cdot p_{x,a}$$ Equation 5.3

k_n	=	Food ingestion rate constant	[$kg_{food}.kg^{-1}$ body wet weight$\cdot d^{-1}$]
$p_{x,a}$	=	Assimilation efficiency of cadmium from food (empirical value, see Appendix 5) (0.51	[%]

The food ingestion rate constant (k_n) can be estimated according to Equation 5.4. Food assimilation efficiencies (p_a) for shrews and herbivorous voles are set at typical values of 0.8 and 0.4 for carnivores and herbivores, respectively (Hendriks, 1999).

$$k_n = \frac{q_{T:c} \cdot \gamma_1}{p_p \cdot p_a} \cdot w^{-\kappa}$$ Equation 5.4

γ_1	=	Food ingestion coefficient ($7.5 \cdot 10^{-4}$)	[$kg^{\kappa} \cdot d^{-1}$]
p_p	=	Production efficiency for warm-blooded vertebrates (0.02)	
p_a	=	Food assimilation efficiency for carnivores (0.8) and herbivores (0.4)	
$q_{T:c}$	=	Temperature correction factor (3.5)	

5.3.2 Data collection and treatment

Field accumulation data (17 locations, 8 studies) were collected for carnivorous and two predominantly herbivorous small mammal species (Appendix Table A5.1 and A5.2). Carnivorous species were represented by the common shrew, Sorex araneus, and the herbivorous voles by the bank vole, *Clethrionomys glareolus*, and the field vole, *Microtus agrestis*. These field data comprise several studies and locations, which were mainly diffusively polluted floodplain soils and former mining areas. Data were selected based on two main criteria. First, we selected studies that report metal concentrations in small mammals and co-located total soil concentrations or concentrations in food items. *Sorex araneus* preferentially feeds on Lumbricid earthworms (Ma et al., 1991) whereas *C. glareolus* and *M. agrestis* are predominantly herbivorous. *Clethrionomys glareolus* predominantly feeds on grasses (± 30% of its diet), seeds (± 30%), and composite ground cover (5%). Soil ingestion contributes another 5% to its diet (Hunter et al., 1987; Milton et al., 2003). *Microtus agrestis* preferentially feeds on the green stems and leaves of grasses (Ma et al., 1991). We considered earthworms to be representative food items for shrews, and plants representative as a diet for voles. Studies that report Cd concentrations in other food items, as seeds or macroinvertebrates, e.g., Andrews

and Cooke (1989) were therefore not included in the food chain accumulation part of the present study. Second, to ensure comparability of data, only studies in which metal analyses are based on concentrated acid extraction methods, for example aqua regia or concentrated nitric acid, in both soil and biota are included. Field studies that did not meet these selection criteria were not included in the present study.

A geometric mean of total soil concentrations and biota concentrations was calculated for each area, representing a typical concentration for this location rather than metal accumulation in individual organisms. If small mammals were monitored in different periods on the same location, a geometric mean concentration for these periods was obtained to minimize variance due to temporal variations. Some studies measured cadmium concentrations in the above ground parts of different plant species (Appendix 5). In these cases data were pooled and a general plant concentration was used. The model OMEGA is a food chain accumulation model and therefore predicts internal metal concentrations on a whole-body basis. To compare field data with model predictions, empirical cadmium concentrations in small mammals were expressed on a whole-body basis. Not all studies provide these data and in these cases total body concentrations were estimated from both kidney and liver concentrations according to Equation 5.5 (Kooistra et al., 2005). The equation applies to both shrews and voles, but species specific weight fractions of the liver (F_l) and kidney (F_k) are used. The Cd fraction in liver and kidney combined is considered to be 80% of the total Cd burden for both voles and shrews (Kooistra et al., 2005).

$$C_{i,x} = \frac{1}{F_b} \cdot \left(F_l \cdot C_{l,x} + F_k \cdot C_{k,x} \right) \qquad \text{Equation 5.5}$$

$C_{i,x}$	=	total body concentration	[$mg_x \cdot kg^{-1}$body dry weight]
$C_{l,x}$	=	empirical cadmium concentration in liver of shrews or voles	[$mg_x \cdot kg^{-1}$dry wt]
$C_{k,x}$	=	empirical cadmium concentration in kidney of shrews or voles	[$mg_x \cdot kg^{-1}$dry wt]
F_b	=	fraction of Cd total body burden in liver and kidney, equal fraction for shrews and voles (0.8)	[/]
$F_{l,vole}$	=	fraction of liver in total vole body weight (0.054)	[/]
$F_{k,vole}$	=	fraction of kidney in total vole body weight (0.013)	[/]
$F_{l,\ shrew}$	=	fraction of liver in total shrew body weight (0.07)	[/]
$F_{k,\ shrew}$	=	fraction of kidney in total shrew body weight (0.019)	[/]

In Hamers et al. (2006) and Hendriks et al. (1995) cadmium concentrations in the liver were not measured. For these studies whole-body concentrations could not be calculated.

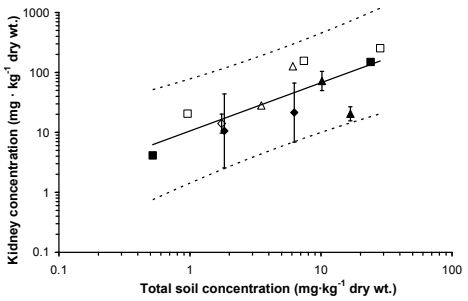
Linear regression analysis of log transformed data was performed for cadmium organ and whole-body concentrations versus total soil concentrations and for whole-body concentrations versus cadmium concentrations in food. In the regression analysis of whole-body concentrations with cadmium concentrations in food items, different studies were included than in the analysis of whole-body concentrations with total soil levels. Therefore these regressions are not directly comparable. The regression equations were optimized using a linear least squares fit to find appropriate values for the slope (a) and intercept (b) of the regression. Apart from regression parameters a and b, the correlation coefficient (r^2) and the residual standard error (SE) were derived. A paired two-sample t test, assuming unequal variances, was performed to determine whether model predictions significantly differ from field data. All data were log-transformed prior to the statistical test. The difference between model predictions and field data was considered significant if $p(H_0 = 0) \leq 0.05$.

Empirical assimilation efficiencies were collected from experimental studies using $CdCl_2$ salts as well as Cd incorporated in the food matrix as Cd-MT or bound to unknown ligands (Appendix 5 Table A5.3). Some studies determined the fractional accumulation in kidney plus liver. This is considered to be a good representation of the intestinal assimilation efficiency as more than 70% of the Cd whole-body burden accumulates in kidney plus liver (Andersen et al., 2004). A geometric mean assimilation efficiency of 0.51% was used in the model calculations.

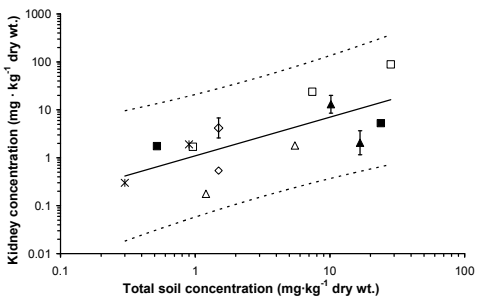
5.4 Results

Cadmium concentrations in the kidney and liver of small mammals are statistically significantly related to total soil concentrations ($p < 0.01$) (Figure 5.1a-d, Table 5.1). In particular for Cd accumulation in the liver and whole-body of carnivorous shrews a large amount of variation is explained by soil concentration ($r^2 > 0.84$) (Table 5.1). For herbivores, a statistically non-significant increase is observed between whole-body Cd concentrations and total soil levels ($p = 0.08$). Cadmium concentrations in liver, kidney and whole-body of shrews are typically more than an order of magnitude greater than concentrations in herbivorous species.

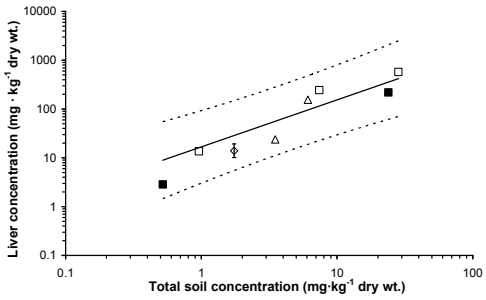
a. Carnivorous shrew (Cd kidney concentrations)



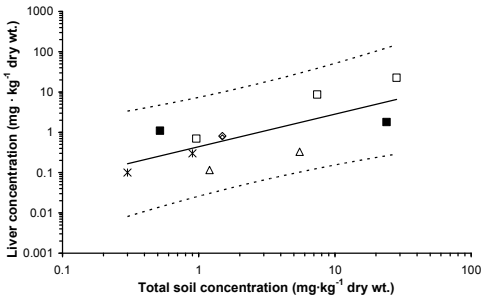
b. Herbivorous voles (Cd kidney concentrations)



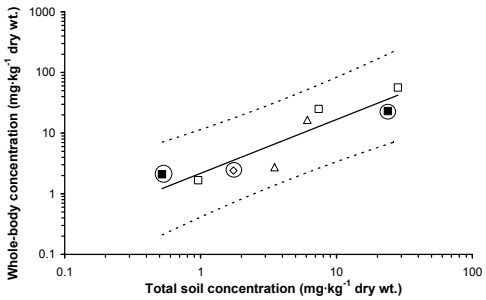
c. Carnivorous shrew (Cd liver concentrations)



d. Herbivorous voles (Cd liver concentrations)



e. Carnivorous shrew (Cd whole-body concentrations)



f. Herbivorous voles (Cd whole-body concentrations)

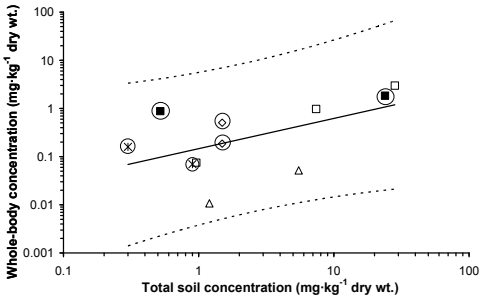


Figure 5.1 Cadmium accumulation in kidneys of carnivorous shrew (**a**) and herbivorous voles (**b**) (mg·kg^{-1} dry wt) compared to total soil concentrations (mg·kg^{-1} dry wt), 1c and 1d: Cd accumulation in liver of carnivorous shrew (**c**) and herbivorous voles (**d**) (mg·kg^{-1} dry wt), (**e**) and (**f**) Cd whole-body concentrations for (**e**) carnivorous shrews and (**f**) herbivorous voles compared to total soil concentrations. ▲ Biesbosch (Hamers et al., 2006; Notten et al., 2005), ◆ Rhine (Hendriks et al., 1995), ◇ Afferdensche and Deestsche Waarden [Luoma and Rainbow, 2005; Wijnhoven et al. 2007; van Vliet et al., 2005], △ near closed smelter (Budel) and industrially polluted area (Arnhem) (Ma et al., 1991), □ near Cd / Cu refinery, 1 km from refinery and reference location (Hunter et al., 1987a,b,c; Hunter et al., 1989) ■ near mine and reference location in the United Kingdom (Shore, 1995; Andrews et al.,

1989), ● Niepolomica Forest, Ojcow National Park, Ratanica Watershed, Olkusz - Pomorzany in Poland (Laskowski and Maryanski, 1993), ∗ lead mine (Frongoch) and reference site (Milton et al., 2003). Encircled figures represent measured whole-body concentrations; other data represent calculated whole-body concentrations from liver and kidney levels. Full line represents empirical regression. Upper and lower dotted lines represent the 97.5th and 2.5th percentile of the field data, respectively. The error bars represent the 95% confidence intervals and were plotted when possible.

Table 5.1. Regression analysis of internal concentrations in small mammals and soil concentrations at small mammal sampling locations

Species	**Tissue / whole body**	**Log ($C_{small\ mammal}$)**	**r^2**	**SE[a]**	**p**	**n**
Carnivorous	Kidney	$0.80 \cdot Log(C_{soil}) + 1.02$	0.64	0.35	<0.01	12
Herbivorous	Kidney	$0.81 \cdot Log(C_{soil}) + 0.04$	0.51	0.53	<0.01	13
Carnivorous	Liver	$0.96 \cdot Log(C_{soil}) + 1.22$	0.90	0.27	<0.001	8
Herbivorous	Liver	$0.81 \cdot Log(C_{soil}) - 0.36$	0.54	0.51	<0.01	11
Carnivorous	Whole-body	$0.89 \cdot Log(C_{soil}) + 0.34$	0.84	0.26	<0.01	8
Herbivorous	Whole-body	$0.63 \cdot Log(C_{soil}) - 0.84$	0.30	0.66	0.08	11

[a] Standard error.

Earthworm Cd burdens and plant cadmium concentrations are statistically significantly related to total soil concentrations ($p < 0.01$), but cadmium concentrations in above ground parts of plants, mostly referring to leaves (Appendix 5, Table 5.1), are generally lower than concentrations in earthworms at equal soil levels (Figure 5.2, Table 5.2). This is in agreement with observations for small mammals, as Cd concentrations in herbivores are generally lower than in carnivores.

a. Cadmium concentrations in earthworms

b. Cadmium concentrations in various plant species

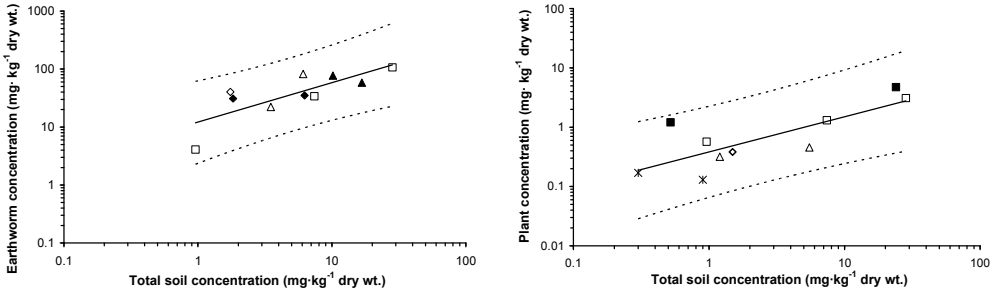


Figure 5.2: Empirical cadmium concentrations in (**a**) earthworms (mg·kg^{-1} dry wt) and (**b**) plants (mg·kg^{-1} dry wt) versus total soil concentrations (mg·kg^{-1} dry wt). ▲ Biesbosch (Hamers et al., 2006) ◆ Rhine (Hendriks et al., 1995), ◇ Afferdensche and Deestsche Waarden (Van Vliet et al., 2005), △ near closed smelter (Budel) and industrially polluted area (Arnhem) (Ma et al., 1991), □ near Cd / Cu refinery, 1 km from refinery and reference location (Hunter et al., 1987a,b) ■ near mine and reference location in the United Kingdom (Shore, 1995; Andrews et al., 1989), ∗ lead mine (Frongoch) and reference site (Milton et al., 2003). Full line represents empirical regression. Upper and lower dotted lines represent the 97.5th and 2.5th percentile of the regression model, respectively.

Table 5.2. Regressions for empirical above ground plant parts (predominantly leaves) and earthworm concentrations with total soil concentrations

Relation	**Log (C_x)**	**r^2**	**SE[a]**	**p**	**n**
$C_{earthworm}$ vs C_{soil}	$0.68 \cdot Log(C_{soil}) + 1.09$	0.61	0.26	<0.01	10
C_{plant} vs C_{soil}	$0.59 \cdot Log(C_{soil}) - 0.42$	0.62	0.32	<0.01	11

[a] Standard error.

Cadmium whole-body concentrations in shrews are statistically significantly related to concentrations in earthworms ($p < 0.05$) Figure 5.3a, Table 5.3). The data of Hunter et al. (1987; 1989) especially locations near the Cu / Cd refinery, show a higher Cd accumulation in shrews than other field data. The t-test indicates that OMEGA predictions (dashed line a in Figure 5.3a) are not significantly different from field data ($p > 0.05$) and deviations are generally within a factor of five. Cadmium concentrations in herbivorous voles are statistically significantly related to empirical plant concentrations ($p < 0.01$, $r^2 = 0.58$). However, the variability in field data is relatively large (Figure 5.3b, Table 5.3). Model predictions are not significantly different from field data (t test, $p > 0.05$) (dashed line a in Figure 5.3b). Deviations between model estimations and empirical data are generally within a

factor of five, with exception of one data point for which the model overestimates the Cd concentration in voles by a factor 20.

a. Carnivorous shrew b. Herbivorous voles

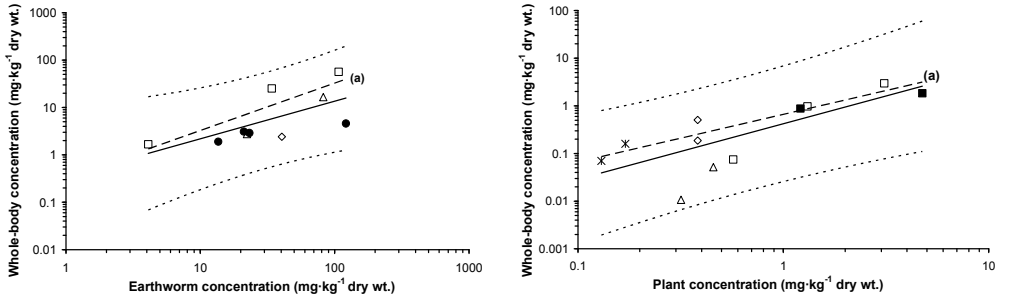


Figure 5.3 (**a**) Cadmium concentrations in *Sorex araneus* (whole-body) versus concentration in food (*Lumbricus rubellus,* earthworms), (**b**) Cadmium concentrations in *Clethrionomys glareolus* (whole-body) and *Microtus agrestis* (whole-body) versus concentration in above ground parts of various plant species (generally leaves, see Appendix 5), ◇ Afferdensche and Deestsche Waarden in The Netherlands (Wijnhoven et al., 2006; Wijnhoven et al. 2007; van Vliet et al., 2005), △ near closed smelter (Budel) and industrially polluted area (Arnhem) in The Netherlands (Ma et al., 1991), □ near Cd / Cu refinery, 1 km from refinery and reference location in The United Kingdom (Hunter et al., 1987a,b,c; Hunter et al., 1989), ■ near mine and reference location in the United Kingdom (Shore, 1995; Andrews et al., 1989), ● Niepolomice Forest, Ojcow National Park, Ratanica Watershed, Olkusz - Pomorzany in Poland (Laskowski and Maryanski, 1993), ∗ lead mine (Frongoch) and reference site in the United Kingdom (Milton et al., 2003). Full line represents empirical regression. Upper and lower dotted lines represent the 97.5th and 2.5th percentile of the field data, respectively. Dashed line (a) represents model predictions using a fixed assimilation efficiency (0.51%).

Table 5.3. Regressions for field measurements and model predictions: Cadmium concentrations in *Sorex araneus* (whole-body) versus empirical concentrations in food (*Lumbricus rubellus*, earthworms) and cadmium concentrations in *Clethrionomys glareolus* and *Microtus agrestis* (whole-body) versus empirical concentrations in food (various plant species, cadmium analyzed in above ground parts of plants, generally leaves)

Relation	**Log ($C_{small\ mammal}$)**	**r^2**	**SE[a]**	**p**	**n**
Empirical: C_{shrew} vs $C_{earthworm}$	$0.79 \cdot Log(C_{earthworm}) - 0.45$	0.45	0.42	0.03	10
OMEGA[b]: predicted C_{shrew} vs empirical $C_{earthworm}$	$1 \cdot Log(C_{earthworm}) - 0.49$				
Empirical: C_{voles} vs C_{plant}	$1.16 \cdot Log(C_{plant}) - 0.37$	0.58	0.51	<0.01	11
OMEGA[b]: predicted C_{voles} vs empirical C_{plant}	$1 \cdot Log(C_{plant}) - 0.18$				

[a]Standard error.

[b]Optimal Modeling for Ecotoxicological Applications.

5.5 Discussion

5.5.1 Empirical data

Results show a significant positive relationship between cadmium concentrations in the kidney and liver of small mammals and total soil levels. This is in agreement with Shore (1995) and can be explained by the fact that cadmium predominantly accumulates in kidney and liver of small mammals, both organs accounting for approximately 80% of total Cd burdens (Shore, 1995). Exposure concentrations are therefore clearly reflected in kidney and liver concentrations. Consequently, whole-body concentrations in *S. araneus*, which are partly estimated from liver and kidney concentrations, are also significantly related to total soil levels.

Cadmium concentrations in carnivorous shrews are typically one order of magnitude higher than concentrations in herbivorous voles. This is due to the higher Cd concentration in the diet of shrews. Earthworms accumulate more cadmium than plants, as in most plants, metals tend to become immobilized in roots and other below-ground storage tissues and undergo limited translocation to aboveground structures, including leaves (Stolz and Greger, 2002; Dahmani-Muller et al., 2000). Earthworms, on the other hand, are known accumulators of metals and store these metals in detoxified forms, i.e., bound to metallothionein and incorporated in granules (Vijver et al., 2004).

Field data on cadmium accumulation in herbivorous and carnivorous small mammals show considerable variability both between locations and within individuals from one location (represented by 95% confidence intervals). Some studies explain this variability in accumulation within a species by heterogeneity in age of the sampling population. Increasing internal (kidney and liver) Cd concentrations with age have been reported for *S. araneus* (Hunter et al., 1989) and *C. glareolus* (Milton et al., 2003). The average age of common shrew population strongly depends on season and varies form 3 to 10 months (Crowcroft, 1957). Field data of Hunter et al. (1989), Hamers et al. (2006), and Andrews and Cooke (1989) include juvenile small mammals, whereas data included from Wijnhoven et al. (2006; 2007) consist of adult specimens only. Heterogeneity in age within the sampling population may explain part of the variability in Cd accumulation observed between locations and within individuals from one location. Additionally, spatial variability in exposure concentrations may add to variability within individuals from one location (Kooistra et al., 2005).

The variability in Cd accumulation in kidney and liver of herbivores is higher compared to carnivores, which is partly explained by a higher variation in cadmium concentrations in the voles' diet (Figure 5.2b). First, Hunter et al. (1987c) and

Andrews and Cooke (1989) measured metal accumulation in unwashed leaves, whereas others used thoroughly rinsed leaves. Hunter et al. (1987c) showed that at the refinery site vegetation, approximately 40% of total cadmium in unwashed plants can be attributed to leaf surface deposits. In these studies higher cadmium concentrations in leaves of plants are found compared to other field data used in the present study (Figure 5.2b). Second, the leaf / root metal translocation of cadmium may show large variations between plant species (Stolz and Greger, 2002; Dahmani-Muller et al., 2000) attributing to the observed variability in plant concentrations. Total soil concentrations may therefore not be reflected in concentrations in shoots and leaves. Finally, in the present study accumulation data of the voles *C. glareolus* and *M. agrestis* are combined, although the diet of *C. glareolus* is more diverse compared to *M. agrestis*, as *C. glareolus* feeds on seeds and composite ground cover in addition to grasses. These food items may have different Cd concentrations than grasses and combining field accumulation data of *M. agrestis* and *C. glareolus* can therefore add to the observed variability.

Cadmium concentrations in shrews are significantly related to earthworm Cd burdens. The fact that internal concentrations reported by Hunter et al. (1987c) are substantially higher than other field data may be explained by an observed reduced earthworm density near the cadmium / copper refinery at issue (Hunter al., 1987a; Hunter et al., 1987b; Hunter et al., 1987c). Therefore, the authors assumed that the diet of carnivorous species shifted from predominantly earthworms to Collembola (*Orchesella villosa*) and isopods (*Oniscus asellus*) (Hunter et al., 1987b). Although cadmium concentrations in Collembola are lower than concentrations in earthworms, isopod concentrations are substantially higher than earthworm Cd burdens. Such differences in diet may partly explain the deviating data of Hunter et al. (1987a,b,c, 1989).

5.5.2 Model predictions

Elimination rates were predicted by OMEGA assuming loss via growth dilution only, yielding a minimum clearance rate. Liu et al. (2001) observed that cadmium is more easily eliminated in MT-null mice (transgenic mice incapable of synthesizing MT) than in normal mice species. They concluded that metallothionein plays a major role in elimination of Cd and the persistence of cadmium in the body is at least partially due to Cd-binding to MT in tissues. Comparing predicted minimum elimination rates with empirical values shows that the model accurately predicts cadmium clearance rates (Table 5.4). These elimination rate constants are similar for herbivorous and carnivorous species, as they are independent of the food source. A previous study (Veltman et al., 2007a) showed that Cd elimination rate

constants were also accurately predicted for the earthworm *Lumbricus rubellus*, by assuming release via growth dilution only. This suggests that cadmium elimination rate constants are predictable for other species as well, provided that binding to metallothionein is the predominant detoxification mechanism.

Table 5.4. Predicted elimination rate constants ($k_{x,ex}$ in d^{-1}) compared to empirical rate constants (d^{-1})

Species	**Empirical $k_{x,ex}$ in d^{-1}**	**Predicted $k_{x,ex}$ in d^{-1}**	**Reference**
Sorex araneus		$6.8 \cdot 10^{-3}$	
Microtus agrestis		$5.1 \cdot 10^{-3}$	
Clethrionomys glareolus		$5.3 \cdot 10^{-3}$	
Mus musculus	$9.4 \cdot 10^{-3}$	$5.5 \cdot 10^{-3}$	Jörgensen, 1979
Rattus norvegicus	$2.3 \cdot 10^{-3}$	$2.7 \cdot 10^{-3}$	Jörgensen, 1979
rodents	$3.5 \cdot 10^{-3}$ – $9.0 \cdot 10^{-4}$		Friberg et al., 1986
mice	$2.9 \cdot 10^{-3}$		Engström and Nordberg, 1979

The observed agreement between model predictions and field data and the accurate prediction of elimination rates, suggests that the metal uptake rate constant ($k_{x,n,in}$) is correctly estimated as well. However, correct conclusions can only be drawn if the food ingestion rate constant (k_n) and the metal assimilation efficiency ($p_{x,a}$) are validated separately with empirical data. Unfortunately, these parameters have not been determined in a field situation at present. Laboratory feeding trials estimated food ingestion rate constants of *S. araneus* at approximately 2.5 $g \cdot d^{-1}$ (Hunter et al., 1987b; Churchfield, 1982), which corresponds to an ingestion rate constant of 1.4 $kg_{food\ wet\ wt}$ / $kg_{body\ wet\ wt} \cdot d^{-1}$. This lab based food ingestion rate constant is higher than the ingestion rate constant predicted by OMEGA (k_n = 0.52 $kg_{food\ wet\ wt}$ / $kg_{body\ wet\ wt} \cdot d^{-1}$). Field accumulation data suggest, however, that the ingestion rate constant is correctly predicted. Shore et al. (1995) determined the food ingestion rate constant of *C. glareolus* at 0.17 $kg_{food\ wet\ wt}$ / $kg_{body\ wet\ wt} \cdot d^{-1}$, whereas Hunter et al. (1987b) obtained an feeding rate of 12 $g_{food\ dry\ wt} \cdot d^{-1}$ ($\cong$ 2 $kg_{food\ wet\ wt}$ / $kg_{body\ wet\ wt} \cdot d^{-1}$) for *M. agrestis*. The modeled ingestion rate constant for voles is within the range of these values (1.0 $kg_{food\ wet\ wt}$ / $kg_{body\ wet\ wt} \cdot d^{-1}$).

The metal assimilation efficiency is based on long-term, laboratory retention experiments, and exposure to various metal species in food (i.e., $CdCl_2$, Cd bound to MT or other organic Cd complexes), which is supposed to resemble field situations.

However, it has been shown that the net intestinal uptake of Cd decreases by a factor of 5 to 8, if mice are fed standard rodent pellets with a high content of natural fibers and trace elements (Andersen et al., 2004) suggesting that field metal assimilation efficiencies may be larger than lab values. This higher field metal assimilation efficiency is not in agreement with field data from the present study.

The empirical assimilation efficiencies show that bioavailability of organic bound cadmium in plants is not very different from Cd bound to metallothionein. Hunter et al. (1987b) found that cadmium concentrations in *S. araneus* and *A. sylvaticus* were very different, which they attributed to changes in bioavailability of cadmium ingested as invertebrate material as opposed to vegetation, or an interspecific physiological difference in metal metabolism that facilitates greater cadmium absorption and retention by *S. araneus* than by other species. Our results indicate that metal assimilation efficiencies are comparable for carnivorous and herbivorous species, even though cadmium is bound to different ligands in plants and invertebrates. Consequently, higher Cd accumulation in shrews compared to voles mainly results from the higher concentrations in earthworms opposed to plants.

Preferably metal uptake by ingestion should be modeled fully mechanistically based on well-defined biochemical transport principles instead of using an empirical assimilation efficiency, as this allows extrapolation to other species and metals. At present, experimental studies relating metal assimilation efficiencies to their subcellular distribution in prey are sparse for terrestrial species. Recently, studies have been published on this subject for aquatic species (Cheung and Wang, 2005; Rainbow et al., 2006) that may allow further improvement of the model structure and parameter validation.

5.6 Conclusions

A significant positive relationship is found between cadmium concentrations in kidney and liver of small mammals and total soil levels. In addition, whole-body concentrations in shrews and voles are significantly related to cadmium concentrations in their diet, which exists of earthworms and plants, respectively. The fact that Cd levels in carnivorous shrews are typically one order of magnitude higher than concentrations in herbivorous voles is attributed to the higher Cd accumulation in earthworms compared to plants. Model predictions are in good agreement with field data. Elimination rate constants are accurately predicted by the model assuming cadmium is only being released via growth dilution. An empirical, laboratory based assimilation efficiency was used to predict the cadmium uptake rate constant in combination with an estimated food ingestion rate. Our results suggest good predictability of this metal uptake rate constant. The

model can however, be improved with empirical information on relations between the assimilation efficiency and subcellular distribution of metals in prey, allowing a more thorough mechanistic understanding and prediction of the metal assimilation efficiency.

5.7 Acknowledgements

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Chapter 6.

Metal bioaccumulation in aquatic species: quantification of uptake and elimination rate constants using physico-chemical properties of metals and physiological characteristics of species

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6.1 Abstract

Mechanistic bioaccumulation models are powerful tools in environmental risk assessment as they provide insight in varying accumulation patterns across species, contaminants and conditions, and they are applicable beyond tested cases. In these models key parameters, as absorption and elimination rate constants, are predicted based on chemical specific properties and physiological characteristics. However, due to the complex environmental behavior of metals, the development of mechanistic bioaccumulation models has lagged behind that for organic chemicals. Absorption and elimination rate constants of organic substances have long been linked to their octanol-water partition coefficient, yet no equivalent quantitative relationships exist for metals. In the present study, we successfully related metal absorption rate constants to a metal specific property, the covalent index, and a species-characteristic, the ventilation rate. This quantitative relationship holds for a wide range of organisms and metals, i.e. 17 aquatic species and 10 metals, suggesting that a generic modeling approach of metal uptake kinetics is feasible for aquatic organisms. In contrast, elimination rate constants show no metal – specific character. Average, weight-corrected elimination rate constants are relatively similar among metals and species, suggesting that a single weight-corrected elimination rate constant can be used in bioaccumulation studies on aquatic species.

6.2 Introduction

Bioaccumulation is a fundamental process in environmental toxicology and risk assessment, as it determines the internal dose of potential toxicants. Mechanistic bioaccumulation models are widely used in the environmental risk assessment of organic chemicals. These models are called “mechanistic” as they describe uptake and elimination kinetics of contaminants based on chemical specific properties and physiological processes, rather than using empirical rate constants that are measured for each chemical and organism. Although the latter approach does provide accurate predictions of accumulation, the advantage of the mechanistic approach is that it allows extrapolation to a wide range of contaminants, organisms and conditions without case-specific calibration.

Metal bioaccumulation kinetics show substantial variability depending on the species, metals and ecosystems investigated (Luoma and Rainbow, 2005). Due to this complex behavior, mechanistic bioaccumulation models for metals are largely absent. Recently, bioaccumulation models have been developed that predict metal concentrations in aquatic and terrestrial species (Luoma and Rainbow, 2005;

Hendriks and Heikens, 2001; Veltman et al., 2007a; Veltman et al., 2007b). However, these models often require empirical rate constants as input parameters (Luoma and Rainbow, 2005). To allow extrapolation to other species and metals, absorption and elimination rate constants need to be based on metal-properties and species – characteristics.

Baines et al. (2006) showed that variability in metal absorption rate constants between mussels is primarily a function of the filtration rate. Additionally, weight has been used to explain variance in metal elimination rates across species (Hendriks and Heikens, 2001). At present, metal accumulation kinetics have not been quantitatively linked to metal-specific properties. In contrast, uptake and elimination rate constants of neutral organic substances have long been related to their octanol-water partition coefficient. Accumulation kinetics of neutral, organic substances are primarily driven by passive diffusion, whereas accumulation of metals is more complicated and includes various uptake and elimination pathways. This may hamper the development of a quantitative relationship between metal absorption rate constants and a single, metal specific property.

Metals can be taken up by passive diffusion, facilitated transport, active transport or endocytosis (Simkiss and Taylor, 1995). Passive diffusion across membranes is only possible for neutral, lipid-soluble metal species such as organotins, methylmercury and tetraethyllead. Most ions are extremely hydrophilic and may be taken up via three types of membrane transport proteins, i.e. channels, carriers and / or pumps. These transporters have been designed for essential nutrients as sodium, potassium, calcium, copper and zinc. Non-essential metals as cadmium, mercury and lead can be taken up by ionic mimicry of essential analogues. Membrane transport proteins may base their selectivity on metal charge and size, and on coordination- and ligand-preferences (Bell et al., 2002; Handy and Eddy, 2004). As these types of transport all involve interaction with proteins, one may expect uptake rate constants from the dissolved phase to be related to metal characteristics that reflect affinity for proteins. Consistently, empirical studies have suggested that the high absorption efficiency of Ag, Hg and Cd may qualitatively be explained by their binding to membrane transport proteins (Bryan, 1984). At present, a quantitative explanation for this theory has not been developed.

For metal toxicity, quantitative structure activity relationships (QSARs) exist that relate various metal-ion characteristics to chronic and acute toxicity in several species as bacteria, crustaceans (*Daphnia magna*), nematodes (*Caenorhabditis elegans*), amphipods and mice (Jones and Vaughn, 1978; Newman and McCloskey, 1996; McCloskey et al., 1996; Tatara et al., 1997; Walker et al., 2003). In these QSARs, metal-ion characteristics included were thought to represent the affinity of

metals for biological ligands in general. The covalent index ($\chi^2_m r$) is a physico-chemical property that was found to be significantly related to metal ion toxicity in several studies (Newman and McCloskey, 1996; McCloskey et al., 1996; Tatara et al., 1997). This covalent index may also have an explaining power for uptake and elimination kinetics in biota. The aim of the present study is two-fold: 1) to develop quantitative relationships for metal absorption and elimination rate constants with the covalent index of the metal ion and 2) to determine whether species-specific characteristics, i.e. ventilation rates and weight, can explain differences in metal absorption and elimination rate constants between species belonging to different phyla. This is done to determine if a generic modeling approach of absorption and elimination rate constants for these aquatic species is possible and if extrapolation to other species is feasible.

For this purpose we collected empirical absorption and elimination rate constants of various metals for 17 aquatic species, belonging to different phyla including mollusks, crustaceans and fish species. For each species, absorption and elimination rate constants were related to the metal–ion covalent index using linear regression analysis. Additionally, metal uptake rate constants were corrected for species–specific ventilation or filtration rates, to obtain absorption efficiencies. Similarly, species–specific elimination rate constants were corrected for species-weight. Average absorption efficiencies and average weight–corrected elimination rate constants were calculated for each phylum (i.e. mollusks, arthropoda, chordata) and for all species together. These average absorption efficiencies and average weight-corrected elimination rate constants were also related to the covalent index using linear regression analysis.

6.3 Methods

6.3.1 Metal accumulation kinetics

We collected 362 absorption rate constants ($k_{x,w,in}$ in $L \cdot kg^{-1}_{wet\ wt.} \cdot d^{-1}$) measured in 36 studies and 155 elimination rate constants ($k_{x,ex}$ in d^{-1}) measured in 29 studies on several species, including five mollusks, four crustaceans and eight fish species (Appendix 6). Species were included if uptake rate constants were available for at least four different metals. However, for fish this selection criterion appeared to be too strict and single metal measurements were included as well.

The collected absorption rate constants are converted to absorption efficiencies as they are directly comparable among species (Wang, 2001). Metal absorption efficiencies are calculated by dividing metal uptake rate constants by the specific water pumping rates (Eqn. 6.1) (Wang, 2001).

$$p_{x,w,in} = \frac{k_{x,w,in}}{k_{w,in}}$$ Equation 6.1

$p_{X,w,in}$	=	Metal absorption efficiency	[%]
$k_{X,w,in}$	=	Metal absorption rate constant	$[L \cdot kg^{-1}_{wet\ weight} \cdot d^{-1}]$
$k_{w,in}$	=	Water pumping rate (i.e. filtration or ventilation rate)	$[L \cdot kg^{-1}_{wet\ weight} \cdot d^{-1}]$

Species-specific filtration rates are found for various mollusks (*P. viridis*, *M. edulis*, *R. phillipinarum*, *D. polymorpha*) and the crustacean *D. magna*. Water ventilation rates for fish, however, are hardly available. To circumvent this lack of data an allometric relationship was used for fish species, relating fish weight to water ventilation rates (Hendriks, 1995) (Eqn. 6.2). In the case that the weight of fish species was not given in a study, these were estimated from fish lengths. If length was not provided, average, adult weights were used.

$$k_{w,in} = \gamma_0 \cdot w^{-\kappa}$$ Equation 6.2

γ_0	=	Water absorption coefficient (200)	$[kg^{\kappa} \cdot d^{-1}]$
κ	=	Rate exponent (0.25)	[/]
w	=	Species weight	$[kg_{wet\ weight}]$

Species-specific elimination rate constants can be compared when corrected for species weight (Hendriks and Heikens, 2001) (Eqn. 6.3). Metals can be eliminated via excretion with urine, egestion with feces and “growth dilution”. The sum of these three elimination rates represents the actual rate constant of loss, which is related to species weight according to Hendriks and Heikens (2001).

$$k_{x,ex}(w) = k_{x,ex} \cdot w^{\kappa}$$ Equation 6.3

$k_{x,ex}(w)$	=	Weight corrected elimination rate constant	$[kg^{\kappa}_{wet\ weight} \cdot d^{-1}]$
$k_{x,ex}$	=	Elimination rate constant	$[d^{-1}]$
w	=	Species weight	$[kg_{wet\ weight}]$
κ	=	Rate exponent (0.25)	[/]

6.3.2 Metal ion characteristics

Generally, metal ions can be classified into three different groups based on their preferred ligand binding, i.e. (1) oxygen (O)-binding, (2) sulfur (S)- or nitrogen (N)-binding metals and (3) borderline metals binding to both O, N and S-donors (Nieboer and Richardson, 1980; Pearson, 1963) (SI). This classification suggests that soft "Class B" metals such as Cu(I), Ag and Hg tend to occupy N and S ligand sites before O sites in the normal pH region of 5 – 8 (Williams and Frausto di Silva, 2000). Following Nieboer and Richardson (1980), the covalent index represents "Class B" character of the metal – ion, i.e. a high covalent index indicates a high affinity of the metal ion for sulfur ligands.

Covalent indices ($\chi^2_m r$) were calculated using Pauling`s electronegativity values (χ_m) provided by Allred (1961) and effective ionic radii (r) (in Ångstrom units) corresponding to octahedral coordination from Shannon and Prewitt (1969, 1970). Electronegativity values for the correct oxidation state of the metal-ion were used, except for Cr (III) and Cu (II) as χ_m is provided for Cr (II) and Cu (I) only. For lead an ionic radius of 0.94 Å, corresponding to tetrahedral coordination, was used as the resulting covalent index is more in line with the known solution coordination chemistry of Pb^{2+} (Nieboer and Richardson, 1980). Electronegativity data, ionic radii and covalent indices compiled per metal included in this study are presented in the Appendix (Table A6 6.1).

6.3.3 Quantitative Structure Activity Relationship development

For each species, metal uptake rate constants and elimination rate constants were related to the covalent index using linear regression analysis. For this purpose, a geometric mean of the collected absorption rate constants was determined for each metal and each species. Similarly, metal-specific geometric means were calculated from elimination rate constants for each species. All uptake and elimination rate constants were log-transformed prior to regression analysis in order to normalize their distribution. Absorption and elimination rate constants were plotted against the covalent index for each species separately. However, for fish species insufficient data were found to obtain species-specific regressions. Therefore, uptake data of the marine fish *Acanthopagrus schlegeli* and *Lutjanus argentimaculatus* were combined. These species have near equal weight, i.e. 0.4 and 0.37 gram respectively, which suggest similar water ventilation rates and thus similar metal absorption rate constants (Eqn. 6.2).

Metal absorption efficiencies ($p_{X,w,in}$) for each phylum, i.e. mollusks, arthropoda (crustaceans only) and chordata (osteichthyes only) were related to the covalent

index using a logistic – regression because $p_{X,w,in}$ is a fraction (Everitt and Dunn, 2001) (Eqn. 6.4).

$$Log\left[\frac{p_{x,w,in}}{1-p_{x,w,in}}\right] = a \cdot \left[\chi_m^2 r\right] + b \qquad \text{Equation 6.4}$$

Therefore, an average phylum-specific absorption efficiency was calculated from the species - specific absorption efficiency. Additionally, a generic absorption efficiency is calculated per metal from the absorption efficiencies of all species. Metal absorption efficiencies for mollusks were mainly calculated using filtration rates and metal absorption rate constants measured in the same study. If these filtration rates were not available, a species-specific typical filtration rate was used. For *Daphnia magna*, filtration rates were collected from literature and a mean filtration rate of 3.3×10^4 $L \cdot kg^{-1} \cdot d^{-1}$ was used (Appendix Table A6 6.2).
Finally, weight – corrected elimination rate constants were related to the covalent index for each phylum using linear regression analysis. Average phylum – specific elimination rate constants were calculated similar to absorption efficiencies. Weight – corrected elimination rate constants were also log – transformed prior to regression analysis in order to normalize the distribution.
Note that in our approach absorption rate constants determined at different exposure concentrations were combined to calculate mean species – specific absorption rate constants. This is only allowed if absorption rate constants are independent of the exposure concentration in water. We found that this assumption is justified under laboratory conditions (Appendix Figure A6 6.1, Table A6 6.3). Absorption rate constants determined at different exposure concentrations can therefore be combined.

6.4 Results

The results show that measured absorption rate constants are significantly related to the metal covalent index for the mussels *P. viridis* and the clams *R. phillipinarum* ($p = 0.01$) (Table 6.1a,c). For *M. edulis*, *D. polymorpha* and *M. balthica* a non-significant increase is observed between the uptake rate constant and the covalent index (Table 6.1b,d,e). Generally, metal absorption rate constants in mollusks decrease in the order Ag > Hg > Zn > Cd > Co > Cr (III) > Cs, which is consistent with a decreasing covalent index.
Significant, positive relationships between the uptake rate constant from water and the covalent index are also found for crustaceans (Table 6.1f,h). For *D. magna*, 99%

of the variability is explained by the model, yet this regression is based on four metals. A non-significant (p = 0.07) increase of the absorption rate constant with $\chi^2_m r$ is observed for the juvenile, marine fish species *L. argentimaculatus* and *A. schlegeli* (Table 6.1j). Metal absorption rate constants for fish species are approximately a factor of 10 lower than the metal absorption rate constants for mollusks and crustaceans.

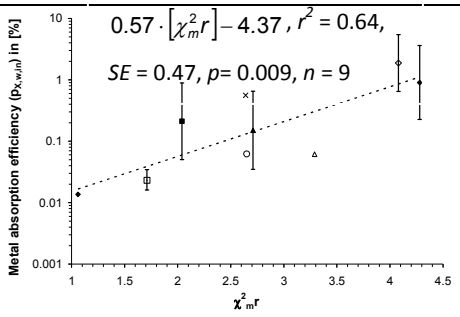
Table 6.1. Regression analysis of species–specific absorption rate constants ($k_{X,w,in}$ in $L \cdot kg^{-1} \cdot d^{-1}$) with the covalent index ($\chi^2_m r$ in Å).

	Species	Metal	Parameter	Regression	r^2	SE	p	n
a	*Perna viridis*	Cs, Cr, Zn, Cu, Cd, Pb, Hg, Ag	Log ($k_{X,w,in}$)	$0.66 \cdot [\chi^2_m r] + 0.05$	0.67	0.56	0.01	8
b	*Mytilus edulis*	Cs, Cr, Zn, Co, Cd, Ag	Log ($k_{X,w,in}$)	$0.58 \cdot [\chi^2_m r] + 0.04$	0.63	0.56	0.06	6
c	*Ruditapes phillipinarum*	Cr, Zn, Cd, Hg, Ag	Log ($k_{X,w,in}$)	$0.68 \cdot [\chi^2_m r] - 0.56$	0.92	0.28	0.01	5
d	*Dreissena polymorpha*	Cr(III), Ag, Hg, Cd	Log ($k_{X,w,in}$)	$0.19 \cdot [\chi^2_m r] + 1.88$	0.86	0.11	0.07	4
e	*Macoma balthica*	Cd, Zn, Ag, Co	Log ($k_{X,w,in}$)	$0.42 \cdot [\chi^2_m r] - 0.17$	0.58	0.41	0.24	4
f	*Daphnia magna*	Zn, Cd, Hg, Ag	Log ($k_{X,w,in}$)	$0.40 \cdot [\chi^2_m r] + 1.47$	0.99	0.05	0.004	4
g	*Chaeto-gammarus marinus**	Ni, Co, (Cu), Cd, Pb	Log ($k_{X,w,in}$)	$1.11 \cdot [\chi^2_m r] - 2.0$	0.89	0.16	0.06	4
h	*Gammarus oceanicus**	Pb, Cd, Ni, (Cu)	Log ($k_{X,w,in}$)	$1.41 \cdot [\chi^2_m r] - 3.53$	1	0.03	0.02	3
i	*Temora longicornis*	Ag, Co, Cd, Zn	Log ($k_{X,w,in}$)	$0.38 \cdot [\chi^2_m r] + 1.48$	0.39	0.56	0.38	4
j	*L.argenti-maculatus* and *A. schlegeli*	Cs, Zn, Cd, Ag	Log ($k_{X,w,in}$)	$0.41 \cdot [\chi^2_m r] - 0.89$	0.86	0.27	0.07	4

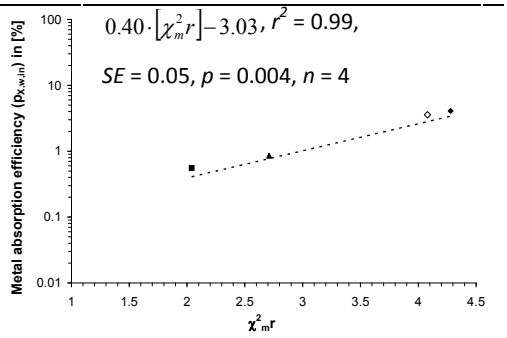
* Copper absorption rate constants are considered to be outliers for *C. marinus* and *G. oceanicus* and therefore excluded from the regression analysis (see discussion on data variability)

A significant, positive relationship is found between metal absorption efficiencies of mollusks and the metal covalent index (Figure 6.1a). Absorption efficiencies of fish are also significantly related to the covalent index and a high explained variance (r^2 = 0.93) is found (Figure 6.1c). For crustaceans, the absorption efficiency relationship with $\chi^2_m r$ is based on data for *D. magna* only and thus similar to the relationship with absorption rate constants for this species (Figure 1b). The slopes of the regression lines for fish, mollusks and crustaceans are very similar (Figure 6.1a,b,c) and therefore the absorption efficiency of all species combined is significantly related to the covalent index (Figure 6.1d).

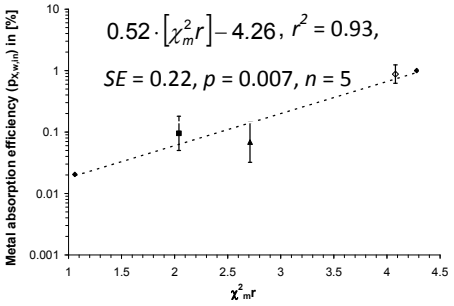
a. Mollusks (Cs, Cr, Zn, Co, Cu, Cd, Pb, Hg, Ag)



b. Crustaceans* (Zn, Cd, Hg, Ag)



c. Fish** (Cs, Zn, Cd, Hg, Ag)



d. All species (Cs, Cr, Zn, Co, Cu, Cd, Pb, Hg, Ag)

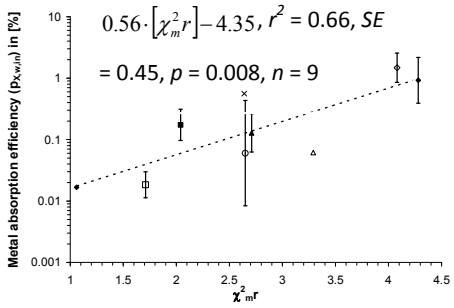


Figure 6.1: Average metal absorption efficiencies ($p_{X,w,in}$ in %) plotted against the metal ion covalent index ($\chi^2_m r$ in Å). Species included: 1a Mollusks: *P. viridis*, *M. balthica*, *R. phillipinarum*, *M. edulis*, *D. polymorpha*, 1b. Crustacea: *D. magna*. 1c. Fish: *A. schlegeli*, *L. argentimaculatus*, *T. jarbua*, *O. mykiss*, *P. platessa*, *P. gibbosus* and *G. affinis*. 1d. All species included. 95% Confidence intervals represent the variability between species. The logistic regression analysis relates Log [$p_{X,w,in}$ / (1-$p_{X,w,in}$)] to [$\chi^2_m r$] ◆ Silver (Ag), ▲ Cadmium (Cd), □ Chromium (III) (Cr), ■ Zinc (Zn), ◇ Mercury (Hg), △ Lead (Pb), ● Cesium (Cs), × Copper (Cu), ○ Cobalt (Co). * Absorption efficiencies for *D. magna* only, ** Confidence intervals of Ag too large to plot

Elimination rate constants are not significantly ($p > 0.18$) related to the covalent index (Table 6.2). Weight – corrected elimination rate constants for various mollusks, crustaceans and fish species, are not consistently different between metals and between species as is shown in Figure 6.2.

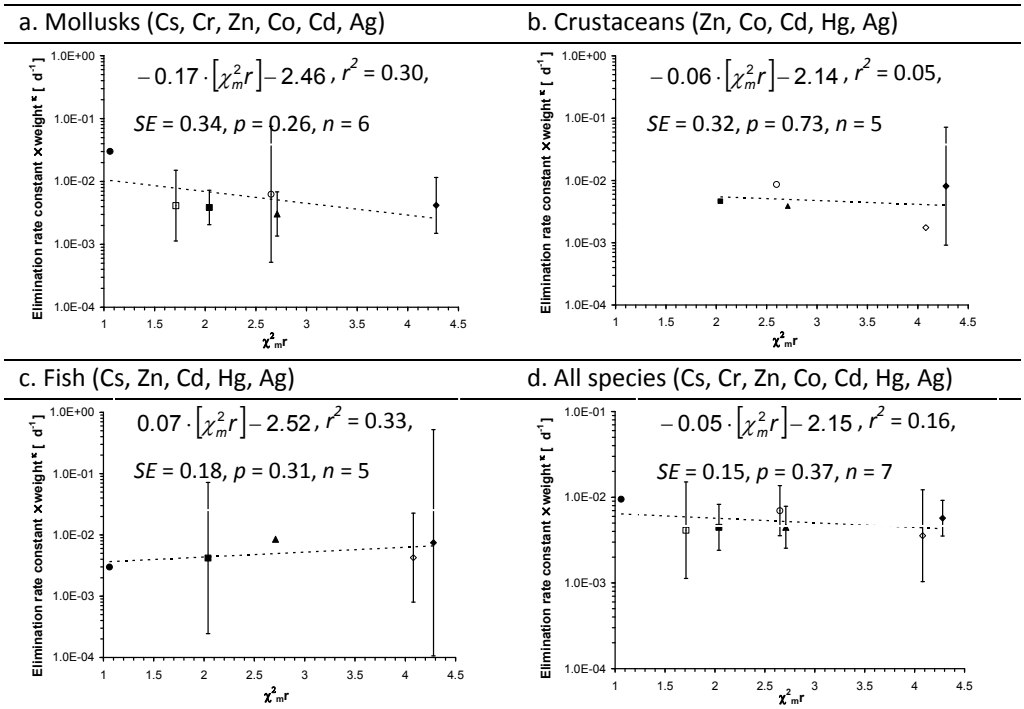


Figure 6.2: Average weight-corrected elimination rate constants ($k_{X,ex}$ (w) in $kg^{\kappa} \cdot d^{-1}$) plotted against the covalent index ($\chi^2_m r$ in Å). Species included are 2a. Mollusks: *P. viridis*, *M. edulis*, *M. balthica*, *R. phillipinarum*, *D. polymorpha*, 2b. Crustaceans: *D. magna*, *T. longicornis*, 2c. Fish: *L. argentimaculatus*, *P. platessa*, *T. jarbua*, *O. mykiss*, *G. affinis*, *P. gibbosus*, *L. microlophys*, *P. cantonensis*, 2d. All species included. 95% Confidence intervals represent the variability between species. The regression analysis relates Log [$k_{X,ex}$ (w)] to [$\chi^2_m r$] ◆ Silver (Ag), ▲ Cadmium (Cd), □ Chromium (III) (Cr), ■ Zinc (Zn), ◇ Mercury (Hg), △ Lead (Pb), ● Cesium (Cs), × Copper (Cu), ○ Cobalt (Co)

Table 6.2. Regression analysis of species – specific elimination rate constants ($k_{X,ex}$ (w)) with the covalent index ($\chi^2_m r$ in Å).

	Species	Metal	Parameter	Regression	r^2	SE	p	n
a	*Perna viridis*	Cd, Cr, Zn, Cd, Ag	Log ($k_{X,ex}$)	$-0.24 \cdot [\chi^2_m r] - 0.94$	0.50	0.34	0.18	5
b	*Mytilus edulis*	Cr, Zn, Co, Cd, Ag	Log ($k_{X,ex}$)	$0.13 \cdot [\chi^2_m r] - 1.71$	0.15	0.37	0.52	5
c	*Ruditapes phillipinarum*	Cr, Zn, Cd	Log ($k_{X,ex}$)	$-0.03 \cdot [\chi^2_m r] - 1.63$	0.07	0.08	0.83	3
d	*Dreissena polymorpha*	Ag, Cd	Log ($k_{X,ex}$)	$0.58 \cdot [\chi^2_m r] - 3.52$				2
e	*Macoma balthica*	Cd, Zn, Ag, Co	Log ($k_{X,ex}$)	$-0.10 \cdot [\chi^2_m r] - 1.52$	0.21	0.22	0.54	4
f	*Daphnia magna*	Zn, Cd, Hg, Ag	Log ($k_{X,ex}$)	$-0.07 \cdot [\chi^2_m r] - 0.66$	0.04	0.48	0.81	4
g	*Chaetogammarus marinus*	Ni, Co, Cu, Cd, Pb	Log ($k_{X,ex}$)	$-0.27 \cdot [\chi^2_m r] + 0.05$	0.21	0.19	0.44	5
h	*Gammarus oceanicus*	Pb, Cd, Ni, Cu	Log ($k_{X,ex}$)	$-0.23 \cdot [\chi^2_m r] + 0.29$	0.05	0.42	0.77	4
i	*Temora longicornis*	Ag, Co, Cd, Zn	Log ($k_{X,ex}$)	$0.14 \cdot [\chi^2_m r] - 1.17$	0.29	0.28	0.46	4
j	*Lutjanus argentimaculatus* and *Acanthopagrus schlegeli*	Cs, Zn, Cd	Log ($k_{X,ex}$)	$0.19 \cdot [\chi^2_m r] - 1.96$	0.38	0.28	0.58	3

6.5 Discussion

6.5.1 Relationship of uptake and elimination rate constants with the covalent index

A significant, positive relationship is observed between metal absorption rate constants and the covalent index of the metal – ion for various species. This can be explained by an increasing affinity for sulfur ligands in membrane transport proteins and higher stability of formed complexes with increasing $\chi^2_m r$, which results in a more efficient uptake. The assumed binding to sulfur ligands in transport proteins is in agreement with experimental data of Wang and Fisher (1999), who showed that the uptake of Ag, Cd and Zn in mollusks was inhibited by N-ethylmaleimide, which specifically binds to sulfhydryl groups thereby reducing the uptake of these metals.

The lowest absorption rate constant found is that for the alkali metal cesium, which corresponds well with the covalent index of cesium. In Pearsons classification cesium belongs to the "sulfur-binding" metals, which is in line with our results. The highest observed absorption rate constants in all species are for silver and mercury. Some studies have explained the efficient uptake of Ag and Hg as a result of the formation of neutral AgCl and $HgCl_2$-complexes that cross membranes by passive diffusion (Reinfelder and Chang, 1999; Bienvenue et al., 1984). This questions the relationship with the covalent index. However, these complexes probably have too much ionic character to make passive diffusion a significant pathway (Bell et al., 2002) and studies with algal species conclude passive diffusion of AgCl is insignificant compared to other uptake routes (Fortin and Campbell, 2000). Consistently, Wang and Fisher (1999) concluded that Ag uptake in the common mussels was dominated by facilitated transport.

The good predictability of absorption rate constants based on one metal-specific property is surprising, as it is believed that metals use various uptake pathways (Bury and Wood, 1999; Grosell and Wood, 2002). For example, Ag and Cu are assumed to cross membranes using Na-channels, whereas Cd is assumed to enter largely via Ca-channels (Bury and Wood, 1999; Grosell and Wood, 2002). The relationship between uptake rate constants and the covalent index does not necessarily exclude metal uptake via other pathways or channels. It indicates that a dominant uptake pathway across the gill membrane involves metal interaction with S-ligands. Other uptake pathways may exist, but these may be less abundant or uptake via these routes is substantially slower than the routes involving S-ligands.

Metal absorption efficiencies are comparable for the crustacean *D. magna* and various mollusks and fish species. This indicates that the high uptake rate constants of metals in mollusks compared to fish species, is due to their high filtration rate and can not be attributed to a higher metal uptake efficiency in mollusks compared to fish. Thus, the metal absorption efficiency of fish, mollusks and crustaceans can be estimated with a single physico-chemical property of the metal-ion, i.e. the covalent index. The results also indicate that metal affinity for membrane transport proteins is not considerably different between fish, crustaceans and mollusks. This is consistent with findings of several biotic ligand modeling (BLM) studies (DiToro et al., 2001; Heijerick et al., 2002; De Schamphelaere and Janssen, 2002). In these studies, conditional stability constants for the binding of a metal to sensitive sites at the cell surface (e.g. channels and carriers) are found to be similar for a range of organisms, including fish species (*Oncorhynchus mykiss* (rainbow trout), *Pimephales promelas* (fathead minnow) and *D. magna* (crustaceans). The similarity of the metal affinity for transport proteins in different species facilitates a generic modeling approach of metal absorption efficiencies.

Metal elimination rate constants show no metal specificity. Elimination rate constants are comparable for different metals and various species after correction for species weight. Various species, including fish and mollusks, rely on sequestration of metals to metallothionein or other intracellular ligands as detoxification mechanism (Hamilton and Mehrle, 1986; Amiard et al., 2006; Ng and Wang, 2004). Measured elimination rate constants are therefore an integral of metal-loss from various compartments. At present, mechanisms of metal elimination are not completely understood. Our results suggest that the affinity of different metals for biological material is, on average, comparable, yet a detailed mechanistic explanation can not be provided.

6.5.2 Variability in accumulation kinetics

Although a large part of the variability in absorption rate constants between metals and species is explained by the covalent index and the ventilation rate, some variability remains. This variability may arise from several factors including metal essentiality, variability in filtration rates of mollusks, combining marine and fresh water fish species and variability in water chemistry. Absorption rate constants for the essential metals, zinc and copper, are generally higher than expected based on their covalent index. Apparently, additional metal properties contribute to the selective uptake of essential metals as well. In particular for the crustaceans, *G. oceanicus* and *C. marinus*, copper absorption rate constants are substantially higher than expected. Copper requirements of these species are relatively high as oxygen-

transport in these crustaceans occurs by the copper-based, respiratory protein haemocyanin (Spicer and Taylor, 1994). Molluscs also use haemocyanin for oxygen transport and Cu uptake rate constants in *P. viridis* are also higher than expected based on the covalent index, although deviations from the regression line are not as large as for the two crustaceans.

Baines et al. (2006) showed that variability in metal absorption rate constants between mussels is primarily a function of the filtration rate. These filtration rates are species-specific and temperature dependent (Baines et al., 2006; Wang, 2001). Correcting metal absorption rate constants for empirical filtration rates (e.g., absorption efficiency) considerably reduces this variability (Figure 6.2). However, some variability in metal absorption efficiencies of mollusks remains. Gill permeability and gill surface area (or size) may also account for inter-specific difference in metal absorption efficiency (Wang, 2001). Additionally, in some cases filtration rates were not measured in the same study as metal uptake rate constants. In these cases typical, species-specific filtration rates were used to calculate metal absorption efficiencies, which may differ from actual filtration rates.

In the present study we included both marine fish and freshwater fish species. Results indicate that metal absorption efficiencies in marine fish are comparable to freshwater fish species, although marine fish drink water to maintain osmotic balance, whereas freshwater fish drink very little water (Wood et al., 1999). Ventilation rates are substantially higher than drinking rates, i.e 18000 versus 3 $mL \cdot kg^{-1} \cdot h^{-1}$ (Wood et al., 1999). Additionally, Wood et al. (2004) and Grosell and Wood (2001) showed that uptake via the gills is the dominant uptake route of Ag (~80%) even at high salinities. Therefore drinking rates probably do not contribute significantly to the total water absorption of marine fish, and marine and freshwater fish species can be combined.

Finally, water chemistry, especially salinity, can induce additional variability in uptake kinetics. Generally, metal absorption rate constants are higher at low salinity (~5 psu) compared to high salinity waters (~30 psu) (Luoma and Rainbow, 2005). Although most absorption rate constants were compiled from experiments with similar experimental conditions (salinity of 30 psu), some studies were carried out under low salinity. This may result in variability in absorption rate constants.

Average, weight-corrected elimination rate constants are relatively constant across metals, as indicated by the standard error (SE = 0.15) of the regression for all species combined. However, metal elimination rate constants do show species-specific variability as indicated by the 95%-confidence intervals. Several factors may influence elimination rates, like temperature, population source, species-specific detoxification mechanisms, and regulation of elimination rates (Luoma and

Rainbow, 2005; Baines et al., 2006). There are, however, no consistent differences observed between species or between metals.

6.5.3 Applicability of the relationship and relevance for environmental risk assessment

In the present study, absorption rate constants were related to the metal ion covalent index, which confines the application of the developed relationship. The relationship is thought to be applicable to all metal ions that cross membranes by binding to sulfur- or nitrogen transport proteins in membranes. This includes all "Class B" and borderline metals from the Nieboer and Richardson-classification, but also includes Cr (III), a "Class A" metal. The relationship is not applicable to anions, as chloride or sulfate, and small, essential metals as sodium, potassium and calcium that are transported by highly, selective transport channels.

Absorption rate constants are predicted independent of the metal concentration in the water-phase. This assumption is justified at low environmental metal concentrations. The concentration of free binding sites on transport proteins remains sufficient and reasonably constant, implying that metal uptake is a linear function of the metal concentration in water and inter-metal competition for free binding sites is assumed to be negligible (Wilkinson and Buffle, 2004). This linearity between absorption rate constants and exposure concentrations has been confirmed by field accumulation data of pristine to relatively contaminated ecosystems (Luoma and Rainbow, 2005). At high exposure concentrations, however, saturation of channels or carriers may occur and therefore competition between metals for binding sites increases. This can be taken into account considering the absorption rate constant to depend on the exposure concentration, as confirmed by field bioconcentration factors (Hendriks and Heikens, 2001). In the present study, we did not include metal uptake from food, as insufficient empirical metal ingestion rate constants were found in literature. Metal uptake from food may be an important accumulation pathway for aquatic organisms (Luoma and Rainbow, 2005). Therefore, this is an important direction for further research.

Our results indicate that metal absorption rate constants can be predicted based on the metal–specific covalent index and the species-specific water ventilation or filtration rate. The developed relationship can facilitate environmental risk assessment as it quantitatively explains a large part of the observed variability across metals and species in absorption rate constants in field and laboratory studies. Implementation of the relationship in bioaccumulation models allows for a more realistic, metal-specific prediction of accumulation, in cases that empirical uptake rate constants are not available. Additionally, extrapolation to other aquatic

species is possible as metal absorption efficiencies are shown to be comparable among various species, including mollusks, crustaceans and fish. Weight-corrected elimination rate constant are shown to be relatively constant among metals, which suggests that a single weight-corrected elimination rate constant can be used in bioaccumulation studies on aquatic species. This relationship is based on average elimination rate constants and variability may exist between species and conditions. Yet, in absence of empirical data, the weight-corrected elimination rate constant is probably the best estimate of elimination.

Chapter 7.

Bioaccumulation potential of air contaminants: combining biological allometry, chemical equilibrium and mass-balances to predict accumulation of air pollutants in various mammals

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7.1 Abstract

In the present study we develop and test a simple, mechanistic model that addresses the accumulation of organic chemicals via inhalation and exhalation of air in mammals. The new aspects of the model are the integration of biological allometry with fugacity-based mass-balance theory to describe exchange of contaminants with air. The developed model is applicable to various mammalian species and a range of chemicals, while requiring few, well-known input parameters, such as the adult mass and composition of the species, and the octanol-water and air-water partition coefficient of the chemical.

Accumulation of organic chemicals is typically considered to be a function of the chemical affinity for lipid components in tissues. Here, we use a generic description of chemical affinity for neutral and polar lipids and proteins to estimate blood-air partition coefficients (K_{ba}) and tissue-air partition coefficients (K_{ta}) for various mammals. This provides a more accurate prediction of blood-air partition coefficients, as proteins make up a large fraction of total blood components.

The results show that 75% of the modeled inhalation and exhalation rate constants are within a factor of 2 from independent empirical values for humans, rats and mice, and 87% of the predicted blood-air partition coefficients are within a factor of 5 from empirical data. At steady-state, the bioaccumulation potential of air pollutants is shown to be mainly a function of the tissue-air partition coefficient and the biotransformation capacity of the species and depends weakly on the ventilation rate and the cardiac output of mammals.

7.2 Introduction

Volatile organic compounds (VOCs) are ubiquitous contaminants of air in both the indoor environment and ambient, urban and regional, environments. A large number of indoor-sources exist including consumer products, building materials, tobacco products, furniture, cleaning products, vehicles in attached garages, water supplies, and outdoor air (Kim et al. 2001; Wallace 2001). VOCs in ambient air largely originate from mobile and industrial sources, including vehicle emissions, industrial operations, gasoline fueling stations, landfills and storage facilities (Wallace 2001; Sexton et al. 2004a; Sexton et al. 2004b). Chronic exposure to relatively low levels of airborne VOCs is an inescapable reality for most people (Sexton et al. 2004a) and (urban) wildlife (Archbold et al., 2007). Many of these compounds are labeled as “hazardous” as they are known or suspected to cause chronic, adverse health effects in exposed populations (Sexton et al. 2004b). They are readily absorbed through the respiratory tract and then transported by blood

to critical organs as the central nervous system. Ashley et al. (1996) have shown that for many VOCs, even when most of the internal dose of these compounds is quickly eliminated, there is a small fraction that is only slowly removed, resulting in potentially important bioaccumulation. Bioaccumulation of persistent VOCs is therefore an issue that must be confronted in human and environmental risk assessment.

Two types of models have been developed to address human and environmental exposure to chemicals. Physiologically based pharmacokinetic (PBPK) models have been widely applied in risk assessment of human exposure to air and water contaminants (Andersen 2003). PBPK models describe the relationship between external exposures and internal concentrations of the biologically effective dose, e.g. the amount biotransformed or the concentration in a target organ (Clewell 1995). Characteristic features of these models are the mass-balance description of chemical distribution within the body based on knowledge of mammalian physiology (e.g. blood flows), biological processes (e.g. metabolic rates as ventilation rate and cardiac output) and physicochemical properties (e.g. tissue-blood partition coefficients). PBPK-models are particularly well-suited to calculate tissue doses of chemicals and their metabolites over a wide range of exposure conditions and can be scaled from one animal species to another (Andersen 2003). However, they are often chemical specific and empirically calibrated, and two major concerns have been the model-complexity and the large amount of biological data that is required to parameterize them (Chiu and White 2006).

A second type of exposure models consists of bioaccumulation models. These models are useful tools in the environmental risk assessment of chemicals because they relate external environmental exposures to internal, possibly toxic, concentrations and provide an estimate of accumulation when no empirical data are available (Hendriks et al. 2001). Bioaccumulation models have traditionally been developed for the aquatic environment, but applications have recently been extended to air-breathing mammals (Czub and MacLachlan 2004; Armitage and Gobas 2007; Kelly et al. 2007). Generally, these models are fugacity-based, one-compartment models, and their major advantage is their relative simplicity and their ability to be consistently applied to a wide range of chemicals. Yet, these models are often species-specific. Human and environmental risk assessment can benefit from a model that can be consistently applied to various species and a wide range of chemicals without case-specific calibration.

One of the most important properties in determining the respiratory kinetics of volatile chemicals in mammals is the blood-air partition coefficient (Lin et al. 2002). In bioaccumulation modeling, it is common practice to assume that chemical

affinity for tissues is a function of the octanol-water partition coefficient of the chemical and the fat content of the organism. Tissue-air partition coefficients are often estimated based on the chemical affinity for lipids only (Kelly and Gobas, 2003; Czub and MacLachlan, 2004; Armitage and Gobas, 2007). However, for blood, where proteins make up a large fraction of total blood components, it is important to consider protein-affinity in predictions of the blood-air partition coefficient. Recently, Hendriks et al. (2005) developed a general description for chemical accumulation in various tissues, distinguishing between neutral lipids and polar lipids and including non-lipid fractions such as proteins. It is important to investigate whether this generic approach can be used to accurately predict blood-air partition coefficients for various mammals and a range of chemicals.

The aim of this study is two-fold:

1. to develop and test, a generic, one-compartment bioaccumulation model for air exposure that describes uptake and elimination kinetics as a function of chemical specific properties, physiological processes, and species-based allometric scaling.
2. to investigate whether a generic description of substance accumulation in various blood components can be used to predict blood-air partition coefficient for various mammalian species including humans, rats, mice, rabbits, pigs, guinea pigs and dogs.

To this end we combined steady-state equations of physiologically-based pharmacokinetic models for volatile chemicals with established allometric relationships for ventilation rate and cardiac output. Allometric scaling of these physiological processes as a power of body weight (¾) allows extrapolation from one species to another. We use the resulting model to estimate chemical uptake from air as a function of species weight and the chemical-specific blood-air and tissue-air partition coefficients. These partition coefficients are estimated from physiological characteristics, such as the fraction of neutral lipids, polar lipids, proteins and the water content, and well-documented physicochemical properties such as the octanol-water and air-water partition coefficients. The model performance is evaluated with an independent dataset of measured chemical inhalation rate constants and measured exhalation rate constants for a variety of chemical substances and different mammals, including humans, rats and mice. Additionally, we compare predicted blood-air partition coefficients to empirical data collected from 29 studies.

7.3 Methods

7.3.1 Internal chemical concentration

At steady-state the chemical concentration in an organism can be estimated as the total uptake via air, divided by the total elimination via exhalation ($k_{x,a,ex}$), excretion with urine ($k_{x,w,ex}$), egestion with food ($k_{x,n,ex}$), growth dilution (k_g) and biotransformation ($k_{x,m}$) (Hendriks et al. 2001).

$$C_i = \frac{k_{x,a,in} \cdot C_A}{k_{x,a,ex} + k_{x,w,ex} + k_{x,n,ex} + k_g + k_{x,m}} \qquad \text{Equation 7.1}$$

C_i	=	Internal concentration	[kg·kg^{-1} $_{\text{wet wt.}}$]
C_A	=	Chemical concentration in air	[kg·dm^{-3}]
$k_{x,a,in}$	=	Chemical inhalation rate constant	[dm^3·kg^{-1}·d^{-1}]
$k_{x,a,ex}$	=	Chemical exhalation rate constant	[d^{-1}]
$k_{x,w,ex}$	=	Chemical excretion rate constant	[d^{-1}]
$k_{x,n,ex}$	=	Chemical egestion rate constant	[d^{-1}]
k_g	=	Growth dilution rate constant	[d^{-1}]
$k_{x,m}$	=	Chemical biotransformation rate constant	[d^{-1}]

Bioaccumulation of chemicals via exposure to contaminated air can be characterized by a bioaccumulation factor, which equals the total internal concentration (C_i) divided by the concentration in air (C_A). If exposure occurs only via inhalation of air, this bioaccumulation factor can be estimated as the inhalation rate constant divided by the total elimination rate (Eqn. 7.2). The equations for excretion with urine, egestion with faeces and growth dilution have been developed by Hendriks et al. (2001) and are provided in the Supporting Information. The development of the inhalation and exhalation rate constants is described in the next section.

$$BAF = \frac{C_i}{C_A} = \frac{k_{x,a,in}}{k_{x,a,ex} + k_{x,w,ex} + k_{x,n,ex} + k_g + k_{x,m}} \qquad \text{Equation 7.2}$$

At present, biotransformation rates ($k_{x,m}$) can not be predicted accurately enough based on species characteristics and chemical properties. Because several hazardous air pollutants are readily transformed it is important to take biotransformation into account as an elimination pathway. But it is also important to note that for the internal mass balance of many VOCs, elimination by exhalation

is very large compared to biotransformation. To address this issue, we tested model sensitivity to biotransformation by incrementally increasing the rate constants for biotransformation. This evaluation will give us information on how values for biotransformation range that will have a strong impact on model results. Biotransformation rates of volatile organic compounds are concentration dependent and are generally considered to follow Michaelis-Menten kinetics (Filser et al. 2000). However, at low, environmentally relevant concentrations, biotransformation rates attend to follow linear, first-order kinetics, allowing the use of a concentration-independent biotransformation rate ($k_{x,m}$).

7.3.2 Chemical inhalation and exhalation rate constants

Chemical uptake and loss by respiration is inversely proportional to a series of resistances and flow delays. The equilibrium rate constant for chemical uptake via inhalation ($k_{x,a,in}$) can be modeled as three transport processes occurring in series (Cahill et al. 2003) (Eqn. 7.3). First, the chemical is inhaled into/by the lungs. This airflow carries the chemical from the external air to the alveolar region in the lungs. The corresponding flow delay is quantified as the inverse of the alveolar ventilation rate (G_A). The alveolar ventilation rate is the amount of air that reaches the alveoli and is available for gas exchange with the blood per unit time. Second, the chemical diffuses across the blood-air barrier and enters the capillaries. The diffusion resistance is a function of the thickness of the blood-air barrier (β_A), the alveolar surface area (A_A), the chemical diffusivity in the cytosol (d_w) and the blood-air partition coefficient (K_{BA}). Finally, the chemical is transported from the lungs to the main blood circulation. It is generally assumed that volatile chemicals equilibrate very rapidly with capillary blood in the lungs (Ramsey and Andersen 1984). This equilibrium ratio can be expressed by the blood-air partition coefficient of the chemical (Ramsey and Andersen 1984). The total flow delay is a function of the full cardiac output (G_B) and the chemical-specific blood-air partition coefficient (K_{BA}).

Chemicals entering the lungs in the venous blood stream can be transferred to lung air and be exhaled. This transport term is opposite of the inhalation and absorption process and works to remove chemicals from the blood stream. It works well for removing chemicals with a sufficiently high vapor pressure that are not accumulated in tissues such as body fat (Eqn. 7.4). The latter is expressed by the accumulation ratio (K_{TA}), which reflects the affinity of substances for different body compartments, including neutral and polar lipids, protein fractions and water.

$$k_{x,a,in} = \frac{1}{\left(\frac{1}{G_A} + \frac{\beta_A}{d_w \cdot A_A \cdot K_{BA}} + \frac{1}{G_B \cdot K_{BA}}\right)}$$ Equation 7.3

$k_{x,a,in}$	=	Chemical inhalation rate constant	[$dm^3 \cdot kg^{-1} \cdot d^{-1}$]
w	=	Species wet weight	[kg]
G_A	=	Alveolar ventilation rate	[$dm^3\ kg^{-1} \cdot d^{-1}$]
d_w	=	Aqueous diffusion in cytosol	[$dm^2 \cdot d^{-1}$]
A_A	=	Alveolar surface area	[$dm^2\ kg^{-1}$]
β_A	=	Diffusion distance across alveolar cells	[dm]
G_B	=	Cardiac output	[$dm^3 \cdot kg^{-1} \cdot d^{-1}$]
K_{BA}	=	Blood –air partition coefficient	[/]

$$k_{x,a,ex} = \frac{1}{K_{TA}} \cdot \frac{1}{\left(\frac{1}{G_A} + \frac{\beta_A}{d_w \cdot A_A \cdot K_{BA}} + \frac{1}{G_B \cdot K_{BA}}\right)}$$ Equation 7.4

Table 7.1: Physiological parameters and allometric relationships for ventilation rate ($dm^3 \cdot kg^{-1} \cdot d^{-1}$), cardiac output and alveolar surface area of mammals

Parameter		**Value**	r^2	***n***	**Unit**	**Reference**
Minute ventilation rate	G_M	$6.7 \cdot 10^2 \cdot w^{-0.25}$	0.96	19	$dm^3 \cdot kg^{-1} \cdot d^{-1}$	Lindstedt and Schaeffer, 2002
Full cardiac output	G_B	$3.2 \cdot 10^2 \cdot w^{-0.25}$	0.99	21	$dm^3 \cdot kg^{-1} \cdot d^{-1}$	Lindstedt and Schaeffer, 2002
Alveolar surface area	A_A	$293 \cdot w^{-0.03}$	0.99	11	$dm^2 \cdot kg^{-1}$	Weibel, 1979
Blood-air barrier	β_A	$2.7 \cdot 10^6 \cdot (w \cdot 1000)^{0.06}$	0.81		dm	Mania and West, 2005

n represents the number of species included, species weight (w) in kg. Alveolar ventilation rate (G_A) equals 67% of minute ventilation rate (G_M) (EPA, 1988)

In the prediction of the inhalation and exhalation rate constants, the allometric relationships for the minute ventilation rate and cardiac output were taken from a review by Lindstedt and Schaeffer (2002) and are based on relationships for various mammals (Table 7.1). The alveolar ventilation rate is assumed to be 67% of the

minute ventilation rate (EPA 1988). The mass-specific flow rates, such as ventilation rate and cardiac output, typically scale to body weight with a power -¼ (Peters 1983). The lung's diffusing capacity for O_2 has, however, repeatedly been shown to be independent of body weight revealing no scale factor (Weibel 1979; Hughes 1984; Lindstedt 1984). Additionally, the alveolar surface area is independent of body weight (Weibel 1979; Hughes 1984) and the diffusion distance across the blood-air barrier is comparable across various mammalian species (Mania 2002; Maina and West 2005; Weibel 1979). Consequently, the diffusion resistance scales to the power of 1 instead of 0.75. The pure water diffusion coefficient (d_w) for neutral organic substances is calculated following Schwarzenbach (1993) (Appendix 7).

7.3.3 Physiological partition coefficients

The chemical affinity for both neutral lipids and polar lipids is approximately linearly related to the affinity for octanol (Hendriks et al. 2005). Substance affinity for proteins can also be related to the octanol-water partition coefficient, but the relationship is less than linear due to the polar nature of proteins (Hendriks et al. 2005). The blood-air partition coefficient can thus be estimated based on the physiological characteristics of blood and the affinity for these different blood components divided by the temperature-corrected air-water partition coefficient (Eqn. 7.5).

$$K_{BA} = \frac{p_{nl,bl} \cdot K_{ow} + p_{pl,bl} \cdot K_{ow}^{0.94} + p_{p,bl} \cdot K_{ow}^{0.63} + p_{H2O,bl} \cdot K_{ow}^{0}}{K_{aw}} \qquad \text{Equation 7.5}$$

K_{BA}	=	Blood-air partition coefficient
K_{aw}	=	Air-water partition coefficient (37°C)
K_{ow}	=	Octanol-water partition coefficient (25°C)
p_{nl}	=	Neutral lipid percentage of blood (bl) or tissues (t)
p_{pl}	=	Polar lipid percentage of blood (bl) or tissues (t)
p_{p}	=	Protein percentage of blood (bl) or tissues (t)
p_{H2O}	=	Water percentage of blood (bl) or tissues (t)

Similarly, equation 7.5 can be used to estimate the tissue-air partition coefficient (K_{ta}). Predicted blood–air partition coefficients were compared to empirical blood–air partition coefficients. Species-specific blood parameters were used to predict the blood-air partition coefficient, except for mice, guinea pigs and pigs. For these species we used typical mammalian values (Table 7.2).

7.3.4 Data collection and treatment

Physiological parameters such as neutral lipid fraction, polar lipid fraction, protein fraction and water fraction of blood were collected for various mammalian species, including humans, rats, dogs, mice and rabbits (Table 7.2; Appendix 7). Typical values for the polar lipid content, neutral lipid content and protein fraction in tissues of mammals were obtained from Hendriks et al. (2005).

Physical-chemical properties, i.e. octanol–water partition coefficient (K_{ow}), vapor pressure (V_P) and water solubility (C_W) were obtained from Mackay et al. (2006). Air–water partition coefficients (K_{aw}) were calculated from the vapor pressure and the water solubility using the ideal gas law (Appendix 7). Generic physical–chemical properties (measured at T = 25ºC) were corrected for temperature (T = 37ºC) following MacLeod et al. (2007) and Beyer et al. (2002) (Appendix 7).

The empirical blood–air partition coefficients were collected from 29 studies (Abbas and Fisher 1997; Barton et al. 1995; Batterman et al. 2002; Béliveau et al. 2001; Dallas et al. 1994; Dills et al. 1993; Fang et al. 1997; Fiserova-Bergerova and Diaz 1986; Fisher et al. 1991; Fisher et al. 2004; Gargas et al. 1989; Gearhart et al. 1993a; Gearhart et al. 1993b; Jarnberg and Johansson 1995; Johanson and Filser 1993; Kaneko et al. 2000; Lin et al. 2001; Liu et al. 1994; Nihlén et al. 1995; Pierce et al. 1996; Reitz et al., 1996; Robinson et al. 2000; Sato et al. 1975; Sato et al. 1974; Sato and Nakajima 1979; Smith et al. 2005; Tardif et al. 1997; Thrall et al. 2004; Wiester et al. 2002). Most studies have focussed on determining K_{BA} for rats and humans. A few additional data were available for mice, dogs, rabbits, guinea pigs and pigs. These blood–air partition coefficients were included to check whether the approach allows extrapolation to other mammalian species.

Empirical inhalation rate constants were obtained from 8 studies and for three species, including rats, mice and human (Andersen 1981; Andersen et al. 1984; Filser et al. 1993; Bolt et al. 1984; Johanson and Filser 1992; Filser and Bolt 1979; Filser et al. 1987; Bolt et al. 1981). Measured exhalation rate constants were obtained from 6 studies and for three species (rats, mice and human) (Andersen et al. 1980; Bolt et al. 1981; Filser and Bolt 1979; Filser et al. 1987; Filser et al. 1993; Yoshida et al. 1998). Where possible chemical-specific geometric means were calculated for inhalation rate constants and exhalation rate constants.

Table 7.2. Species-specific blood and tissue parameters and for mammals: water content (p_{H2O} in $g \cdot mL^{-1}$), neutral lipid content (p_{nl} in $g \cdot mL^{-1}$), polar lipid content (p_{pl} in $g \cdot mL^{-1}$) and protein content (p_p in $g \cdot mL^{-1}$) of whole blood and tissues (SI). The values for "mammals" are average values for mammal blood and tissues (SI). These average values were calculated based on species-specific data (minimum and maximum values for these fractions and SE are provided in SI).

Species	**Body part**	**p_{H2O}**	**p_{nl}**	**p_{pl}**	**p_p**	**References**
		$mL \cdot mL^{-1}$	$g \cdot mL^{-1*}$	$g \cdot mL^{-1*}$	$g \cdot mL^{-1*}$	
Human	Blood	80.6%	0.33%	0.24%	17.4%	Altman, 1961; Davies and Morris, 1993, Haddad et al., 2000
Rat	Blood	81.6%	0.23%	0.23%	17.9%	Altman, 1961; Davies and Morris, 1993; Nelson, 1967; Nelson, 1972
Rabbit	Blood	81.7%	0.23%	0.19%	17.9%	Altman, 1961; Davies and Morris, 1993; Nelson, 1967; Nelson, 1972
Dog	Blood	80.1%	0.34%	0.28%	19.3%	Altman, 1961; Davies and Morris, 1993; Nelson, 1967; Nelson, 1972
Mammals	Blood	80.4%	0.23%	0.20%	19.8%	Altman, 1961; Davies and Morris, 1993; Nelson, 1967; Nelson, 1972; Haddad et al., 2000
Mammals	Tissue	70%	9%	1%	21%	Hendriks et al., 2005

*The density of blood is approximately 1 $g \cdot mL^{-1}$ (1.06 $g \cdot mL^{-1}$)

7.4 Results

Figure 7.1 shows that 87-88% of the predicted blood-air partition coefficients for rats and humans are within a factor of five of empirical values. 67% and 51% of the predictions are within a factor of two from measurements for humans and rats, respectively. For the other mammals all predictions are within a factor of five from empirical blood-air partition coefficients, except the prediction for 2,4-dimethylpentane (dogs). Several deviations between empirical K_{BA}s and predicted

partition coefficients are observed. Two issues stand out: first, blood-air partition coefficients are underestimated for chemicals with a combination of a relatively low octanol-water partition coefficient, $\log K_{ow}<2$, and a relatively high air-water partition coefficient, $\log K_{aw}>0.32$. Deviations between predicted values and empirical data are observed for several low $\log K_{ow}$-high $\log K_{aw}$-chemicals: vinyl chloride (for human, rat and mouse), methylchloride, 1-bromopropane and 1,3-butadiene (for both humans and rats) and diethyl ether (for rats). Second, blood-air partition coefficients for humans are slightly, with a factor of 2-3, overestimated and measured partition coefficients for humans are generally lower than partition coefficients for rats.

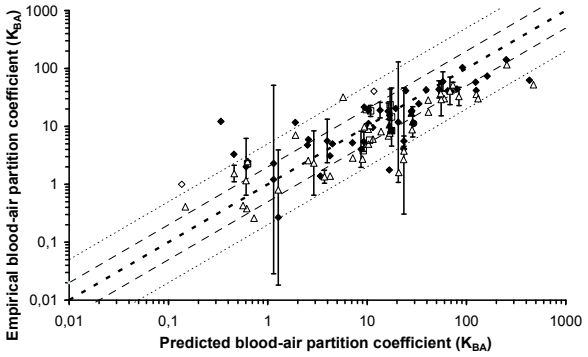

Figure 7.1. Predicted blood-air partition coefficients (K_{BA}) versus independent empirical blood-air partition coefficients. △Human □ Mice ◇ Dog ◆ Rat ● Pig ▲ Rabbit, ■ Guinea pig. Geometric means with 90% confidence intervals are presented were possible. Dashed line represents the theoretical 1:1-relationship
Dotted lines represent a factor of 2 and a factor of 5 above and below the 1:1 line. $r^2 = 0.67$, n = 100, SE = 0.36. Regression analysis was performed on the total dataset for log-transformed values of empirical K_{BA} vs. predicted K_{BA}

Figure 7.2a and b show that 75% of the predicted inhalation and exhalation rate constants are within a factor of 2 from empirical data. All modelled inhalation rate constants are within a factor of five from measurements (Figure 7.2a) with exception of the predicted vinyl chloride inhalation rate constant for rats. The underestimation of the inhalation rate constant for this chemical can be attributed to the underestimation of the blood-air partition coefficient for low $\log K_{ow}$-high $\log K_{aw}$-chemicals, such as vinyl chloride. The K_{BA} of vinyl chloride is underestimated by a factor of 3.3 and model predictions are within a factor of 3 from empirical data if the measured K_{BA} for rats is used (results not shown). In figure 7.2b it is shown that chemical exhalation rate constants are well predicted by the model ($r^2 = 0.82$).

a. Inhalation rate constants ($k_{x,a,in}$) [$dm^3 \cdot kg^{-1} \cdot d^{-1}$]: model predictions compared to empirical data	b. Exhalation rate constant ($k_{x,a,ex}$) [d^{-1}]: model predictions compared to empirical data

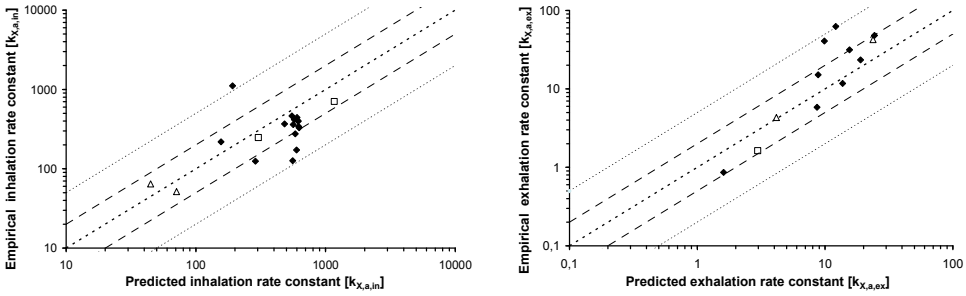

Figure 7.2. Model predictions of exchange with air compared to measurements △Human□ Mice ◆ Rat. Dashed line represents the theoretical 1:1-relationship, Dotted lines represent a factor of 2 and a factor of 5 above and below the 1:1 line. A regression analysis was performed of log-transformed inhalation and exhalation rate constants versus log-transformed empirical rate constants. For inhalation rate constants (Fig. 7.2a): $r^2 = 0.41$, SE = 0.28, n = 19 and for exhalation rate constants (Fig. 7.2b) $r^2 = 0.82$, SE = 0.22, n = 13

The overall rate constant for chemical exchange with air is limited by the slowest process. Evaluation of the model equation for exchange via air shows that the diffusion resistance through the blood–air barrier is negligible compared to the combined resistance of the alveolar ventilation rate and blood flow. The chemical uptake and elimination via air is therefore flow-limited and the diffusion term can be neglected in the equations for inhalation and exhalation. In figure 3a model predictions for chemical uptake in rats and empirical inhalation-data are plotted against the estimated blood-air partition coefficient (K_{BA}). For vinyl chloride and 1,3-butadiene empirical values of the K_{BA} were used, as these substances belong to the group of chemicals with a low $\log K_{ow}$ and high $\log K_{aw}$. The K_{BA}-model was shown to have less predictability for this chemical group. This figure shows that inhalation rate constants depend on the blood-air partition coefficient at values of K_{BA} lower than 1.6, whereas at high K_{BA} a maximum chemical inhalation rate constant is observed. This trend is partly confirmed by empirical data. A maximum chemical inhalation rate constant is observed, yet empirical data do not show a clear decline in inhalation rate constants with decreasing K_{BA}. However, insufficient empirical inhalation rate constant data for low K_{BA}-substances are available to evaluate model predictions in this range. The model slightly overestimates the maximum inhalation rate constant for rats by approximately a factor of 1.7. For most compounds exhalation rate constants are somewhat underestimated (by a factor of 2.2) compared to empirical rate constants of loss.

Exhalation rate constants decrease with increasing K_{BA} and similarly, these rate constants decrease with increasing K_{TA} (Figure 7.3b).

a. Inhalation rate constants for rats ($k_{x,a,in}$ in $dm^3 \cdot kg^{-1} \cdot d^{-1}$) plotted against the predicted blood-air partition coefficient (K_{ba})	b. Predicted and measured exhalation rate constants for rats ($k_{x,a,ex}$ in d^{-1}) plotted against the predicted tissue-air partition coefficient (K_{ta})

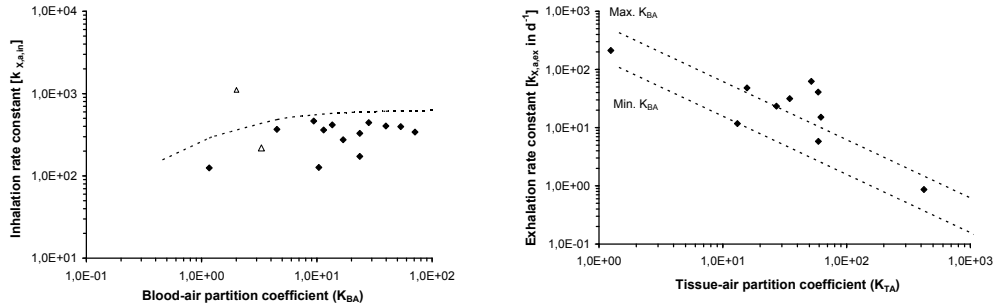

Figure 7.3 (a) ◆ Empirical inhalation rate constants for rats plotted against predicted K_{ba}, △ Empirical inhalation rate constants of vinyl chloride and 1,3-butadiene are plotted against measured blood-air partition coefficients as these substances belong to the class of chemicals with a low $logK_{ow}$ and high $logK_{aw}$. The K_{ba}-model has less predictability for this class of chemicals. (b) ◆ Empirical exhalation rate constants for rats plotted against predicted K_{ta}. Model predictions are shown for a minimum and maximum value of the blood-air partition coefficient, i.e. a value of 0.46 and 71, respectively. This minimum and maximum K_{ba} are representative for the included substances.

Bioaccumulation of chemicals in an organism occurs if the rate of uptake from air is higher than the total rate of elimination, i.e. the sum of excretion with urine, egestion with faeces, growth dilution and biotransformation. A quantitative analysis of the different elimination rate constants shows that persistent volatile organic compounds are predominantly eliminated via exhalation. This finding is illustrated in figure A7.3 of Appendix 7. The contribution of other pathways of loss, such as excretion with water, egestion with faeces and growth dilution, to the total elimination rate is typically less than <10%. Therefore, the steady-state bioaccumulation factor (C_i/C_A) of air pollutants can be estimated as the inhalation rate constant divided by the exhalation rate constant. Figure 7.4 shows that persistent air pollutants with a blood-air partition coefficient of 5 or higher, have a high bioaccumulation potential (BAF $\geq$ 60 $dm^3 \cdot kg^{-1}$ wet wt.) unless they are biotransformed at a sufficiently rapid rate (i.e. $k_m > 500\ d^{-1}$ obtains a BAF of 1). This ratio equals the equilibrium tissue-air partition coefficient, if the biotransformation rate is negligible.

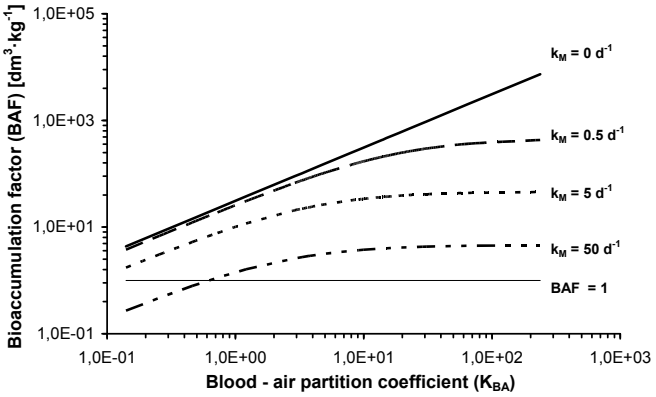

Figure 7.4. Predicted bioaccumulation factors (BAF) using different metabolization rate constants (k_M in d^{-1}) plotted against the blood-air partition coefficient.

7.5 Discussion

7.5.1 Blood-air partition coefficients

One of the objectives of the present study was to investigate whether a generic description of substance accumulation in various blood components can be used to predict blood-air partition coefficient for various mammalian species including humans, rats, mice, rabbits, pigs, guinea pigs and dogs. Our results suggest that in general blood-air partition coefficients can be reliably predicted for various mammals. However, blood-air partition coefficients of substances with a combination of a relatively low octanol-water partition coefficient, $\log K_{ow}<2$, and a relatively high air-water partition coefficient, $\log K_{aw}>0.32$ are underestimated. DeBruyn and Gobas (2007) concluded that low K_{ow}-chemicals have a higher affinity for blood proteins such as albumin than expected based on their K_{ow}. Our results suggest that especially chemicals with a combination of a lowK_{ow} and a high $\log K_{aw}$ have a higher affinity for blood proteins than expected.

Also blood-air partition coefficients for humans are slightly overestimated and measured partition coefficients for humans are generally lower than partition coefficients for rats. This observation is consistent with results from several studies (Lin 1998; Gargas et al. 1989; Lam et al. 1990) and has been attributed to a weaker binding to plasma-proteins such as albumin and/or hemoglobin in humans compared to rats (Lin 1998; Gargas et al. 1989; Wiester et al. 2002; Lam et al. 1990). This suggests that the sorptive capacity of human blood proteins is overestimated by our model.

Other deviations between model predictions and empirical values may be due to variation in blood parameters, especially in neutral lipid content of blood, and due

to differences in experimental techniques between studies. Although inter-species differences in blood composition are relatively small (Nelson 1972; Nelson 1967), partition coefficients have been found to vary with changes in hematocrit and blood lipids (Fiserova-Bergerova 1983; Lin et al. 2002). There is considerable variation in reported blood-air partition coefficients of a chemical between studies as indicated by 90% confidence intervals in figure 7.1. Differences in experimental techniques, for example differences in equilibration time may also contribute to the observed variability in empirical blood-air partition coefficients (Wiester et al. 2002).

7.5.2 Contaminant exchange via air

The blood-air partition coefficient is often assumed to be one of the most important properties in determining the respiratory kinetics of volatile chemicals in humans (Lin et al. 2002). Evaluation of the model equations for contaminant exchange with air shows that inhalation rate constants are K_{BA}-dependent at low values for K_{BA}, whereas at high K_{BA} values a maximum chemical inhalation rate constant is observed. This is due to a flow-limitation imposed by the ventilation rate, which results in comparable inhalation rate constants for different chemicals. Compared to inhalation rate constants, exhalation rate constants are a more complex function of the tissue-air partition coefficient (K_{TA}), the blood-air partition coefficient (K_{BA}) and the flow delays imposed by ventilation and cardiac output. Consequently, the exhalation rate constants show more variation than inhalation rate constants, depending on chemical-specific properties and species characteristics.

Our results show that the maximum inhalation rate constant for rats is generally slightly overestimated. There are two possible explanations for this overestimation. First, model performance for high K_{BA} substances strongly depends on an accurate prediction of the ventilation rate. A ventilation rate of 180 $dm^3 \cdot d^{-1}$ is predicted for a 250 gram rat. This prediction is in close agreement (within a factor of 1.1) with standard physiological parameters for ventilation (168 $dm^3 \cdot d^{-1}$ for a 250 gram rat) (Arms and Travis 1988). Second, in our modelling approach we assumed that there is no exchange of substances between air and tissue in parts of the respiratory tract other than the alveolar region. However, gases with a high water-solubility will also be absorbed to the tissues of the conducting airways during inhalation and desorbed upon exhalation, so-called wash-in/wash-out effects. This may result in lower uptake rate constants than expected based on the ventilation rate, cardiac output and blood-air partition coefficient of the chemical (Johanson and Filser 1995). This wash-in/wash-out effect is particularly important for rodents, and is

thought to be less important in humans, due to the relative smaller surfaces of the human nasal cavity (Filser et al. 1993). In contrast, exhalation rate constants may be underestimated compared to measured rates of loss due to wash-out of chemicals from tissues in the respiratory tract. This may explain our observation that exhalation rate constants for rats are slightly underestimated.

7.5.3 Bioaccumulation potential of air pollutants

It is shown that air pollutants with a blood-air partition coefficient of 5 or higher, have a high bioaccumulation potential (BAF $\geq$ 60 $dm^3 \cdot kg^{-1}$ wet wt.) unless they are biotransformed at a sufficiently rapid rate. This bioaccumulation potential is similar in humans and rodents as variation in tissue-composition is relatively small across mammals (Hendriks et al. 2005). However, as is shown in figure 7.4 the bioaccumulation potential strongly depends on the biotransformation capacities. This biotransformation capacity is largely chemical- and species-specific and differences in bioaccumulation potential between humans and rodents will arise due to variation in biotransformation rates (Pastino et al. 2000).

7.6 Model applicability in human and environmental risk assessment

Following calls for integration of human and ecological risk assessment, we have presented a mechanistic bioaccumulation model for chemical exchange via air, which is applicable to neutral organic chemicals and various mammals. This model uses chemical- and species-specific inhalation rate constants and exhalation rate constants that are estimated based on chemical properties, physiological partition coefficients and species characteristics. We have evaluated the performance of this model for accumulation kinetics of volatile organic compounds in rats, mice and humans. Options to evaluate the model for other animal species were limited. Yet, the combination of biological allometry with chemical fugacity theory makes it feasible to extrapolate the model to other species and chemicals beyond the tested range. The advantage of the model is the relative simplicity and the minimum amount of input-data required, i.e. adult mass of the species, and the octanol-water and air-water partition coefficient of the chemical. In contrast, PBPK-models require many parameters to be set on substance-specific and species specific values. While size of organs and affinity of chemicals for tissues have been occasionally related to chemical and biological properties using empirical regressions, these relationships have not been incorporated into a consistent and mechanistic framework. However, as organ sizes and affinity of chemicals for different tissues follows the same allometric and fugacity principles, the approach laid down in the present paper can be extended to multi-compartment models

without additional data on parameters for body composition of individual species and organ-specific partitioning. As the present one-compartment version of the model already produces fits within a factor of 5, such an extension to a multi-compartment is likely to yield even better predictions. Obviously, the validity of such an approach is to be tested, which is beyond the scope of the present paper.

For both one-compartment and multi-compartment modelling availability of information on biotransformation rates is and will be a limiting factor. This applies, however to all risk assessment models in this field. While improvements of “in vivo”, “in vitro” and “in silico” methods for predicting metabolism are promising, the present model can, just as other risk assessment models be useful for a conservative screening-level risk assessment, in which comparisons are made between large sets of substances and various species.

Chapter 8.

Synthesis and Discussion

8.1 Introduction

Bioaccumulation has become a critical consideration in chemical regulation worldwide (EC, 2006; EU-WFD, 2000; OSPAR, 2004; UNEP, 2006). Yet, most of the thousands of substances and species that are of interest for environmental management have not been monitored at relevant locations and periods, because of financial, ethical and technical constraints (Hendriks et al., 1994; Hendriks and Van de Guchte, 1997; Arnot and Gobas, 2006; Weisbrod et al., 2007). Environmental risk assessment therefore requires quantitative tools that can reliably estimate the bioaccumulation potential of various chemicals, including metals and non-chlorine organics, for a range of species in different ecosystems. The last decade, mass-balance models have been successfully applied to estimate accumulation of organic compounds and metals in mostly aquatic ecosystems (Thomann et al., 1992; Gobas, 1993; Traas et al., 1994; Thomann et al., 1995; Campfens and Mackay, 1997; Luoma and Rainbow, 2005; Arnot and Gobas, 2004). Accumulation kinetics of nonpolar organic chemicals has been related to substance properties as the octanol-water partition coefficient, and to species-characteristics as the organism's lipid content, the gill ventilation rate and the food ingestion rate. These mechanistic models show good predictability of organochlorine bioaccumulation in aquatic organisms, including mussels, crustaceans and fish (Gobas and Wilcockson, 2003; Arnot and Gobas, 2004). Similar models for other ecosystems, in particular terrestrial environments, and other substances, i.e. non-chlorine organics and metals, are largely absent. Although most models estimate accumulation based on substance properties and species characteristics, key physiological parameters are often obtained by species-specific calibration or parameterization. Therefore, these models are often not easily scaled from one species to another.

The unique characteristics of the bioaccumulation model OMEGA are the combination of classical mass-balance approach with biological allometry to predict chemical accumulation in biota. Internal chemical concentrations are estimated as a function of well-known properties of species and chemicals, such as the adult mass (w), body composition and trophic level of the species, and the octanol-water partition coefficient (K_{ow}) of the chemical (Hendriks et al., 2001; Hendriks and Heikens, 2001). The model can be applied to both nonpolar organic chemicals and metals. Accumulation kinetics of metals is estimated analogous to accumulation kinetics of organic compounds, i.e. uptake and elimination rate constants are a function of species characteristics, as adult mass, body composition and trophic

level, and a generic tissue-water partition coefficient. Metal uptake rate constants from water and food are additionally assumed to be dependent on the exposure concentration and follow saturable uptake kinetics analogous to Michaelis-Menten kinetics for enzymes (Hendriks and Heikens, 2001). This approach allows for model application to a wide range of species, both aquatic and terrestrial environments, and various chemicals, without case-specific calibration. The model has been validated for PCB and metal accumulation in species of the Rhine–Meuse delta (Hendriks et al., 2001; Hendriks and Heikens, 2001). To further evaluate the generic applicability of the model, the model principles should be validated for other ecosystems, such as marine environments, and for a broader range of substances, including non-organochlorines. Additionally, a further refinement of the model concept is necessary for metal accumulation kinetics. While uptake and elimination rate constants of organic compounds have for long been linked to their octanol-water partition coefficient, no equivalent relationships exist for metals. A mechanistic estimation of metal accumulation is therefore not yet possible. To advance science of metal bioaccumulation modeling, mechanistic estimation routines need to be developed that relate metal uptake and elimination kinetics to a metal specific property. Finally, chemical inhalation and exhalation rate constants have not been predicted based on allometric relationships and chemical properties. It is therefore useful to extend the model concept to exposure via air and develop quantitative relationships for chemical exchange via lung epithelia in mammals, analogous to the OMEGA-concept for chemical exchange via gill epithelia in aquatic organisms. The advantage for bioaccumulation modeling in general is the applicability of these relationships to a wide range of (terrestrial) organisms and various chemicals, without increasing model-complexity or input-parameter requirements. Additionally, these relationships provide insight in species-specific differences in chemical uptake and elimination rate constants from air. The aim of this thesis was to validate and improve the generic applicability of elementary accumulation concepts, in particular those incorporated in the OMEGA model, for a wide range of species, chemicals and exposure routes.

8.2 Model validation

The first chapters of this thesis focus on validation of the model-concept with independent field accumulation data. The model is tested for:

- accumulation of PCBs and brominated flame retardants in estuarine and marine species (Chapter 2)
- organotin accumulation in an estuarine food chain of the Western Scheldt (Chapter 3)

- metal accumulation in earthworms and small mammals, including herbivorous voles and carnivorous shrews (Chapter 4 and 5)

8.2.1 PCB and brominated flame retardant accumulation in an estuarine food-chain

In Chapter 2, the model is validated with field accumulation data of PCBs and brominated flame retardants in species of the Western Scheldt estuary and the Wadden Sea. This study showed that OMEGA accurately predicts accumulation of both PCBs and brominated diphenyl ethers in lower trophic level species, if biotransformation rates are negligible. Substantial deviations between model predictions and field data are observed if species are capable of metabolic transformation of the contaminant.

For lower trophic level species, i.e. for herbi-detritivores, crustaceans and primary and secondary carnivorous fish, PCBs generally behave as expected based on their K_{ow} and accumulation is predicted well by the model. For some congeners, deviations between model predictions and empirical data are observed, however, at the upper end of the food chain. Generally, field biomagnification ratios for piscivorous birds (*Sterna hirundo*) and marine mammals (*Phoca vitulina*) are lower than predicted by the model. This is attributed to biotransformation of meta-para unsubstituted PCB congeners (Boon et al., 1994; Boon et al., 1997). Biomagnification ratios for some persistent PCB congeners are, however, also lower than expected by the model. For birds, this may be explained by feeding in less polluted areas.

Accumulation of brominated diphenyl ethers (BDE) in crustaceans and herbi-detritivores as mollusks and lugworms, is accurately predicted by OMEGA. Generally, these species have poorly developed metabolic capacities and accumulation ratios are in line with model predictions based on the substances K_{ow}. For higher trophic level species, bioaccumulation of brominated diphenyl ethers is found to be highly congener- and species-specific as a result of biotransformation and bioformation. BDE-47 is the predominant congener in fish species and accumulation ratios of BDE-99 and BDE-100 can be substantially lower than expected based on their respective K_{ow}s. In contrast, high accumulation ratios of BDE-99 and BDE-100 are found in marine mammals and birds. Empirical evidence exists that fish are capable of metabolic transformation of BDE-99 and BDE-100 (Stapleton et al., 2004a,b,c; Tomy et al., 2004) and biotransformation probably results in formation of BDE-47 as a result of debromination (Stapleton et al., 2004b; Ghandi et al., 2006). Incorporation of these biotransformation and bioformation

processes is necessary to improve model predictions for fish and piscivorous birds, and to understand accumulation kinetics of brominated diphenyl ethers. Incorporation of biotransformation is not yet feasible: biotransformation rates have seldom been measured, due to difficulties in experimental analysis (Tomy et al., 2004). Recently, a multichemical foodweb model has been developed for fish that estimates bioaccumulation of several brominated diphenyl ethers incorporating biotransformation and bioformation (Bhavsar et al., 2008; Gandhi et al., 2006). At present, this model is empirically calibrated using lab data and applicable to fish only (Bhavsar et al., 2008). An important direction for future research is therefore to establish biotransformation rates and bioformation rates for various species. These rates can subsequently be incorporated in mechanistic bioaccumulation models to provide a more accurate prediction of bioaccumulation of brominated diphenyl ethers.

8.2.2 Organotin accumulation in an estuarine food-chain

In Chapter 3 the bioaccumulation model is used to explore organotin accumulation in various species of the Western Scheldt estuary, including molluscs, polychaeta, crustaceans, various fish species and eggs of a piscivorous bird. Organotins, as tributyltin and triphenyltin, are ionizable, organometallic compounds that form complexes with various ligands present in the abiotic and biotic environment (Arnold et al., 1997, Buck et al., 2003; Elliott et al., 1979; Nishikimi et al., 2001; Hunziker et al., 2001). Speciation of organotin compounds strongly determines their environmental fate and accumulation kinetics, which therefore differs from nonpolar organic substances. As complexation to biotic ligands is also known for metals, it was hypothesized that elimination rates of organotins may be more comparable to elimination kinetics of metals than to elimination kinetics of nonpolar organic substances. Therefore, elimination kinetics were modeled following two approaches:

1) comparable to nonpolar organic substances
2) comparable to metals

Additionally, measured K_{oc}s, specific for marine sediments were used, as sediment-water partitioning is strongly dependent on the speciation of organotins and likely underestimated using the traditional equilibrium partitioning approach.

The results show low accumulation ratios (BSAF <1) for all species, except for the shrimp *C. crangon*. This is attributed to a strong sorption of organotins to suspended matter. No magnification of tributyltin in the food chain is observed, probably due to substantial biotransformation (Reader et al., 1996; Stäb et al., 1996; Mensink et al., 1997). In contrast, triphenyltin shows biomagnification potential in

fish species, with field-BMFs approaching those of PCBs in the same location. Accumulation kinetics of organotins are, however, different from nonpolar organic compounds. Our modeling study suggests that uptake of organotins mainly occurs via hydrophobic, passive diffusion, while elimination is more comparable to elimination kinetics of metals. In particular for triphenyltin, model predictions of metal elimination in fish and molluscs are highly comparable (within a factor of 2) to measured elimination rate constants derived from laboratory experiments. In contrast, elimination rate constants are substantially underestimated for tributyltins, possibly as a result of biotransformation (Reader et al., 1996; Stäb et al., 1996; Mensink et al., 1997). The octanol-water partition coefficient is probably not a good indicator of membrane-water partitioning of organotins. Organotins possibly form complexes with ligands present in membrane constituents (Hunziker et al., 2001), which may results in lower uptake rate constants than expected based on the substance K_{ow}. This can also explain the overestimation of organotin uptake rate constants by the model compared to empirical data.

This study shows that the model cannot be directly applied to organometallic compounds as organotins and methylmercury, as accumulation kinetics of these compounds are not solely driven by passive diffusion, and not directly related to the substance K_{ow}. Further refinement is necessary to characterize uptake and elimination kinetics of organometallic compounds. This refinement should include the characterization of membrane – water partitioning coefficients and the substance affinity for proteins. This research does illustrate, however, how a mechanistic bioaccumulation model can be used to provide quantitative information concerning specific parts of the bioaccumulation process, i.e. uptake and elimination kinetics, allowing a more mechanistic explanation of field bioaccumulation ratios.

8.2.3 Metal accumulation in a terrestrial food chain

In the third model validation study model predictions of metal accumulation are compared to field data of various terrestrial species, including earthworms, herbivorous voles and carnivorous shrews. Although mechanistic bioaccumulation models are widely used in the environmental risk assessment of nonpolar, organic chemicals, similar models are largely absent for metals. This is due to the complexity of metal bioaccumulation processes including saturable uptake kinetics, existence of homeostatic mechanisms to control internal body concentrations, natural background accumulation, essentiality of some metals and the species-specific ability to detoxify, store and excrete excess accumulated metal (White and Rainbow, 1982; Bury and Wood, 1999; Lock and Janssen, 2001; Rainbow, 2002;

Vijver et al., 2004; Schlekat et al., 2007). In Chapter 4 and 5 we show that a mechanistic modeling approach is feasible for cadmium bioaccumulation in several terrestrial species, including earthworms, voles and shrews, when accounting for the following key processes that govern Cd bioaccumulation:

1) Geochemical bioavailability of metals
2) Saturable uptake kinetics for earthworms (Hendriks and Heikens, 2001)
3) Elimination via growth dilution only

Earthworms are soft-bodied, soil-dwelling organisms that predominantly accumulate metals via pore-water mediated absorption through their skin (Vijver et al., 2003). Metal pore-water concentrations were calculated from total soil levels with a competitive absorption model that accounts for (pore-water) pH and organic carbon content (Sauvé et al., 2000). This is an improvement compared to the earlier model version, where pore-water concentrations were estimated using average soil-water partition coefficients that are independent of geochemical conditions as pH that govern metal bioavailability (Hendriks and Heikens, 2001).

Metal uptake kinetics were assumed to be dependent on the exposure concentration and follow saturable uptake kinetics analogous to Michaelis-Menten kinetics for enzymes (Hendriks and Heikens, 2001). This model-approach is consistent with Lock and Janssen (2001), who concluded that uptake rate constants for cadmium decrease with increasing metal concentrations in soil, based on experimental results on two terrestrial oligochaetes, *Eisenia fetida* and *Enchytraeus albidus*. Earthworms mainly detoxify non-essential metals by binding these metals to metallothionein. Therefore, elimination rate constants were modeled as "growth dilution" only, as strongly bound substances are probably hardly eliminated via excretion and egestion. Consequently, steady-state internal concentrations were calculated as the influx via water divided by the elimination via growth dilution.

A comparison of model predictions with field data shows that Cd accumulation is accurately predicted by OMEGA in earthworms and deviations between model predictions and empirical data are generally within a factor of 3. Measured earthworm cadmium concentrations are less than linearly related to pore-water concentrations (r^2 = 0.37, $p < 0.001$), i.e the slope < 1. The observed inverse relationship between BSAF and metal pore-water concentrations is correctly portrayed by OMEGA. The slope of the empirical regression line is highly comparable to the model-line, i.e 0.60 and 0.53 respectively. Additionally, predicted elimination rate constants are compared to measured elimination rate constants. This comparison shows that cadmium elimination in earthworms is accurately predicted by assuming "loss" via growth dilution only.

In Chapter 5, model predictions were compared to independent field data of cadmium accumulation in herbivorous voles and carnivorous shrews. Analogous to the model approach for earthworms, elimination was modeled as "loss" via growth dilution only, as small mammals detoxify Cd by binding this metal to metallothionein, similar to earthworms. Small mammals predominantly take up metals via food and therefore steady-state, internal concentrations were predicted as the total uptake from food divided by the elimination via growth dilution. Carnivorous shrews were assumed to feed on earthworms, whereas herbivorous voles were assumed to feed solely on plants. Measured cadmium concentrations in food were used as input for the model.

A comparison of model predictions with field data shows that Cd accumulation is accurately predicted by OMEGA in voles and shrews. Deviations between model predictions and empirical data are generally within a factor of 5. Additionally, a two-paired t-test indicated that model predictions were not significantly different from field data ($p > 0.05$ and $p(H_0=0) < 0.05$). Internal cadmium concentrations in both voles and shrews are less than linearly related to cadmium concentrations in plants and earthworms, respectively. Elimination rate constants are accurately predicted by the model assuming that cadmium is only being released via growth dilution.

These two validation studies indicate that saturable uptake kinetics for earthworms are tenable, and suggest that Cd accumulation is predictable for different contaminant levels and other species as well. These species, should however, have similar cadmium elimination kinetics as earthworms, voles and shrews, i.e. binding to metallothionein is the main detoxification mechanism and elimination occurs via "growth dilution" only.

These model validation studies also provide three important directions for further research. Firstly, although cadmium accumulation in earthworms is accurately predicted, the model has less predictability for essential metals, as copper and zinc, and the non-essential metal lead. For lead, this can probably be attributed to uncertainty of lead pore water concentrations (Sauvé et al., 2000). However, for Cu and Zn, more insight in regulation of uptake and elimination kinetics of essential metals is necessary to improve model predictions (Loos et al., in prep).

Secondly, for small mammals, an empirical, laboratory based assimilation efficiency was used to predict the cadmium uptake rate constant from food. Preferably, metal uptake by ingestion should be modeled mechanistically based on well-defined biochemical transport principles instead of using an empirical assimilation efficiency. At present, experimental studies relating metal assimilation efficiencies

to their subcellular distribution in prey are sparse for terrestrial species. Recently, studies have been published on this subject for various aquatic species (Croteau and Luoma, 2008; Cheung and Wang, 2005; Rainbow et al., 2006), that may allow further improvement in the model structure and parameter validation.
Finally, total dissolved metal concentrations in pore-water are considered to be bioavailable for uptake by earthworms. However, this bioavailability of metals is influenced by speciation and various metal species may have different contributions to the total uptake rate (Chuang and Wang, 2006; Vink, 2002). A further improvement may be obtained by considering different metal species instead of total dissolved pore water concentrations.

8.3 Model development

In the second part of this thesis two model developments are described:

- specification and calibration of metal accumulation kinetics via gill epithelia for aquatic organisms
- specification of organic chemical accumulation kinetics via lung epithelia for various mammals

The approach followed in the development of these estimation routines was analogous to the model-concept for organic chemical exchange via the gills in aquatic organisms. Hence, accumulation kinetics were related to a minimum number of well-known chemical properties and species characteristics. Chemical uptake and elimination rate constants were assumed to be a function of the resistances in the unstirred aqueous layer and the lipid layer, and the delays imposed by metabolic flows, as the ventilation rate (aquatic organisms), and the inhalation rate and perfusion rate (mammals).

8.3.1 Specification of metal accumulation kinetics via gill epithelia in aquatic organisms

The objective of the research described in Chapter 6 was to further improve the estimation of metal exchange via the gills by relating uptake and elimination rate constants to a metal-specific property and the adult mass of species. Although bioaccumulation kinetics of nonpolar, organic substances have long been linked to their octanol-water partition coefficient, metal accumulation has not been quantitatively linked to a metal-specific property, which is necessary for a more mechanistic estimation of uptake and elimination rate constants.
Accumulation kinetics of neutral organic chemicals is primarily driven by passive diffusion, whereas metal accumulation is more complicated and includes various uptake and elimination pathways involving different transport proteins (Bury et al.,

1999; Grosell and Wood, 2002). This may hamper the development of a quantitative relationship between metal absorption rate constants and a single, metal specific property.

In Chapter 6 it is shown that:

- Metal absorption rate constants of aquatic species can be predicted based on the species-specific ventilation rate and the metal-specific covalent index ($\chi^2_m r$), which is a measure of metal-affinity for sulphur-ligands
- Elimination kinetics show no metal-specific behavior and bodyweight-corrected elimination rate constants are relatively similar across various aquatic species

For this study, 362 absorption rate constants and 155 elimination rate constants for several aquatic species, including five molluscs, four crustaceans, and eight fish species, were collected. A significant, positive relationship is observed between metal absorption rate constants and the covalent index of the metal-ion for various species. This can be explained by an increasing affinity for sulphur-ligands in membrane transport proteins and higher stability of formed complexes with increasing $\chi^2_m r$, which results in a more efficient uptake. The relationship is thought to be applicable to all metal ions that cross membranes by binding to sulfur- or nitrogen transport proteins in membranes. This includes all "Class B" and borderline metals from the Nieboer and Richardson-classification, but also includes Cr (III), a "Class A" metal. The relationship is not applicable to anions, as chloride or sulfate, and small, essential metals as sodium, potassium and calcium that are transported by highly, selective transport channels.

Metal elimination rates show no metal-specific character, suggesting that the affinity of different metals for biological material is, on average, comparable. It should be noted, however, that metal elimination represents an integral of loss from various different "storage" compartments. This complex elimination kinetics are not properly reflected in a single, metal-generic elimination rate and deviations from this single rate constant will probably occur as a result of enhanced regulation and / or detoxification mechanisms at high exposure concentrations. Yet, in absence of empirical data, the weight-corrected elimination rate constant is probably the best estimate of elimination.

Absorption rate constants are predicted independent of the metal concentration in the water-phase. This may seem contradictory to results described in Chapters 4 and 5, however, this assumption is justified at low environmental metal concentrations (Appendix 6). At high exposure concentrations saturation of

channels or carriers may occur, however, and therefore competition between metals for binding sites increases. This can be taken into account considering the absorption rate constant to depend on the exposure concentration, as confirmed by field bioconcentration factors (Hendriks and Heikens, 2001).

8.3.2 Specification of organic chemical accumulation kinetics via lung epithelia in mammals

The last aim of this thesis was to develop a generic modeling approach for contaminant exchange via lung epithelia based on chemical-specific properties and species-based allometric scaling, analogous to accumulation kinetics via the gills. In Chapter 7 it is shown that:

- Accumulation kinetics of nonpolar organic chemicals via air exchange, can be succesfully predicted based on two chemical properties, the octanol-water partition coefficient (K_{ow}) and the air-water partition coefficient (K_{aw}), and few species characteristics, as adult mass and body composition (lipid fraction, protein fraction, water fraction).

Chemical inhalation rate constants are predicted as a function of two flow delays: the alveolar ventilation and the cardiac output. The latter flow delay is additionally dependent on the substance blood-air partition coefficient (K_{ba}). This blood-air partition coefficient is predicted based on the blood-composition of the species and the K_{ow} and K_{aw} of the substance. Diffusion through the lung membrane is found to be insignificant compared to the two flow delays and therefore this diffusion term is omitted in the final equation. The chemical elimination via exhalation is estimated as the reverse process except that chemicals that are accumulated in body tissues, such as fat, are not easily eliminated. This is represented by the tissue-air partition coefficient (K_{ta}), which is a function of the chemical K_{ow} and K_{aw}, and the body composition of species.

The model is validated with independently measured uptake and elimination rate constant of various volatile organic chemicals for three mammalian species, i.e. humans, rats and mice. The results show that 75% of the modeled inhalation and exhalation rate constants are within a factor of 2 from measured values. Additionally, 87% of the predicted blood-air partition coefficients are within a factor of 5 from empirical data. Options to evaluate the model for other species were limited. Yet, the combination of biological allometry with chemical thermodynamic diffusion theory makes it feasible to extrapolate the model to other species and chemicals beyond the tested range. The availability of empirical biotransformation rates is, however, still a limiting factor. The use of substance specific properties as K_{ow} and K_{aw}, confines the chemical application domain of the

model. The octanol-water partition coefficient is a good indicator for bioaccumulation of non-polar organic substances, for which accumulation kinetics is mainly driven by passive diffusion. Therefore, the model can be applied to other nonpolar organic chemicals than the ones tested, but it cannot be directly applied to other chemicals as organometallic compounds, ionic compounds and surfactants.

8.3.3 Comparison of the new estimation routines with previously established model-equations

The approach followed in the development of these estimation routines was analogous to the model-concept for organic chemical accumulation kinetics via gill exchange in aquatic organisms. Some important differences can be noted as well. For contaminant exchange via the lungs, uptake and elimination rate constants are flow-limited. The delay imposed by blood flow is an important rate-limiting factor and the diffusion resistance through the lung membrane is found to be insignificant compared to the flow delays. In contrast, it can be shown here that the role of blood flow in regulating the overall rate of chemical uptake and elimination is insignificant in aquatic species, whereas the lipid layer resistance is rate-limiting for chemicals with a $\log K_{ow} < 5.5$. For substances with a $\log K_{ow} > 5.5$ the resistance in the aqueous layer and the flow delay imposed by ventilation become rate-limiting (Appendix 8).

Additionally, while accumulation kinetics of organic chemicals by fish is a function of one chemical property, the octanol-water partition coefficient (K_{ow}), the accumulation kinetics of organic chemicals by mammals is also related to the chemical air-water partition coefficient (K_{aw}).

Within the range of exposure conditions tested, the estimation of the metal absorption rate constants is analogous to the estimation of the absorption rate constant of organic contaminants, and is a function of the resistances in the unstirred aqueous layer and the lipid layer and a flow delay imposed by the ventilation rate. The resistance in the lipid layer membrane is related to the metal-ion's covalent index and reflects the metal-affinity for transport proteins in the lipid membrane. Similar to uptake of organic substances, metal exchange via the gills is predominantly limited by the transport over the membrane, which is a function of the metal covalent index. This resistance is, however, more than a factor of 1000 higher than the lipid layer resistance for organic chemicals (with a $K_{ow} > 10$), and results in low metal uptake rate constants compared to organic chemical uptake rate constants.

Also, unlike elimination of organic chemicals, metal elimination kinetics could not be related to a metal-specific property. Metal elimination rates represent the integral of loss from various compartments and can probably not be adequately predicted using first-order kinetics.

8.4 Implications for environmental risk assessment

8.4.1 Organic compounds

The results described in Chapter 2 on bioaccumulation of PCBs have implications for monitoring surveys and field studies. It was shown that chemical concentrations in suspended solids are a better basis to predict internal concentrations in aquatic biota than concentrations in sediment. PCB accumulation was generally underestimated using chemical concentrations in sediment. This can be attributed to higher chemical concentrations in sediment compared to suspended solids, because of historically higher emissions. These residues are probably less available for uptake due to aging (Burkhard et al., 2005). Abiotic samples must be representative for the recent exposure history in aquatic biota and it is suggested that chemical concentrations in suspended solids should be monitored too.

There is growing concern over the bioaccumulation potential and global distribution of brominated flame retardants in the environment (de Boer et al., 1998; Hites, 2004; Jenssen et al., 2007). Brominated diphenyl ethers are high K_{ow} compounds with a similar molecular structure as PCBs. The results described in Chapter 2 indicate that the bioaccumulation potential of brominated diphenyl ethers can not solely be assessed on the compounds octanol – water partition coefficient (K_{ow}). This because biotransformation and bioformation, results in largely species-specific and congener-specific accumulation in higher trophic levels species, as fish and birds. Bioaccumulation factors may be higher or lower than expected based on the substance K_{ow}. In future risk assessment studies it is therefore important to consider biotransformation processes of brominated diphenyl ethers.

8.4.2 Metals

Bioaccumulation is often used as a criterion for prioritization and risk assessment of metals (McGeer et al., 2003). One of the primary assumptions that make BCF and BAF values suitable as indicators of bioaccumulation for neutral organic compounds is that they are independent of exposure concentrations. In other words, uptake and elimination rate constants are invariant over a range of exposure

concentrations. The results described in Chapter 4 and Chapter 5 indicate that use of a generic, constant bioaccumulation factor, as common in risk assessment is not jusitified for metal contamination in terrestrial ecosystems. BSAFs of earthworms, voles and shrews, decrease substantially with increasing total soil concentrations, probably due to saturable uptake kinetics. These results are consistent with decreasing BCFs observed for aquatic species (McGeer et al., 2003).

This research may also have implications for biotic ligand (BLM) models. The BLM of acute metal toxicity to aquatic organisms is based on the idea that to invoke a biological effect, the metal must first react with sensitive sites on the membrane (Di Toro et al., 2001; Slaveykova and Wilkinson, 2005). These sensitive sites can be transporters, channels or ion-carriers. At present, BLM-models are developed for each species separately. The results described in this thesis indicate that metal affinity for membrane transport proteins may not be considerably different between fish, crustaceans and mollusks. Thus metal affinity for membrane transport proteins might be comparable for various aquatic species, allowing a generic BLM-modeling approach.
Additionally, metal absorption rate constants were shown to be dependent on the gill ventilation rate, whereas in BLM-models it is often assumed that ventilation rates are not a rate-limiting factor (Slaveykova and Wilkinson, 2005).

8.5 Conclusions

Uptake and elimination rate constants are key parameters in mechanistic bioaccumulation models. Various studies have shown that these rate constants can be successfully predicted based on the substance octanol-water partition coefficient. Yet, accumulation kinetics have usually not been related to organism characteristics and available models often do not allow extrapolation to other species. The unique characteristics of the OMEGA model concept are the combination of classical thermodynamic diffusion theory with biological allometry, i.e. uptake and elimination rate constants are estimated as a function of well-known properties of species and chemicals, such as the adult mass (w), body composition and trophic level of the species, and the octanol-water partition coefficient (K_{ow}) of the chemical (Hendriks et al., 2001; Hendriks and Heikens, 2001). The advantage of this approach is that the model can be extrapolated to other chemicals, species and areas without case-specific calibration of key parameters. The model approach has previously been successfully validated with field accumulation data of PCBs in terrestrial and freshwater aquatic species (Hendriks et al., 2001). The aim of this thesis was to validate and improve the generic

applicability of elementary accumulation concepts, in particular the combined allometry and mass-balance approach, as employed in OMEGA.
The main conclusions of this thesis are that the model-concept can be applied to predict:

- accumulation of nonpolar organic chemicals, including non-organochlorines as brominated diphenyl ethers and various volatile organic compounds, if biotransformation is negligible
- accumulation in estuarine and marine organisms, as crustaceans, mollusks and fish species
- cadmium accumulation in earthworms and shrews, and possibly other terrestrial species, provided that the metal detoxification mechanisms are comparable to those of earthworms and shrews
- uptake of several metals (Cs, Co, Cr^{3+}, Cu, Zn, Cd, Ag, Hg) via gill epithelia in aquatic organisms, as crustaceans, mollusks and fish, based on the metal-specific covalent index and the species-specific ventilation rate
- exchange of nonpolar organic chemicals via lung epithelia in various mammals, including humans, rats and mice, based on two chemical specific properties, K_{aw} and K_{ow}, and species weight

The results also indicate that some additional refinements and/or validation studies are necessary to improve the predictability for other classes of compounds and exposure routes.
At present, the model cannot be directly applied to predict accumulation of:

- labile compounds, if measured biotransformation rates are unavailable
- essential metals, as Cu and Zn, if accumulation kinetics are regulated by the organism
- metals via ingestion of food and egestion with feces
- organometallic compounds, as organotins

These current model limitations point out the following directions for further research for bioaccumulation modelling in general:

- at present, many bioaccumulation assessments are limited by available empirical biotransformation rates for various labile chemicals and a range of species. These data are needed for a more adequate prediction of bioaccumulation of labile subtances. Current development of mechanistic estimation routines that predict biotransformation rates based on chemical properties and species characteristics should be encouraged.

- although metal absorption rate constants of aquatic species were successfully related to the species-specific ventilation rate and the metal-specific covalent index ($\chi^2_m r$), further refinements are necessary to accurately estimate metal elimination kinetics.
- a better understanding of metal uptake from food is necessary, this in terms of the subcellular distribution in prey and the effect of this subcellular distribution on metal assimilation efficiency
- at present, the model does not account for species-specific regulation and/or detoxification strategies of metals. This is necessary for a more accurate estimation of uptake and elimination kinetics of essential metals, as copper and zinc.

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Appendix 2

A2.1 Substance specific data

Table A2.1 Octanol-water partition coefficients (K_{ow}) for PCBs and brominated flame retardants

Organochlorines		
Compound	**Abbreviation**	**K_{ow}**
2,3',5-trichlorobiphenyl	PCB26	4.5×10^5
2,4,4'-trichlorobiphenyl	PCB28	5.1×10^5
2,4',5-trichlorobiphenyl	PCB31	4.8×10^5
2,2',3,5-tetrachlorobiphenyl	PCB44	5.4×10^5
2,2',4,5'-tetrachlorobiphenyl	PCB49	2.3×10^5
2,2',5,5'-tetrachlorobiphenyl	PCB52	6.2×10^5
3,3',4,4'-tetrachlorobiphenyl	PCB77	4.3×10^5
2,2',3,3',6-pentachlorobiphenyl	PCB84	4.0×10^5
2,2',3,5',6-pentachlorobiphenyl	PCB95	8.3×10^5
2,2',4,5,5'-pentachlorobipheny	PCB99	1.4×10^6
2,3',4,4',5-pentachlorobiphenyl	PCB114	3.7×10^6
3,3',4,4',5-pentachlorobiphenyl	PCB123	1.1×10^7
2,2',3,3',4,4'-hexachlorobiphenyl	PCB126	2.1×10^7
2,2',3,4,4',5-hexachlorobiphenyl	PCB137	5.2×10^6
2,2',3,4',5',6-hexachlorobiphenyl	PCB149	2.6×10^6
2,2',4,4',5,5'-hexachlorobiphenyl	PCB153	6.3×10^6
3,3',4,4',5,5'-hexachlorobiphenyl	PCB169	2.6×10^7
2,2',3,3',4,4',5-heptachlorobiphenyl	PCB171	6.8×10^6
2,2',3,3',4,5,6'-heptachlorobiphenyl	PCB177	3.7×10^7
2,2',3,4,4',5,5'-heptachlorobiphenyl	PCB182	3.6×10^6
2,2',3,4,4',5,6'-heptachlorobiphenyl	PCB183	4.5×10^7
2,2',3,4',5,5',6-heptachlorobiphenyl	PCB189	8.3×10^6
2,2',3,3',4,4',5,5'-octachlorobiphenyl	PCB196	4.2×10^7
2,2',3,3',4,4',5,5',6,6'-decachlorobiphenyl	PCB209	1.9×10^8
hexachlorobenzene	HCB	5.4×10^5
pentachlorophenol	PCP	1.5×10^5

Table A2.1 Octanol-water partition coefficients (K_{ow}) for PCBs and brominated flame retardants (*continued*)

Brominated flame retardants		
Compound	**Abbreviation**	**K_{ow}**
2,4,4'-tribromodiphenylether	BDE28	8.7×10^{5}
2,2',4,4'-tetrabromodiphenylether	BDE47	6.5×10^{6}
2,2',4,5'-tetrabromodiphenylether	BDE49	5.9×10^{6}
2,3',4,4'-tetrabromodiphenylether	BDE66	3.7×10^{6}
2,3',4',6-tetrabromodiphenylether	BDE71	5.9×10^{6}
2,4,4',6,-tetrabromodiphenylether	BDE75	5.9×10^{6}
2,2',3,4,4'-pentabromodiphenylether	BDE85	4.6×10^{7}
2,2',4,4',5-pentabromodiphenylether	BDE99	2.1×10^{7}
2,2',4,4',6-pentabromodiphenylether	BDE100	1.7×10^{7}
2,3',4,4',6-pentabromodiphenylether	BDE119	1.5×10^{7}
2,2',3,4,4',5'-hexabromodiphenylether	BDE138	3.5×10^{8}
2,2',4,4',5,5'-hexabromodiphenylether	BDE153	7.9×10^{7}
2,2',4,4',5,6'-hexabromodiphenylether	BDE154	6.6×10^{7}
2,2',3,4,4',5',6-heptabromodiphenylether	BDE183	2.8×10^{9}
2,3,3',4,4',5,6-heptabromodiphenylether	BDE190	2.8×10^{8}
2,2',3,3',4,4',5,5',6,6'-decabromodiphenylether	BDE209	9.3×10^{9}
tetrabromobisphenol A	TBBPA	6.0×10^{6}
1,2,5,6,9,10-hexabromocyclododecane	HBCD	6.5×10^{6}

Appendix 3

A3.1 Equations for uptake and elimination rate constants

Uptake

The rate constant for absorption of neutral organic compounds from water $k_{x,w,in}$ ($\mu g \cdot kg^{-1}$ wet wt / $\mu g \cdot L^{-1} \cdot d^{-1}$) (Hendriks et al., 2001):

$$k_{x,w,in} = \frac{w^{-\kappa}}{\rho_{H2O,0} + \frac{\rho_{CH2}}{K_{ow}} + \frac{1}{\gamma_0}}$$

Rate constant for ingestion of neutral organic compounds with food $k_{x,n,in}$ ($\mu g \cdot kg^{-1}$ wet wt / $\mu g \cdot kg^{-1} \cdot d^{-1}$):

$$k_{x,n.in} = \frac{f_{as}}{1-f_{as}} \frac{1}{p_{CH2,i-1} \cdot (K_{ow} - 1) + 1} \cdot \frac{w^{-\kappa}}{\rho_{H2O,food} + \frac{\rho_{CH2}}{K_{ow} \cdot q_{T:c}} + \frac{1}{p_{CH2,i-1} \cdot K_{ow} \cdot (1-f_{as}) \cdot q_{T:c} \cdot \frac{(\gamma_2 + \gamma_{resp} \cdot q_{ap})}{f_{as}}}}$$

Elimination rate constants

The total elimination rate, equals the sum of elimination via excretion, egestion and growth dilution:

$$\sum_{0}^{2} k_{x,j,ex} = k_{x,w,ex} + k_{k,n,ex} + k_g$$

1) Neutral organic compounds (Hendriks et al., 2001):

Rate constant for excretion via water $k_{x,w,ex}$ (d^{-1}):

$$k_{x,w,ex} = \frac{1}{p_{CH2,i} \cdot (K_{ow} - 1) + 1} \cdot \frac{w^{-\kappa}}{\rho_{H2O,0} + \frac{\rho_{CH2}}{K_{ow}} + \frac{1}{\gamma_0}}$$

Rate constant for egestion with food $k_{x,n,ex}$:(d^{-1}):

$$k_{x,n.in} = \frac{1}{p_{CH2,i} \cdot (K_{ow} - 1) + 1} \cdot \frac{w^{-\kappa}}{\rho_{H2O,food} + \frac{\rho_{CH2}}{K_{ow} \cdot q_{T:c}} + \frac{1}{p_{CH2,i-1} \cdot K_{ow} \cdot (1-f_{as}) \cdot q_{T:c} \cdot \frac{(\gamma_2 + \gamma_{resp} \cdot q_{ap})}{f_{as}}}}$$

Rate constant for dilution of biomass k_g (d^{-1}):

$$k_g = q_{T:c} \cdot \gamma_2 \cdot w^{-\kappa}$$

2) Metals (Hendriks and Heikens, 2001):

Elimination equations for metals are basically similar to those for organic substances. Most important difference is the incorporation of a generic tissue water distribution coefficient (K_{tw}). This coefficient describes the affinity of metals for dry tissue.

Rate constant for metal excretion via water $k_{x,w,ex}$ (d^{-1}):

$$k_{x,w,ex} = \frac{1}{K_{tw} \cdot p_{s,i}} \cdot \frac{w^{-\kappa}}{\rho_{H2O,0} + \rho_{CH2,metal,ex} + \frac{1}{\gamma_0}}$$

Rate constant for metal egestion with food $k_{x,n,ex}$ (d^{-1}):

$$k_{x,n,ex} = \frac{1}{K_{tw} \cdot p_{s,i}} \cdot \frac{w^{-\kappa}}{\rho_{H2O,food} + \frac{\rho_{CH2,metal,ex}}{q_{T:c}} + \frac{1}{K_{tw} \cdot p_{s,i-1} \cdot (1 - f_{as}) \cdot q_{T:c} \cdot \frac{(\gamma_2 + \gamma_{resp} \cdot q_{ap})}{f_{as}}}}$$

Rate constant for dilution of biomass k_g (d^{-1}):

$$k_g = q_{T:c} \cdot \gamma_2 \cdot w^{-\kappa}$$

Table A3.1: Factors used in equations with typical or default values for parameters (Hendriks et al., 2001, Hendriks and Heikens, 2001)

Symbol	Description	Unit	Typical value
$C_{i,x}$	Concentration in organism	$kg \cdot kg^{-1}$ wet weight	
$C_{0w,x}$	Concentration in water	$kg \cdot L^{-1}$	
$C_{i-1,x}$	Concentration in food	$kg \cdot kg^{-1}$ wet weight	
$k_{x,w,in}$	Substance absorption rate constant	$kg \cdot kg^{-1}$ wet wt d^{-1}/ $kg \cdot L^{-1}$	
$k_{x,n,in}$	Substance assimilation rate constant	$kg \cdot kg^{-1}$ wet wt d^{-1} / $kg \cdot kg^{-1}$	
$k_{x,w,ex}$	Substance excretion rate constant	d^{-1}	
$k_{x,n,ex}$	Substance egestion rate constant	d^{-1}	
k_g	Substance dilution rate constant	d^{-1}	
f_{as}	Fraction of ingested food assimilated		20% for herbi-detritivores,80% for fish species, 80% for piscivorous birds
γ_0	Water absorption – excretion coefficient	$kg^{\kappa} \cdot d^{-1}$	200 (water-ventilating) *
γ_2	Biomass (re)production coefficient	$kg^{\kappa} \cdot d^{-1}$	0.00075
γ_{resp}	Average respiration rate coefficient	$kg^{\kappa} \cdot d^{-1}$	0.00075
κ	Rate exponent	/	0.25
K_{tw}	Dry tissue–water partition ratio	$kg \cdot kg^{-1}$ dry weight / $kg \cdot L^{-1}$	$8.3 \cdot 10^3$

Table A3.1: Factors used in equations with typical or default values for parameters (Hendriks et al., 2001, Hendriks and Heikens, 2001) (*continued*)

Symbol	Description	Unit	Typical value
K_{tw}	Dry tissue –water partition ratio	$kg \cdot kg^{-1}$ dry weight / $kg \cdot L^{-1}$	$8.3 \cdot 10^{3}$
$p_{CH2,i-1}$	Lipid fraction of food (i-1)	kg lipid wt / kg wet wt	$0.03 \cdot w^{-0.04}$
$p_{CH2,i}$	Lipid fraction of organism (i)	kg lipid wt / kg wet wt	$0.03 \cdot w^{-0.04}$
$p_{s,i}$	Dry fraction of organism (i)	kg dry wt / kg wet wt	$0.20 \cdot w^{0.03}$
$p_{s,i-1}$	Dry fraction of food (i-1)	kg dry wt / kg wet wt	$0.20 \cdot w^{0.03}$
ρ_{CH2}	Lipid layer resistance for in /efflux of organic substances in animals	$d \cdot kg^{-\kappa}$	68
$\rho_{CH2,metal,\ ex}$	Lipid layer resistance for efflux of inorganic substances	$d \cdot kg^{-\kappa}$	0.3
$\rho_{H2O,0}$	Water layer resistance from / to water	$d \cdot kg^{-\kappa}$	$2.8 \cdot 10^{-3}$
$\rho_{H2O,food}$	Water layer resistance from / to food	$d \cdot kg^{-\kappa}$	$1.1 \cdot 10^{-5}$
q_{ap}	Animal to plant respiration coefficient	/	6.0

A3.2 Empirical data used

Table A3.2: Measured mean organotin concentrations in suspended solids of the Western Scheldt estuary

Organotin	**$C_{s,x}$ (µg·kg^{-1} dw)**
Tributyltin	74.8
Dibutyltin	13.5
Monobutyltin	13.5
Triphenyltin	8.3
Diphenyltin	2.8
Monophenyltin	< 1.8 (d.l.)

OC (%) = Organic carbon percentage of suspended solids (9.15 %)

$C_{s,x}$ = Organotin concentration (on a Sn-basis) in suspended solids (µg·kg^{-1} dry weight)

Table A3.3: Measured mean organotin concentrations ($C_{i,x}$), dry weight (p_s) percentages and fat (p_{CH2}) percentages of species studied

Organotin	**Species**	**p_s (%)**	**p_{CH2} (%)**	**$C_{i,x}$ (µg·kg$^{-1}_{dw}$)**
Tributyltin	*Arenicola marina*	24	1	248.5
Dibutyltin	*Arenicola marina*	24	1	180.4
Monobutyltin	*Arenicola marina*	24	1	155.6
Triphenyltin	*Arenicola marina*	24	1	11.4
Diphenyltin	*Arenicola marina*	24	1	< 1.3 (= d.l.)
Monophenyltin	*Arenicola marina*	24	1	< 1.8 (= d.l.)
Tributyltin	*Cerastoderma edule*	10	1	331.6
Dibutyltin	*Cerastoderma edule*	10	1	40.8
Monobutyltin	*Cerastoderma edule*	10	1	55.1
Triphenyltin	*Cerastoderma edule*	10	1	20.5
Diphenyltin	*Cerastoderma edule*	10	1	< 1.3 (= d.l.)
Monophenyltin	*Cerastoderma edule*	10	1	< 1.8 (= d.l.)
Tributyltin	*Crangon crangon*	26	2	575.2
Dibutyltin	*Crangon crangon*	26	2	10.9
Monobutyltin	*Crangon crangon*	26	2	7.9
Triphenyltin	*Crangon crangon*	26	2	50.1
Diphenyltin	*Crangon crangon*	26	2	< 1.3 (= d.l.)
Monophenyltin	*Crangon crangon*	26	2	< 1.8 (= d.l.)
Tributyltin	*Ammodytes sp.*	27	5	635.0
Dibutyltin	*Ammodytes sp.*	27	5	99.4
Monobutyltin	*Ammodytes sp.*	27	5	33.2
Triphenyltin	*Ammodytes sp.*	27	5	78.3
Diphenyltin	*Ammodytes sp.*	27	5	3.3
Monophenyltin	*Ammodytes sp.*	27	5	< 1.8 (= d.l.)
Tributyltin	*Chasmichthys gulosus*	21	3	80.0
Dibutyltin	*Chasmichthys gulosus*	21	3	14.8
Monobutyltin	*Chasmichthys gulosus*	21	3	5.3
Triphenyltin	*Chasmichthys gulosus*	21	3	34.7
Diphenyltin	*Chasmichthys gulosus*	21	3	1.5
Monophenyltin	*Chasmichthys gulosus*	21	3	< 1.8 (= d.l.)
Tributyltin	*Clupea harengus*	25	4	248.5
Dibutyltin	*Clupea harengus*	25	4	49.1
Monobutyltin	*Clupea harengus*	25	4	15.5
Triphenyltin	*Clupea harengus*	25	4	46.7
Diphenyltin	*Clupea harengus*	25	4	3.8
Monophenyltin	*Clupea harengus*	25	4	< 1.8 (= d.l.)

Table A3.3: Measured mean organotin concentrations ($C_{i,x}$), dry weight (p_s) percentages and fat (p_{CH2}) percentages of species studied (*continued*)

Tributyltin	*Platichthys flesus (juvenile)*	21	2	172.0
Dibutyltin	*Platichthys flesus (juvenile)*	21	2	45.2
Monobutyltin	*Platichthys flesus (juvenile)*	21	2	13.5
Triphenyltin	*Platichthys flesus (juvenile)*	21	2	84.7
Diphenyltin	*Platichthys flesus (juvenile)*	21	2	3.7
Monophenyltin	*Platichthys flesus (juvenile)*	21	2	< 1.8 (= d.l.)
Tributyltin	*Platichthys flesus*	20	3	67.5
Dibutyltin	*Platichthys flesus*	20	3	19.9
Monobutyltin	*Platichthys flesus*	20	3	8.7
Triphenyltin	*Platichthys flesus*	20	3	44.7
Diphenyltin	*Platichthys flesus*	20	3	2.1
Monophenyltin	*Platichthys flesus*	20	3	< 1.8 (= d.l.)
Tributyltin	*Eutriglia gurnardus*	23	3	233.9
Dibutyltin	*Eutriglia gurnardus*	23	3	20.6
Monobutyltin	*Eutriglia gurnardus*	23	3	9.3
Triphenyltin	*Eutriglia gurnardus*	23	3	23.4
Diphenyltin	*Eutriglia gurnardus*	23	3	< 1.3 (= d.l.)
Monophenyltin	*Eutriglia gurnardus*	23	3	< 1.8 (= d.l.)
Tributyltin	*Solea solea*	22	2	30.9
Dibutyltin	*Solea solea*	22	2	9.3
Monobutyltin	*Solea solea*	22	2	2.3
Triphenyltin	*Solea solea*	22	2	26.9
Diphenyltin	*Solea solea*	22	2	< 1.3 (= d.l.)
Monophenyltin	*Solea solea*	22	2	< 1.8 (= d.l.)
Tributyltin	*Merlangius merlangus*	19	2	123.9
Dibutyltin	*Merlangius merlangus*	19	2	12.6
Monobutyltin	*Merlangius merlangus*	19	2	2.0

Table A3.3: Measured mean organotin concentrations ($C_{i,x}$), dry weight (p_s) percentages and fat (p_{CH2}) percentages of species studied (*continued*)

Triphenyltin	*Merlangius merlangus*	19	2	26.0
Diphenyltin	*Merlangius merlangus*	19	2	1.3
Monophenyltin	*Merlangius merlangus*	19	2	< 1.8 (= d.l.)
Tributyltin	*Trisopterus luscus*	19	2	124.7
Dibutyltin	*Trisopterus luscus*	19	2	13.9
Monobutyltin	*Trisopterus luscus*	19	2	2.2
Triphenyltin	*Trisopterus luscus*	19	2	11.3
Diphenyltin	*Trisopterus luscus*	19	2	< 1.3 (= d.l.)
Monophenyltin	*Trisopterus luscus*	19	2	< 1.8 (= d.l.)
Tributyltin	*Pleuronectes platessa*	22	3	97.2
Dibutyltin	*Pleuronectes platessa*	22	3	15.5
Monobutyltin	*Pleuronectes platessa*	22	3	4.3
Triphenyltin	*Pleuronectes platessa*	22	3	29.9
Diphenyltin	*Pleuronectes platessa*	22	3	< 1.3 (= d.l.)
Monophenyltin	*Pleuronectes platessa*	22	3	< 1.8 (= d.l.)
Tributyltin	*Sterna hirundo*	19	13	4.6
Dibutyltin	*Sterna hirundo*	19	13	11.8
Monobutyltin	*Sterna hirundo*	19	13	2.9
Triphenyltin	*Sterna hirundo*	19	13	5.5
Diphenyltin	*Sterna hirundo*	19	13	< 1.3 (= d.l.)
Monophenyltin	*Sterna hirundo*	19	13	< 1.8 (= d.l.)
Tributyltin	*Sterna hirundo*	17	13	4.8
Dibutyltin	*Sterna hirundo*	17	13	16.3
Monobutyltin	*Sterna hirundo*	17	13	5.0
Triphenyltin	*Sterna hirundo*	17	13	11.8
Diphenyltin	*Sterna hirundo*	17	13	< 1.3 (= d.l.)
Monophenyltin	*Sterna hirundo*	17	13	< 1.8 (= d.l.)

d.l. = detection limit

Appendix 4

A 4.1 Field data used

The supporting information includes all field data used, i.e. total soil concentrations, pH, organic matter content and internal metal concentrations in *L. rubellus*, are presented in tables. When possible 95%-confidence intervals are provided.

Table A4.1a: Measured internal zinc concentrations of *Lumbricus rubellus* ($mg \cdot kg^{-1}$ dry body weight), measured total soil concentrations ($mg \cdot kg^{-1}$ dry soil), pH-$CaCl_2$ (-) or pH-pw (-), and organic matter content ($kg \cdot kg^{-1}$) and estimated pore water pH (-), organic carbon content ($kg \cdot kg^{-1}$) and dissolved pore water concentrations ($mg \cdot L^{-1}$)

Area	Location	x	y	date	EMPIRICAL DATA													ESTIMATED DATA			
					pH-CaCl			pH-pw	OM%			C_s			C_l			pH-pw[I]	OC%[II]	Estimated C_{pw}[III]	Reference
					(-)			(-)	(kg / kg)			(mg / kg dw)			(mg / kg dw)				(kg / kg)	(mg / l)	
					geo. mean	95% CI	5% CI	geo. mean	geo. mean	95% CI	5% CI	geo. mean	95% CI	5% CI	geo. mean	95% CI	5% CI				
BB	Lage Hof	110640	415731	Jul-04	7.5	7.6	7.4		17%	19%	16%	1126.1	1216.0	1042.8	939.0	1151.0	766.0	7.7	10.2%	9.9E-02	Hobbelen et al. 2004
BB	Lage Hof	110679	417487	Jul-04	7.5	7.5	7.5		19%	20%	17%	1205.3	1302.8	1115.2	1695.2	2074.7	1385.1	7.7	10.9%	1.0E-01	Hobbelen et al. 2004
BB	Lage Hof	110960	415153	Jul-04	7.5	7.6	7.5		19%	21%	17%	1279.6	1301.3	1258.3	1299.9	1812.8	932.1	7.7	11.2%	1.0E-01	Hobbelen et al. 2004
BB	Lage Hof	110963	418719	Jul-04	7.5	7.6	7.4		24%	25%	22%	1722.3	1764.3	1681.3	978.0	1655.0	577.9	7.7	14.0%	1.3E-01	Hobbelen et al. 2004
BB	Lage Hof	112206	418113	Jul-04	7.6	7.7	7.6		20%	22%	18%	1622.8	1784.8	1475.6	1604.6	2722.1	945.9	7.8	11.6%	1.1E-01	Hobbelen et al. 2004
BB	Lage Hof	112380	418021	Jul-04	7.6	7.8	7.5		21%	24%	19%	1586.2	1822.7	1380.4	1303.7	1660.5	1023.6	7.8	12.4%	1.1E-01	Hobbelen et al. 2004
BB	Lage Hof	112764	417211	Jul-04	7.4	7.5	7.4		21%	22%	20%	1295.9	1334.3	1258.7	1066.2	1304.8	871.3	7.6	12.2%	1.1E-01	Hobbelen et al. 2004
BB	Lage Hof	113633	416438	Jul-04	7.4	7.5	7.3		25%	27%	24%	1381.7	1501.7	1271.4	1128.4	1639.1	776.9	7.6	14.9%	1.2E-01	Hobbelen et al. 2004
BB	Lage Hof	117477	415434	Jul-04	7.3	7.4	7.3		23%	24%	22%	1572.0	1604.1	1540.5	1479.3	2086.0	1049.1	7.5	13.6%	1.5E-01	Hobbelen et al. 2004
BB	Lage Hof	118034	418050	Jul-04	7.5	7.5	7.4		18%	19%	17%	1150.5	1250.0	1058.8	1269.5	1951.3	825.9	7.6	10.6%	1.0E-01	Hobbelen et al. 2004
BB	Lage Hof	118050	417991	Jul-04	7.4	7.5	7.4		16%	19%	14%	748.9	951.9	589.1	1056.7	1283.2	870.1	7.6	9.6%	7.5E-02	Hobbelen et al. 2004
ADW	Deel 3	172354	434008	Nov-00	7.1				10%			492.6			694.0			7.3	5.9%	8.8E-02	Van Vliet et al. 2005
ADW	Deel 3	172337	434005	Nov-00	7.1				10%			540.9			623.0			7.3	6.0%	9.3E-02	Van Vliet et al. 2005
ADW	Deel 3	172316	434007	Nov-00	7.0				13%			669.3			926.0			7.2	7.7%	1.2E-01	Van Vliet et al. 2005
ADW	Deel 3	172283	433998	Nov-00	7.1				13%			743.9			1372.0			7.3	7.8%	1.2E-01	Van Vliet et al. 2005
ADW	Deel 3	172265	433996	Nov-00	6.9				14%			775.6			1096.0			7.2	8.5%	1.4E-01	Van Vliet et al. 2005
ADW	Deel 3	172237	433994	Nov-00	6.9				15%			834.5			1259.0			7.1	8.9%	1.6E-01	Van Vliet et al. 2005
ADW	Deel 3	172210	433983	Nov-00	7.1				14%			796.0			837.0			7.3	8.0%	1.2E-01	Van Vliet et al. 2005
ADW	Deel 3	172185	433981	Nov-00	7.1				15%			845.8			1183.0			7.3	8.6%	1.3E-01	Van Vliet et al. 2005
ADW	Rijswaard	171084	434280	Nov-00	7.5				9%			540.3			1419.0			7.6	5.5%	6.3E-02	Van Vliet et al. 2005
ADW	Rijswaard	171023	434274	Nov-00	7.5				8%			545.8			926.0			7.7	5.0%	6.4E-02	Van Vliet et al. 2005
ADW	Rijswaard	170950	434268	Nov-00	7.5				9%			562.4			1081.0			7.7	5.2%	6.4E-02	Van Vliet et al. 2005
ADW	Rijswaard	170893	434264	Nov-00	7.4				8%			474.3			622.0			7.6	4.6%	6.2E-02	Van Vliet et al. 2005
ADW	Rijswaard	170836	434261	Nov-00	7.4				9%			476.9			597.0			7.6	5.5%	6.2E-02	Van Vliet et al. 2005
Rhine	Ochten 3			Aug-93				7.0	5%			944.9			1010.4				2.9%	2.9E-01	Hendriks et al. 1995
Rhine	Gelderse Poort 2			Aug-93				7.0	9%			731.2			1340.9				5.3%	1.9E-01	Hendriks et al. 1995
Rhine	Gelderse Poort 4			Aug-93				7.0	9%			1026.4			768.6				5.3%	2.6E-01	Hendriks et al. 1995
Rhine	Gelderse Poort 3			Aug-93				7.0	9%			643.8			1097.2				5.3%	1.7E-01	Hendriks et al. 1995
Rhine	Ochten 1			Aug-93				7.0	5%			956.0			1227.9				2.9%	3.0E-01	Hendriks et al. 1995
Rhine	Gelderse Poort 6			Aug-93				7.0	9%			1096.1			1031.9				5.3%	2.8E-01	Hendriks et al. 1995
Rhine	Ochten 2			Aug-93				7.0	5%			277.0			870.1				2.9%	9.3E-02	Hendriks et al. 1995
Rhine	Gelderse Poort 5			Aug-93				7.0	9%			1134.2			992.6				5.3%	2.9E-01	Hendriks et al. 1995
Rhine	Ochten 6			Aug-93				7.0	5%			240.1			900.2				2.9%	8.1E-02	Hendriks et al. 1995
Rhine	Ochten 5			Aug-93				7.0	5%			159.1			765.3				2.9%	5.5E-02	Hendriks et al. 1995
Rhine	Ochten 4			Aug-93				7.0	5%			116.0			716.5				2.9%	4.1E-02	Hendriks et al. 1995
Rhine	Gelderse Poort 1			Aug-93				7.0	9%			792.9			808.2				5.3%	2.0E-01	Hendriks et al. 1995

Table A4.1b Measured internal zinc concentrations of *Lumbricus rubellus* (mg·kg^{-1} dry body weight), measured total soil concentrations (mg·kg^{-1} dry soil), pH-$CaCl_2$ (-) or pH-pw (-), and organic matter content (kg·kg^{-1}) and estimated pore water pH (-), organic carbon content (kg·kg^{-1}) and dissolved pore water concentrations (mg·L^{-1})

					EMPIRICAL DATA												ESTIMATED DATA			
Area	Location	x	y	date	pH-CaCl (-)			pH-pw (-)	OM% (kg /kg)			C_s (mg / kg dw)			C_l (mg/ kg dw)			pH-pw[I]	OC%[II] (kg /kg)	Estimated C_{pw}[III] (mg / l)	Reference
					geo. mean	95% CI	5% CI	geo. mean	geo. mean	95% CI	5% CI	geo. mean			geo. mean	95% CI	5% CI				
	Bossche Broek							6.6	22%			105.4			506.9	676.7	379.7		13.0%	3.2E-02	Peijnenburg (unpublished data)
	Boxtel1							6.8	6%			257.4			1266.7	1510.4	1062.3		3.3%	9.4E-02	Peijnenburg (unpublished data)
	Hank-wilg							7.7	16%			1044.7			1375.7	1703.3	1111.0		9.3%	7.1E-02	Peijnenburg (unpublished data)
	Liempde - wei							5.6	4%			38.9			547.5	776.9	385.9		2.4%	8.2E-02	Peijnenburg (unpublished data)
	Noordpolder (G)							7.4	1%			20.5			340.9				0.7%	6.4E-03	Peijnenburg (unpublished data)
	Oost							7.9	10%			692.4			1227.4	4991.7	301.8		5.7%	4.8E-02	Peijnenburg (unpublished data)
	Ouderkerk a/d IJssel							5.6	61%			166.0			325.0				35.9%	1.4E-01	Peijnenburg (unpublished data)
	Polder Lage Hof							7.4	29%			2846.0			880.1				17.2%	2.3E-01	Peijnenburg (unpublished data)
	Stolberg							7.2	8%			111.0			906.2	1292.1	635.5		4.5%	2.1E-02	Peijnenburg (unpublished data)
	Veenoord							6.0	4%			398.4			690.6				2.6%	3.9E-01	Peijnenburg (unpublished data)
	Zenderpark - Lelystad							7.3	3%			21.8			304.6				1.9%	5.3E-03	Peijnenburg (unpublished data)
BB	Petrusplaat Oost	113170	419659	Mar-04	7.2	7.3	7.1					1215.7	1295.4	1141.0	1504.0	1820.4	1242.6	7.4			Koolhaas (unpublished data)
BB	Lage Hof	110947	418747	Mar-03								2107.7	2181.8	2036.1	1681.8	1985.4	1424.6				Koolhaas (unpublished data)
BB	Lage Hof	110947	418747	Mar-04	7.1	7.2	7.1					1998.8	2039.9	1958.5	1697.3	2053.0	1403.2	7.3			Koolhaas (unpublished data)
BB	Torenvalkweg			Mar-03								144.9	1222.3	746.6	1439.9	1759.7	1178.3				Koolhaas (unpublished data)
BB	Torenvalkweg			Mar-04	7.5	7.5	7.4					133.8	137.9	129.9	1105.2	1315.4	928.5	7.6			Koolhaas (unpublished data)
BB	Nerzienplaatje			Mar-03								1301.9	1664.2	1018.5	1906.0	2281.1	1592.5				Koolhaas (unpublished data)
	BEST-MAST							6.7	-			256.6			1165.1	1590.8	853.4				Peijnenburg (unpublished data)
	Boxteltuin							7.1	-			186.0			636.6	848.1	477.8				Peijnenburg (unpublished data)
	Epen - Berg							7.4	-			425.9			1150.9	1828.1	724.6				Peijnenburg (unpublished data)
	Esch-3							7.5				1645.9			3653.6						Peijnenburg (unpublished data)
	Esch-4							-				1765.8			1155.8						Peijnenburg (unpublished data)
	Gorinchem							7.5				655.6			847.8	1798.5	399.6				Peijnenburg (unpublished data)
	Hank-wei							7.4				301.4			1045.4	3884.9	281.3				Peijnenburg (unpublished data)
	Lepelstraat - bos							5.7				28.7			453.9	717.5	287.2				Peijnenburg (unpublished data)
BB	Lage Hof	117582	418736	Jul-01	6.9				17%			2100.0			155.3	212.7	113.3	7.2	9.7%	3.4E-01	Hobbelen et al. 2004

Table 2a Measured internal copper concentrations of *Lumbricus rubellus* ($mg \cdot kg^{-1}$ dry body weight), measured total soil concentrations ($mg \cdot kg^{-1}$ dry soil), pH-$CaCl_2$ (-) or pH-pw (-), and organic matter content ($kg \cdot kg^{-1}$) and estimated pore water pH (-), organic carbon content ($kg \cdot kg^{-1}$) and dissolved pore water concentrations ($mg \cdot L^{-1}$)

					EMPIRICAL DATA													ESTIMATED DATA			
Area	Location	x	y	date	pH-CaCl (-)			pH-pw (-)	OM% (kg / kg)			C_s (mg / kg dw)			C_l (mg / kg dw)			pH-pw[i]	OC%[ii] (kg / kg)	Estimated C_{pw}[iii] (mg / l)	References
					geo. mean	95% CI	5% CI	geo. mean	geo. mean	95% CI	5% CI	geo. mean	95% CI	5% CI	geo. mean	95% CI	5% CI				
BB	Petrusplaat Oost			Jul-01	6.9				11%			141.1	165.2	120.5	28.6	54.9	14.9	7.1	7%	5.0E-02	Hobbelen et al. 2004
BB	Lage Hof	117582	418736	Jul-01	6.9	7.0	6.9		17%			380.0			57.6	3448.6	1.0	7.2	10%	1.1E-01	Hobbelen et al. 2004
BB	Lage Hof	110640	415731	Jul-04	7.5	7.6	7.4		17%	19%	16%	132.0	140.7	123.8	36.8	46.9	28.8	7.7	10%	3.3E-02	Hobbelen et al. 2004
BB	Lage Hof	110679	417487	Jul-04	7.5	7.5	7.5		19%	20%	17%	169.7	194.3	148.3	46.9	66.2	33.3	7.7	11%	4.1E-02	Hobbelen et al. 2004
BB	Lage Hof	110960	415153	Jul-04	7.5	7.6	7.5		19%	21%	17%	139.9	146.3	133.9	29.7	31.0	28.6	7.7	11%	3.4E-02	Hobbelen et al. 2004
BB	Lage Hof	110963	418719	Jul-04	7.5	7.6	7.4		24%	25%	22%	306.7	321.3	292.7	72.1	100.5	51.7	7.7	14%	6.8E-02	Hobbelen et al. 2004
BB	Lage Hof	112206	418113	Jul-04	7.6	7.7	7.6		20%	22%	20%	168.3	191.2	148.1	34.7	44.1	27.3	7.8	12%	3.8E-02	Hobbelen et al. 2004
BB	Lage Hof	112380	418021	Jul-04	7.6	7.8	7.5		21%	24%	19%	194.2	215.8	174.8	35.2	44.5	27.8	7.8	12%	4.3E-02	Hobbelen et al. 2004
BB	Lage Hof	112764	417211	Jul-04	7.4	7.5	7.4		21%	22%	20%	162.8	167.1	158.7	33.2	44.7	24.7	7.6	12%	4.0E-02	Hobbelen et al. 2004
BB	Lage Hof	113633	416438	Jul-04	7.4	7.5	7.3		25%	27%	24%	153.8	159.4	148.5	34.0	59.1	19.6	7.6	15%	3.7E-02	Hobbelen et al. 2004
BB	Lage Hof	117477	415434	Jul-04	7.3	7.4	7.3		23%	24%	22%	164.6	169.2	160.1	31.1	42.0	23.1	7.5	14%	4.1E-02	Hobbelen et al. 2004
BB	Lage Hof	118034	418050	Jul-04	7.5	7.5	7.4		18%	19%	17%	125.9	136.0	116.6	28.7	35.3	23.4	7.6	11%	3.2E-02	Hobbelen et al. 2004
ADW	Deel 3	172354	434008	Nov-00	7.1				10%			66.6			22.7			7.3	6%	2.4E-02	van Vliet et al. 2005
ADW	Deel 3	172337	434005	Nov-00	7.1				10%			72.8			23.2			7.3	6%	2.5E-02	van Vliet et al. 2005
ADW	Deel 3	172316	434007	Nov-00	7.0				13%			87.7			26.7			7.2	8%	3.1E-02	van Vliet et al. 2005
ADW	Deel 3	172283	433998	Nov-00	7.1				13%			93.2			34.0			7.3	8%	3.1E-02	van Vliet et al. 2005
ADW	Deel 3	172265	433996	Nov-00	6.9				14%			100.0			29.0			7.2	9%	3.4E-02	van Vliet et al. 2005
ADW	Deel 3	172237	433994	Nov-00	6.9				15%			111.9			34.8			7.1	9%	3.8E-02	van Vliet et al. 2005
ADW	Deel 3	172210	433983	Nov-00	7.1				14%			105.5			28.0			7.3	8%	3.4E-02	van Vliet et al. 2005
ADW	Deel 3	172185	433981	Nov-00	7.1				15%			121.9			36.0			7.3	9%	3.8E-02	van Vliet et al. 2005
ADW	Rijswaard	171136	434281	Nov-00	7.5				9%			68.4			35.3			7.6	5%	2.1E-02	van Vliet et al. 2005
ADW	Rijswaard	171084	434280	Nov-00	7.5				8%			61.2			30.9			7.7	5%	1.9E-02	van Vliet et al. 2005
ADW	Rijswaard	171023	434274	Nov-00	7.5				9%			68.4			34.1			7.7	5%	2.1E-02	van Vliet et al. 2005
ADW	Rijswaard	170950	434268	Nov-00	7.4				8%			59.7			27.4			7.6	5%	1.9E-02	van Vliet et al. 2005
ADW	Rijswaard	170893	434264	Nov-00	7.4				9%			62.7			35.6			7.6	5%	2.0E-02	van Vliet et al. 2005

Table A4.2b Measured internal copper concentrations of *Lumbricus rubellus* (mg·kg^{-1} dry body weight), measured total soil concentrations (mg·kg^{-1} dry soil), pH-$CaCl_2$ (-) or pH-pw (-), and organic matter content (kg·kg^{-1}) and estimated pore water pH (-), organic carbon content (kg·kg^{-1}) and dissolved pore water concentrations (mg·L^{-1})

					EMPIRICAL DATA										ESTIMATED DATA			
Area	Location	x	y	date	pH-CaCl			pH-pw	OM%	C_s			C_i			pH-pw[i]	OC%[ii]	Estimated C_{pw}[iii]	References
					(-)			(-)	(kg / kg)	(mg / kg dw)			(mg / kg dw)				(kg / kg)	(mg / l)	
					geo. mean			geo. mean	geo. mean	geo. mean			geo. mean	95% CI	5% CI				
Rhine	Gelderse Poort 3			Aug-93				7.0	9%	119.8			29.1				5%	4.7E-02	Hendriks et al. 1995
Rhine	Ochten 1			Aug-93				7.0	5%	167.4			37.5				3%	7.3E-02	Hendriks et al. 1995
Rhine	Ochten 2			Aug-93				7.0	5%	30.3			23.1				3%	1.5E-02	Hendriks et al. 1995
Rhine	Ochten 6			Aug-93				7.0	5%	32.4			21.8				3%	1.6E-02	Hendriks et al. 1995
Rhine	Ochten 5			Aug-93				7.0	5%	30.4			21.7				3%	1.5E-02	Hendriks et al. 1995
Rhine	Gelderse Poort 1			Aug-93				7.0	9%	166.1			33.2				5%	6.4E-02	Hendriks et al. 1995
Rhine	Ochten 3			Aug-93				7.0	5%	181.9			25.3				3%	7.9E-02	Hendriks et al. 1995
Rhine	Gelderse Poort 4			Aug-93				7.0	9%	199.4			35.4				5%	7.6E-02	Hendriks et al. 1995
Rhine	Gelderse Poort 5			Aug-93				7.0	9%	186.4			32.2				5%	7.1E-02	Hendriks et al. 1995
Rhine	Gelderse Poort 6			Aug-93				7.0	9%	183.5			34.8				5%	7.0E-02	Hendriks et al. 1995
Rhine	Gelderse Poort 2			Aug-93				7.0	9%	134.9			29.8				5%	5.3E-02	Hendriks et al. 1995
Rhine	Ochten 4			Aug-93				7.0	5%	24.8			23.2				3%	1.2E-02	Hendriks et al. 1995
	Boxtel							6.8	6%	10.5			22.6	26.8	19.1		3%	5.5E-03	Peijnenburg (unpublished data)
	Hank-wilg							7.7	16%	100.3			26.7	33.7	21.1		9%	2.3E-02	Peijnenburg (unpublished data)
	Liempde - wei							5.6	4%	16.3			19.3	28.3	13.2		2%	1.6E-02	Peijnenburg (unpublished data)
	Noordpolder (G)							7.4	1%	2.5			16.0				1%	1.5E-03	Peijnenburg (unpublished data)
	Oost							7.9	10%	25.9			21.3	96.6	4.7		6%	6.7E-03	Peijnenburg (unpublished data)
	Ouderkerk a/d IJssel							5.6	36%	49.8			11.2				21%	2.8E-02	Peijnenburg (unpublished data)
	Stolberg							7.2	8%	15.8			22.9	47.6	11.1		4%	6.1E-03	Peijnenburg (unpublished data)
	Veenoord							6.0	3%	17.8			9.5				2%	1.4E-02	Peijnenburg (unpublished data)
	Zenderpark - Lelystad							7.3	3%	4.0			6.0				2%	1.9E-03	Peijnenburg (unpublished data)
BB	Petrusplaat Oost	113170	419659	Mar-03						124.9	161.8	96.4	28.4	32.1	25.1				Koolhaas (unpublished data)
BB	Petrusplaat Oost	113170	419659	Mar-04	7.2	7.3	7.1			113.4	117.8	109.1	21.0	23.5	18.7	7.4			Koolhaas (unpublished data)
BB	Lage Hof	110947	418747	Mar-03						349.2	363.0	335.9	39.3	48.4	31.9				Koolhaas (unpublished data)
BB	Nerzienplaatje			Mar-03						126.8	162.5	98.9	23.2	26.6	20.2				Koolhaas (unpublished data)
BB	Torenvalkweg			Mar-03						15.5	16.8	14.4	13.5	14.9	12.3				Koolhaas (unpublished data)
BB	Lage Hof	110947	418747	Mar-04	7.1	7.2	7.1			399.1	418.8	380.3	28.6	37.1	22.0	7.3			Koolhaas (unpublished data)
	BEST-MAST							6.7		13.5			19.6	58.2	6.6				Peijnenburg (unpublished data)
	Epen - Berg							7.4		17.3			20.6	38.5	11.1				Peijnenburg (unpublished data)
	Gorinchem							7.5		88.5			31.0						Peijnenburg (unpublished data)

Table A4.3a Measured internal cadmium concentrations of *Lumbricus rubellus* ($mg \cdot kg^{-1}$ dry body weight), measured total soil concentrations ($mg \cdot kg^{-1}$ dry soil), pH-$CaCl_2$ (-) or pH-pw (-), and organic matter content ($kg \cdot kg^{-1}$) and estimated pore water pH (-), organic carbon content ($kg \cdot kg^{-1}$) and dissolved pore water concentrations ($mg \cdot L^{-1}$)

					EMPIRICAL DATA													ESTIMATED DATA			
Area	Location	x	y	date	pH-CaCl (-)			pH-pw (-)	OM% (kg / kg)			C_s (mg / kg dw)			C_i (mg / kg dw)			pH-pw[j]	OC%[ll] (kg / kg)	Estimated C_{pw}[lll] (mg / l)	Reference
					geo. mean	95% CI	5% CI	geo. mean	geo. mean	95% CI	5% CI	geo. mean	95% CI	5% CI	geo. mean	95% CI	5% CI				
BB	Lage Hof	117582	418736	Jul-01	6.9	7.0	6.9		17%			19.0			32.1	68.4	15.0	7.2	10%	4.3E-03	Hobbelen et al. (2004)
BB	Lage Hof	110640	415731	Jul-04	7.5	7.6	7.4		17%	19%	16%	15.3	16.5	14.1	81.6	112.8	58.9	7.7	10%	1.9E-03	Hobbelen et al. (2004)
BB	Lage Hof	110960	415153	Jul-04	7.5	7.6	7.5		19%	21%	17%	19.3	19.7	19.0	95.6	245.2	37.3	7.7	11%	2.2E-03	Hobbelen et al. (2004)
BB	Lage Hof	110963	418719	Jul-04	7.5	7.6	7.4		24%	25%	22%	15.9	16.4	15.4	45.3	86.8	23.7	7.7	14%	1.6E-03	Hobbelen et al. (2004)
BB	Lage Hof	112380	418021	Jul-04	7.6	7.8	7.5		21%	24%	19%	18.2	21.0	15.8	73.4	125.9	42.8	7.8	12%	1.7E-03	Hobbelen et al. (2004)
BB	Lage Hof	112764	417211	Jul-04	7.4	7.5	7.4		21%	22%	20%	18.1	19.0	17.3	69.2	134.9	35.5	7.6	12%	2.1E-03	Hobbelen et al. (2004)
BB	Lage Hof	113633	416438	Jul-04	7.4	7.5	7.3		25%	27%	24%	20.2	21.7	18.9	95.6	149.8	61.0	7.6	15%	2.1E-03	Hobbelen et al. (2004)
BB	Lage Hof	117477	415434	Jul-04	7.3	7.4	7.3		23%	24%	22%	24.4	25.4	23.5	154.5	189.2	126.2	7.5	14%	2.9E-03	Hobbelen et al. (2004)
BB	Lage Hof	118034	418050	Jul-04	7.5	7.5	7.4		18%	19%	17%	18.7	19.9	17.6	105.4	156.8	70.8	7.6	11%	2.4E-03	Hobbelen et al. (2004)
BB	Lage Hof	118050	417991	Jul-04	7.4	7.5	7.4		16%	19%	14%	12.3	15.6	9.7	71.2	84.2	60.3	7.6	10%	1.7E-03	Hobbelen et al. (2004)
ADW	Deel 3	172354	434008	Nov-00	7.1				10%			2.5			54.7			7.3	6%	6.4E-04	van Vliet et al. (2005)
ADW	Deel 3	172337	434005	Nov-00	7.1				10%			3.1			39.0			7.3	6%	7.6E-04	van Vliet et al. (2005)
ADW	Deel 3	172316	434007	Nov-00	7.0				13%			3.8			48.7			7.2	8%	9.1E-04	van Vliet et al. (2005)
ADW	Deel 3	172283	433998	Nov-00	7.1				13%			4.1			50.3			7.3	8%	8.8E-04	van Vliet et al. (2005)
ADW	Deel 3	172265	433996	Nov-00	6.9				14%			4.1			41.9			7.2	9%	9.2E-04	van Vliet et al. (2005)
ADW	Deel 3	172237	433994	Nov-00	6.9				15%			4.8			43.5			7.1	9%	1.1E-03	van Vliet et al. (2005)
ADW	Deel 3	172210	433983	Nov-00	7.1				14%			4.6			43.7			7.3	8%	9.5E-04	van Vliet et al. (2005)
ADW	Deel 3	172185	433981	Nov-00	7.1				15%			5.6			52.7			7.3	9%	1.1E-03	van Vliet et al. (2005)
ADW	Rijswaard	171084	434280	Nov-00	7.5				9%			3.0			47.0			7.6	5%	5.7E-04	van Vliet et al. (2005)
ADW	Rijswaard	171023	434274	Nov-00	7.5				8%			2.5			34.7			7.7	5%	5.0E-04	van Vliet et al. (2005)
ADW	Rijswaard	170950	434268	Nov-00	7.5				9%			2.8			42.3			7.7	5%	5.3E-04	van Vliet et al. (2005)
ADW	Rijswaard	170893	434264	Nov-00	7.4				8%			2.3			36.6			7.6	5%	5.0E-04	van Vliet et al. (2005)
ADW	Rijswaard	170836	434261	Nov-00	7.4				9%			2.3			14.0			7.6	5%	4.6E-04	van Vliet et al. (2005)
Rhine	Gelderse Poort 3			Aug-93				7.0	9%			4.9			33.4				5%	1.9E-03	Hendriks et al. (1995)
Rhine	Ochten 4			Aug-93				7.0	5%			0.5			32.9				3%	2.3E-04	Hendriks et al. (1995)
Rhine	Ochten 2			Aug-93				7.0	5%			1.7			25.6				3%	9.6E-04	Hendriks et al. (1995)
Rhine	Ochten 6			Aug-93				7.0	5%			1.1			32.6				3%	6.1E-04	Hendriks et al. (1995)
Rhine	Gelderse Poort 4			Aug-93				7.0	9%			7.1			35.5				5%	2.8E-03	Hendriks et al. (1995)
Rhine	Gelderse Poort 5			Aug-93				7.0	9%			6.9			39.8				5%	2.7E-03	Hendriks et al. (1995)
Rhine	Gelderse Poort 6			Aug-93				7.0	9%			6.9			34.3				5%	2.7E-03	Hendriks et al. (1995)
Rhine	Ochten 1			Aug-93				7.0	5%			6.8			35.7				3%	4.4E-03	Hendriks et al. (1995)
Rhine	Ochten 5			Aug-93				7.0	5%			0.9			40.0				3%	4.8E-04	Hendriks et al. (1995)
Rhine	Gelderse Poort 1			Aug-93				7.0	9%			6.9			32.1				5%	2.7E-03	Hendriks et al. (1995)
Rhine	Ochten 3			Aug-93				7.0	5%			7.3			22.6				3%	4.7E-03	Hendriks et al. (1995)
Rhine	Gelderse Poort 2			Aug-93				7.0	9%			5.7			35.1				5%	2.2E-03	Hendriks et al. (1995)

Table A4.3b: Measured internal cadmium concentrations of *Lumbricus rubellus* (mg·kg^{-1} dry body weight), measured total soil concentrations (mg·kg^{-1} dry soil), pH-$CaCl_2$ (-) or pH-pw (-), and organic matter content (kg·kg^{-1}) and estimated pore water pH (-), organic carbon content (kg·kg^{-1}) and dissolved pore water concentrations (mg·L^{-1})

					EMPIRICAL DATA											ESTIMATED DATA			
Area	Location	x	y	date	pH-CaCl (-)			pH-pw (-)	OM% (kg /kg)	C_s (mg / kg dw)			C_i (mg/ kg dw)			pH-pw[j]	OC%[ii] (kg /kg)	Estimated C_{pw}[iii] (mg / l)	Reference
					geo. mean			geo. mean	geo. mean	geo. mean			geo. mean	95% CI	5% CI				
	Boxtel1							6.8	6%	1.3			74.5	89.7	61.9		3%	9.0E-04	Peijnenburg (unpublished data)
	Hank-wilg							7.7	16%	12.6			152.8	174.3	133.9		9%	1.5E-03	Peijnenburg (unpublished data)
	Oost							7.9	10%	0.7			3.9	25.6	0.6		6%	8.1E-05	Peijnenburg (unpublished data)
	Ouderkerk a/d IJssel							5.6	36%	1.1			4.8				21%	5.7E-04	Peijnenburg (unpublished data)
	Polder Lage Hof							7.4	29%	16.9			57.5				17%	1.9E-03	Peijnenburg (unpublished data)
	Stolberg							7.2	8%	6.4			45.7	107.8	19.4		4%	2.3E-03	Peijnenburg (unpublished data)
	Veenoord							6.0	4%	0.3			2.8				3%	4.2E-04	Peijnenburg (unpublished data)
	West							7.7	7%	0.5			25.9	293383.8	0.002		4%	8.3E-05	Peijnenburg (unpublished data)
	Zenderpark - Lelystad							7.3	3%	0.1			2.0				2%	4.3E-05	Peijnenburg (unpublished data)
	BEST-MAST							6.6		0.2			6.7	9.0	5.0				Peijnenburg (unpublished data)
	Epen - Berg							7.4		4.9			20.8	37.5	11.6				Peijnenburg (unpublished data)
	Esch-3							7.5		10.4			107.0						Peijnenburg (unpublished data)
	Esch-4									11.7			132.7						Peijnenburg (unpublished data)
	Gorinchem							7.5		4.6			37.5	71.4	19.6				Peijnenburg (unpublished data)
	Lepelstraat - bos							5.7		0.6			12.8	33.2	4.9				Peijnenburg (unpublished data)
BB	Petrusplaat Oost	113170	419659	Mar-03						10.4	13.3	8.2	77.1	97.0	61.2				Koolhaas (unpublished data)
BB	Lage Hof	110947	418747	Mar-03						17.5	18.0	17.1	61.9	72.6	52.8				Koolhaas (unpublished data)
BB	Lage Hof	110947	418747	Mar-04	7.1	7.2	7.1			16.3	17.4	15.3	54.6	74.3	40.1	7.3			Koolhaas (unpublished data)
BB	Nerzienplaatje			Mar-03						13.3	18.0	9.8	90.4	110.6	73.8				Koolhaas (unpublished data)
BB	Torenvalkweg			Mar-03						1.9	8.3	0.4	29.8	47.7	18.7				Koolhaas (unpublished data)
BB	Torenvalkweg			Mar-04						0.1	1.2	0.0	7.1	11.2	4.6				Koolhaas (unpublished data)

Table A4.4a Measured internal lead concentrations of *Lumbricus rubellus* (mg·kg^{-1} dry body weight), measured total soil concentrations (mg·kg^{-1} dry soil), pH-$CaCl_2$ (-) or pH-pw (-), and organic matter content (kg·kg^{-1}) and estimated pore water pH (-), organic carbon content (kg·kg^{-1}) and dissolved pore water concentrations (mg·L^{-1})

					EMPIRICAL DATA													ESTIMATED DATA			
Area	Location	x	y	date	pH-CaCl			pH-pw	OM%			C_s			C_l			pH-pw[j]	OC%[ii]	Estimated C_{pw}[iii]	Reference
					(-)			(-)	(kg /kg)			(mg / kg dw)			(mg/ kg dw)				(kg /kg)	(mg / l)	
					geo. mean	95% CI	5% CI	geo. mean	geo. mean	95% CI	5% CI	geo. mean	95% CI	5% CI	geo. mean	95% CI	5% CI				
BB	Lage Hof	117582	418736	Jul-01	6.9	7.0	6.9		17%			560.0			132.6	211.2	83.2	7.2	10%	5.0E-03	Hobbelen et al. 2004
BB	Petrusplaat Oost			Jul-01	6.9	6.9	6.9		11%	12%	11%	277.2	308.3	249.2	31.5	69.2	14.3	7.1	7%	3.5E-03	Hobbelen et al. 2004
ADW	Deel 3	172354	434008	Nov-00	7.1				10%			155.9			19.5			7.3	6%	2.2E-03	van Vliet et al. 2005
ADW	Deel 3	172337	434005	Nov-00	7.1				10%			171.2			19.0			7.3	6%	2.3E-03	van Vliet et al. 2005
ADW	Deel 3	172316	434007	Nov-00	7.0				13%			229.4			30.5			7.2	8%	3.0E-03	van Vliet et al. 2005
ADW	Deel 3	172283	433998	Nov-00	7.1				13%			261.2			70.2			7.3	8%	3.0E-03	van Vliet et al. 2005
ADW	Deel 3	172265	433996	Nov-00	6.9				14%			255.9			31.6			7.2	9%	3.3E-03	van Vliet et al. 2005
ADW	Deel 3	172237	433994	Nov-00	6.9				15%			287.4			45.0			7.1	9%	3.6E-03	van Vliet et al. 2005
ADW	Deel 3	172210	433983	Nov-00	7.1				14%			274.7			39.6			7.3	8%	3.0E-03	van Vliet et al. 2005
ADW	Deel 3	172185	433981	Nov-00	7.1				15%			284.2			42.5			7.3	9%	3.1E-03	van Vliet et al. 2005
ADW	Rijswaard	171084	434280	Nov-00	7.5				9%			174.3			47.9			7.6	5%	1.7E-03	van Vliet et al. 2005
ADW	Rijswaard	171023	434274	Nov-00	7.5				8%			179.2			22.5			7.7	5%	1.7E-03	van Vliet et al. 2005
ADW	Rijswaard	170950	434268	Nov-00	7.5				9%			193.3			38.1			7.7	5%	1.8E-03	van Vliet et al. 2005
ADW	Rijswaard	170893	434264	Nov-00	7.4				8%			141.9			10.2			7.6	5%	1.6E-03	van Vliet et al. 2005
ADW	Rijswaard	170836	434261	Nov-00	7.4				9%			145.4			16.5			7.6	5%	1.7E-03	van Vliet et al. 2005
Rhine	Gelderse Poort, 3			Aug-93				7.0	9%			30.8			12.9				5%	1.1E-03	Hendriks et al. 1995
Rhine	Ochten, 1			Aug-93				7.0	5%			55.7			9.7				3%	1.5E-03	Hendriks et al. 1995
Rhine	Ochten, 2			Aug-93				7.0	5%			308.1			23.8				3%	4.0E-03	Hendriks et al. 1995
Rhine	Ochten, 6			Aug-93				7.0	5%			398.5			17.7				3%	4.6E-03	Hendriks et al. 1995
Rhine	Ochten, 5			Aug-93				7.0	5%			382.6			15.7				3%	4.5E-03	Hendriks et al. 1995
Rhine	Gelderse Poort, 1			Aug-93				7.0	9%			321.9			12.1				5%	4.1E-03	Hendriks et al. 1995
Rhine	Ochten, 3			Aug-93				7.0	5%			240.2			11.7				3%	3.5E-03	Hendriks et al. 1995
Rhine	Gelderse Poort 4			Aug-93				7.0	9%			73.4			10.6				5%	1.8E-03	Hendriks et al. 1995
Rhine	Gelderse Poort, 5			Aug-93				7.0	9%			228.3			12.8				5%	3.4E-03	Hendriks et al. 1995
Rhine	Gelderse Poort, 6			Aug-93				7.0	9%			341.2			23.1				5%	4.2E-03	Hendriks et al. 1995
Rhine	Gelderse Poort, 2			Aug-93				7.0	9%			268.8			19.3				5%	3.7E-03	Hendriks et al. 1995
Rhine	Ochten, 4			Aug-93				7.0	5%			42.9			13.9				3%	1.3E-03	Hendriks et al. 1995

Table A4.4b Measured internal lead concentrations of *Lumbricus rubellus* (mg·kg^{-1} dry body weight), measured total soil concentrations (mg·kg^{-1} dry soil), pH-$CaCl_2$ (-) or pH-pw (-), and organic matter content (kg·kg^{-1}) and estimated pore water pH (-), organic carbon content (kg·kg^{-1}) and dissolved pore water concentrations (mg·L^{-1})

Area	Location	x	y	date	EMPIRICAL DATA													ESTIMATED DATA			
					pH-CaCl (-)			pH-pw (-)	OM% (kg /kg)			C_s (mg / kg dw)			C_i (mg/ kg dw)			pH-pw[I]	OC%[II] (kg /kg)	Estimated C_{pw}[III] (mg / l)	Reference
					geo. mean	95% CI	5% CI	geo. mean	geo. mean	95% CI	5% CI	geo. mean	95% CI	5% CI	geo. mean	95% CI	5% CI				
	BEST-MAST							6.7				25.0			15.0	61.5	3.7			1.3E-03	Peijnenburg (unpublished data)
	Bossche Broek							6.6	22%			65.5			7.5	12.9	4.3		13%	2.5E-03	Peijnenburg (unpublished data)
	Boxtel1							6.8	6%			27.4			10.1	15.3	6.7		3%	1.3E-03	Peijnenburg (unpublished data)
	Boxteltuin							7.1				42.9			10.9	15.2	7.8			1.3E-03	Peijnenburg (unpublished data)
	Epen - Berg							7.4				111.1			24.0	122.5	4.7			1.6E-03	Peijnenburg (unpublished data)
	Gorinchem							7.5				206.8			23.4					2.1E-03	Peijnenburg (unpublished data)
	Hank-wilg							7.7	16%			310.0			28.7	35.5	23.2		9%	2.2E-03	Peijnenburg (unpublished data)
	Liempde - wei							5.6	4%			20.9			8.7	14.1	5.4		2%	3.1E-03	Peijnenburg (unpublished data)
	Noordpolder (G)							7.4	1%			6.2			9.4				1%	3.2E-04	Peijnenburg (unpublished data)
	Oost							7.9	10%			48.1			17.1	2591.7	0.1		6%	6.9E-04	Peijnenburg (unpublished data)
	Ouderkerk a/d IJssel							5.6	36%			187.2			8.9				21%	1.0E-02	Peijnenburg (unpublished data)
	Stolberg							7.2	8%			35.9			41.6	175.4	9.8		4%	1.0E-03	Peijnenburg (unpublished data)
	Veenoord							6.0	4%			38.0			3.8				3%	2.9E-03	Peijnenburg (unpublished data)
	Zenderpark - Lelystad							7.3	3%			6.7			2.5				2%	3.6E-04	Peijnenburg (unpublished data)

BB	=	Biesbosch
ADW	=	Afferdensche and Deestsche Waarden
pH-pw	=	Porewater pH
OM%	=	Organic matter content of soils (kg_{om}·kg^{-1} dry soil)
C_s	=	Total metal concentration in soil (mg·kg^{-1} dry soil)
C_i	=	Internal metal concentration in *L. rubellus* (mg·kg^{-1} dry body weight)
OC%	=	Organic carbon content of soils (kg_{oc}·kg^{-1} dry soil)
C_{pw}	=	Dissolved metal concentration in porewater (mg·L^{-1})
I		Peijnenburg et al. (2001)
II		EC (2004)
III		Sauvé et al. (2000)

Appendix 5

A5.1 Field data used

Table 1: Characteristics of empirical accumulation data of carnivorous and herbivorous small mammals
Number in parenthesis is the sample size.

#	Location	Small mammal species	Tissues analyzed*	Food items	Plant parts analyzed	C_{soil}	Reference
1	Lage Hof, Biesbosch, the Netherlands, floodplain area	*S. araneus*[a]** (18), *C. glareolus*[a] (12)	k	*L. rubellus*[a] (32)		+[a,b] (59)	[a][Hamers et al. 2006], [b][Notten et al., 2005]
2	Petrusplaat Oost, Biesbosch, the Netherlands, floodplain area	*S. araneus*[a]** (19) *C. glareolus*[a] (11)	k	*L. rubellus* (15)[a]		+[a] (12)	[a][Hamers et al., 2006
3	Afferdensche and Deestsche Waarden (ADW), the Netherlands, floodplain area	*S. araneus*[c] (29), *C. glareolus*[c] (23), *M.agrestis*[c] (2)	l, k, w-b	*L. rubellus*[d] (13), mixed grasses[c] (21)	above ground parts	+[c,d] (67)	[c][Wijnhoven et al., 2006; Wijnhoven et al., 2007], [d][Van Vliet et al., 2005]
4	Ochten, Rhine delta, the Netherlands, floodplain area	*S. araneus*[e] (6)	k	*L. rubellus*[e] (6)	-	+[e] (6)	[e][Henriks et al., 1995]
5	Gelderse Poort, Rhine delta, the Netherlands, floodplain area	*S. araneus*[e] (5)	k	*L. rubellus*[e] (5)	-	+[e](5)	[e][Hendriks et al., 1995]
6	Budel, the Netherlands, near closed smelter	*S. araneus*[f] (32), *M. agrestis*[f] (51)	k, l	*L. rubellus*[f], *Agrostis capillaries*[f], *Holcus*	leaves and stems	+[f] (11)	[f][Ma et al., 1991]

				lanatus[f], *Deschampsia fluxuosa*[f]			
7	Arnhem, the Netherlands, industrially polluted area	*S. araneus*[f] (33), *M. agrestis*[f] (43)	k, l	*L. rubellus*[f], *A. capillaries*[f], *H. lanatus*[f], *D. fluxuosa*[f]	leaves and stems	+[f] (7)	[f][Ma et al., 1991]
7	Mining complex, UK, re-vegetated mine waste site	*S. araneus*[g]*** (17), *M. agrestis*[g]*** (21)	k, l	Macroinvertebrates, *Festuca rubra*[g], *H. lanatus*[g], *Dactylis glomerata*[g]	unwashed young shoots of *F. rubra* and *H. lanatus*, unwashed leaves of *D. glomerata*	+[h]	[g][Andrews et al., 1989], [h][Shore, 1995]
8	Reference site. UK	*S. araneus*[g] (13), *M. agrestis*[g] (20)	k, l	Macroinvertebrates[g], *F. rubra*[g], *H. lanatus*[g], *D. glomerata*[g]	unwashed young shoots of *F. rubra* and *H. lanatus*, unwashed leaves of *D. glomerata*	+[h]	[g][Andrews et al., 1989], [h][Shore, 1995]
9	Copper-cadmium refinery, UK	*S.araneus*[k,l](25)****, *M. agrestis*[k,l] (23)	k, l	*earthworms*[j]*****, *A. stolonifera*[i],	unwashed leaves	+ (30)[i]	[i][Hunter et al., 1987a], [j][Hunter et

				F. rubra[l]			al., 1987b], [k][Hunter et al., 1987c] [l][Hunter et al., 1989]
10	1 km from copper-cadmium refinery, UK	*S. araneus*[k,l](20), *M. agrestis*[k,l](20)	k, l	*Earthworms*[j], *A. stolonifera*[i], *F. rubra*[i]	unwashed leaves	+ (30)[i]	[i][Hunter et al., 1987a], [j][Hunter et al., 1987b], [k][Hunter et al., 1987c] [l][Hunter et al., 1989]
11	Control site, UK	*S. araneus*[k,l](21), *M. agrestis*[k,l] (19)	k, l	*earthworms**[j], *A. stolonifera*[i], *F. rubra*[i]	unwashed leaves	+ (30)[i]	[i][Hunter et al., 1987a], [j][Hunter et al., 1987b], [k][Hunter et al., 1987c] [l][Hunter et al., 1989]
12	Frongoch, Wales, UK, abandoned lead mine	*C. glareolus*[m](35)	k, l, w-b	*F. rubra*[m], *Agrostis sp.*[m] (3-9)	leaves	+ (4)[m]	[m][Milton et al., 2003]
13	Reference site, UK	*C. glareolus*[m] (10)	k, l, w-b	*F. rubra*[m], *Agrostis sp.*[m](3-9)	leaves	+ (4)[m]	[m][Milton et al., 2003]

*k = kidney, l = liver, w-b = whole-body

**86% of sampling population of *S. araneus* consisted of juvenile individuals, whereas 35% of *C. glareolus* sampled were juveniles [Hamers et al., 2006]

***All *S. araneus* individuals sampled were not yet sexually mature, i.e. under the age of 6 months

****Population caught around Cu-Cd refinery comprised 19 juvenile and 6 adult individuals [Hunter et al., 1989]

*****Reduced earthworm density at Cu-Cd refinery which is related to highly elevated copper concentrations. With virtually absence of earthworms at the refinery, diet predominantly exists of collembolan (*Orchesella villosa*) [Hunter et al., 1987b]

Table 2: Field data used: cadmium concentrations in soil (C_{soil}), plants (C_{plant}), earthworms ($C_{earthworm}$) and kidney, liver and whole-body of small mammals (C_{i_kidney}, C_{i_liver}, $C_{i\_wholebody}$).

95% confidence intervals are provided when possible. Underlined whole-body concentrations are calculated from kidney and liver concentrations, whereas other values are true measurements. Carnivorous small mammals are represented by the common shrew, *Sorex araneus*, except data from Laskowski and Marjański (1993) which include different shrew species: *S. araneus*, *Sorex minutus*, *Neomys fodiens* and *Sorex alpinus*. Underlined whole-body concentrations are estimated levels from kidney and liver concentrations, other whole-body concentrations are "true" measurements.

a. Carnivorous shrew

Location	C_{soil} mg·kg^{-1} dw	95% CI	5% CI	References	$C_{earthworm}$ mg·kg^{-1} dw	95% CI	5% CI	Species	References	C_{i_kidney} mg·kg^{-1} dw	95% CI	5% CI	C_{i_liver} mg·kg^{-1} dw	95% CI	5% CI	$C_{i\_wholebody}$ mg·kg^{-1} dw	References
Biesbosch, Lage Hof	16,75	17,05	16,46	van Vliet et al. 2005	58,38	68,39	49,82	*Lumbricus rubellus*	Hamers et al. 2006	20,43	26,80	15,58					Hamers et al. 2006
Biesbosch, PetrusplaatOost	10,18	11,81	8,78	van Vliet et al. 2005	77,05	96,99	61,21	*Lumbricus rubellus*	Hamers et al. 2006	72,06	104,36	49,75					Hamers et al. 2006
Budel	6,10			Ma et al. 1991	82,21			*Lumbricus rubellus*	Ma et al. 1991	126,85			154,87			16,56	Ma et al. 1991
Arnhem	3,50			Ma et al. 1991	22,23			*Lumbricus rubellus*	Ma et al. 1991	28,08			23,81			2,75	Ma et al. 1991
Ochten, Rijn	1,83	6,00	0,56	Hendriks et al. 1995	31,00	38,78	24,78	*Lumbricus rubellus*	Hendriks et al. 1995	10,60	43,85	2,56					Hendriks et al. 1995
Gelderse Poort, Rijn	6,26	7,49	5,22	Hendriks et al. 1995	35,07	38,78	31,72	*Lumbricus rubellus*	Hendriks et al. 1995	21,39	66,38	6,89					Hendriks et al. 1995
ADW	1,75	2,25	1,35	Wijnhoven et al. 2006 Wijnhoven et al. 2007 van Vliet et al. 2005	40,45	49,87	32,81	*Lumbricus rubellus*	van Vliet et al. 2005	14,00	20,23	9,69	14,00	19,24	10,18	2,41	Wijnhoven et al. 2006 Wijnhoven et al. 2007
Control	0,96			Hunter et al. 1987a	4,10			various oligochaeta	Hunter et al. 1987b	20,50			13,60			1,68	Hunter et al. 1987c
1 km from Cu/Cd-refinery	7,40			Hunter et al. 1987a	34,00			various oligochaeta	Hunter et al. 1987b	156,00			245,00			25,14	Hunter et al. 1987c
Cu/Cd-refinery	28,30			Hunter et al. 1987a	107,00			various oligochaeta	Hunter et al. 1987b	253,00			578,00			56,56	Hunter et al. 1987c
mine	23,90			Shore, 1995						149,00			219,00			23,20	Andrews et al. 1989
Reference	0,52			Shore, 1995						4,13			2,85			2,10	Andrews et al. 1989
Cu refinery				Hunter et al. 1987a				various oligochaeta	Hunter et al. 1987b	12,60			156,00			13,95	Hunter et al. 1987c
Ojcow National Park					21,10			Lumbricidae	Laskowski and Maryanski, 1993							3,10	Laskowski and Maryanski, 1993
Olkusz-Pomorzany					121,30			Lumbricidae	Laskowski and Maryanski, 1993							4,60	Laskowski and Maryanski, 1993
Niepolomice Forest					23,20			Lumbricidae	Laskowski and Maryanski, 1993							2,90	Laskowski and Maryanski, 1993
Ratanica Watershed					13,60			Lumbricidae	Laskowski and Maryanski, 1993							1,90	Laskowski and Maryanski, 1993

b. Herbivores voles

Location	C_{soil} mg·kg^{-1} dw	95% CI	5% CI	References	C_{plant} mg·kg^{-1} dw	Plant species	References	C_{i_kidney} mg·kg^{-1} dw	95% CI	5% CI	C_{i_liver} mg·kg^{-1} dw	95% CI	5% CI	$C_{i\_wholebody}$ mg·kg^{-1} dw	Small mammal species	References
Budel	5,50			Ma et al., 1991	0,46	*Holcus lanatus*	Ma et al., 1991	1,81			0,33			0,05	*M. agrestis*	Ma et al., 1991
Arnhem	1,20			Ma et al., 1991	0,32	*Deschampsia flexuosa*	Ma et al., 1991	0,18			0,12			0,01	*M. agrestis*	Ma et al., 1991
Control	0,96			Hunter et al., 1987a	0,57	*Agrostis stolonifera*	Hunter et al., 1987a	1,70			0,70			0,07	*M. agrestis*	Hunter et al., 1989
1 km from Cu/Cd-refinery	7,40			Hunter et al., 1987a	1,31	*Agrostis stolonifera*	Hunter et al., 1987a	23,90			8,70			0,98	*M. agrestis*	Hunter et al., 1989
Cu/Cd-refinery	28,30			Hunter et al., 1987a	3,10	*Agrostis stolonifera*	Hunter et al., 1987a	88,80			22,70			2,96	*M. agrestis*	Hunter et al., 1989
Mine	23,90			Shore, 1995	4,75	*F.rubra*, *H. lanatus*, *D. glomerata*	Andrews et al., 1989	5,27			1,80			1,84	*M. agrestis*	Andrews et al., 1989
Reference	0,52			Shore, 1995	1,21	*F.rubra*, *H. lanatus*, *D. glomerata*	Andrews et al., 1989	1,75			1,10			0,88	*M. agrestis*	Andrews et al., 1989
Frongoch	0,90			Milton et al., 2003	0,13	*Agrostis* spp.	Milton et al., 2003	1,90			0,30			0,07	*C. glareolus*	Milton et al., 2003
Reference	0,30			Milton et al., 2003	0,17	*Agrostis* spp.	Milton et al., 2003	0,30			0,10			0,16	*C. glareolus*	Milton et al., 2003
Biesbosch, Lage Hof	16,75	17,05	16,46	Notten et al., 2005				2,06	3,66	1,16					*C. glareolus*	Hamers et al., 2006
Biesbosch, Petrusplaat Oost	10,18	11,81	8,78	Notten et al., 2005				13,07	20,04	8,52					*C. glareolus*	Hamers et al., 2006
ADW	1,49	2,01	1,11	van Vliet et al., 2005; Wijnhoven et al., 2006; Wijnhoven et al., 2007	0,38	above ground grasses	Wijnhoven et al., 2006; Wijnhoven et al., 2007	4,19	6,78	2,59	0,76	1,15	0,50	0,19	*C.glareolus*	Wijnhoven et al., 2006 Wijnhoven et al., 2007
ADW	1,49	2,01	1,11	van Vliet et al., 2005; Wijnhoven et al., 2006; Wijnhoven et al., 2007	0,38	above ground grasses	Wijnhoven et al., 2006; Wijnhoven et al., 2007	0,54	1,5E+08	1,9E-09	0,81	16,05	0,04	0,51	*M. agrestis*	Wijnhoven et al., 2006 Wijnhoven et al., 2007

A5.2 Cadmium assimilation efficiencies compiled from literature

Table 3: Empirical cadmium assimilation efficiencies

Species	Food and metal speciation	(C_0)	Unit	AE (%)	n, o, i	Experimental design	Reference
mice	Incorporated in wheat-bran + semi-synthetic food	0.056	$mg \cdot kg^{-1}$ food	0.20	n	Fractional accumulation in kidney + liver after 9 weeks of exposure	[Lind et al., 1998]
mice	Incorporated in sugar-beet fibre + semi-synthetic food	0.044	$mg \cdot kg^{-1}$ food	0.28	n	Fractional accumulation in kidney + liver after 9 weeks of exposure	[Lind et al., 1998]
mice	Incorporated in carrot + semi-synthetic food	0.042	$mg \cdot kg^{-1}$ food	0.27	n	Fractional accumulation in kidney + liver after 9 weeks of exposure	[Lind et al., 1998]
mice	$CdCl_2$ + synthetic food	0.05	$mg \cdot kg^{-1}$ food	0.29	n	Fractional accumulation in kidney + liver after 9 weeks of exposure	[Lind et al., 1998]
mice	$CdCl_2$ + synthetic food	0.1	$mg \cdot kg^{-1}$ food	0.32	n	Fractional accumulation in kidney + liver	[Lind et al., 2001]
mice	Partly Cd-MT in raw horse kidney + synthetic food	0.06	$mg \cdot kg^{-1}$ food	0.28	n	Fractional accumulation in kidney + liver	[Lind et al., 2001]
mice	Partly Cd-MT in broiled horse kidney + synthetic food	0.06	$mg \cdot kg^{-1}$ food	0.24	n	Fractional accumulation in kidney + liver	[Lind et al., 2001]
mice	$CdCl_2$ + synthetic food	3.7	$mg \cdot kg^{-1}$ food	0.28	n	Fractional accumulation in kidney + liver	[Lind et al., 2001]
mice	Partly Cd-MT in raw horse kidney + synthetic food	3.4	$mg \cdot kg^{-1}$ food	0.29	n	Fractional accumulation in kidney + liver	[Lind et al., 2001]
mice	Partly Cd-MT in broiled horse kidney + synthetic food	3.5	$mg \cdot kg^{-1}$ food	0.27	n	Fractional accumulation in kidney + liver	[Lind et al., 2001]
mice	$CdCl_2$ + synthetic food	0.3	$mg \cdot kg^{-1}$ food	0.32	n	Fractional accumulation in kidney + liver, continuously exposure during a week	[Lind et al., 1997]

mice	$CdCl_2$ + synthetic food	2.1	$mg \cdot kg^{-1}$ food	0.49	n	Fractional accumulation in kidney + liver, exposed for 24hr/wk	[Lind et al., 1997]
mice	$CdCl_2$	0.34 – 3.4	$mg \cdot kg^{-1}$	0.35	o	Whole-body accumulation, determined 1 week after administration	[Liu and Klaassen, 1996]
mice	$CdCl_2$	34	$mg \cdot kg^{-1}$	3.5	o	Whole-body accumulation, determined 1 week after administration	[Liu and Klaassen, 1996]
rat	$CdCl_2$ dissolved in 0.5 ml saline	$1 \cdot 10^{-4}$	$mg \cdot kg^{-1}$ body wt.	0.09	i	Injection into in situ isolated intestinal loop	[Goon and Klaassen, 1989]
rat	$CdCl_2$ dissolved in 0.5 ml saline	0.01	$mg \cdot kg^{-1}$ body wt.	0.14	i	Injection into in situ isolated intestinal loop	[Goon and Klaassen, 1989]
rat	$CdCl_2$ dissolved in 0.5 ml saline	0.1	$mg \cdot kg^{-1}$ body wt.	1.14	i	Injection into in situ isolated intestinal loop	[Goon and Klaassen, 1989]
rat	$CdCl_2$ dissolved in 0.5 ml saline	1	$mg \cdot kg^{-1}$ body wt.	1.80	i	Injection into in situ isolated intestinal loop	[Goon and Klaassen, 1989]
rat	$CdCl_2$ dissolved in 0.5 ml saline	10	$mg \cdot kg^{-1}$ body wt.	3.40	i	Injection into in situ isolated intestinal loop	[Goon and Klaassen, 1989]
mice	$CdCl_2$	0.11	$mg \cdot kg^{-1}$	0.50	o	Systemic absorption in organs other than the gastrointestinal tract	[Liu et al., 2001]
mice	$CdCl_2$	33.7	$mg \cdot kg^{-1}$	1.50	o	Systemic absorption in organs other than the gastrointestinal tract	[Liu et al., 2001]
MT null mice	$CdCl_2$	0.11	$mg \cdot kg^{-1}$	0.50	o	Systemic absorption in organs other than the gastrointestinal tract	[Liu et al., 2001]
MT null mice	$CdCl_2$	33.7	$mg \cdot kg^{-1}$	1.50	o	Systemic absorption in organs other than the gastrointestinal tract	[Liu et al., 2001]

rat	Cd polluted rice + synthetic diet	0.01 - 100	ppm	0.60	*ad lib*.	Retention rate after 3 exposure times (1,4,8 months)	[Hiratsuka et al., 1999]
mice	$CdCl_2$	$1 \cdot 10^{-3}$	$mg \cdot kg^{-1}$ body wt.	0.50	o	Accumulation in whole-body 5 days after exposure	[Engström and Nordberg, 1979]]
mice	$CdCl_2$	0.015	$mg \cdot kg^{-1}$ body wt.	1.40	o	Accumulation in whole-body 5 days after exposure	[Engström and Nordberg, 1979]]
mice	$CdCl_2$	0.75	$mg \cdot kg^{-1}$ body wt.	1.40	o	Accumulation in whole-body 5 days after exposure	[Engström and Nordberg, 1979]]
mice	$CdCl_2$	37.5	$mg \cdot kg^{-1}$ body wt.	3.20	o	Accumulation in whole-body 5 days after exposure	[Engström and Nordberg, 1979]]
mice	$CdCl_2$ + traditional rodent diet	3.85	$mg \cdot kg^{-1}$ food	3.57	o	Total retention in kidney, liver, small intestines and testes. Daily feeding for 14 days. Gastric pH of 2.75	[Waisberg et al., 2005]
mice	Traditional rodent diet	0.057	$mg \cdot kg^{-1}$ food	0.12	o	Total retention in kidney, liver, small intestines and testes. Daily feeding for 14 days. Gastric pH of 3.0	[Waisberg et al., 2005]
mice	Oyster incorporated Cd mixed with synthetic diet (low Zn)	0.4	$mg \cdot kg^{-1}$ food	0.30	n	Accumulation in whole-body excluding gastrointestinal tract, 2 wk feeding study, absorption measured after 2 days fecal excretion	Sullivan et al., 1984
mice	$CdCl_2$ + synthetic food (low Zn)	0.4	$mg \cdot kg^{-1}$ food	0.25	n	Accumulation in whole-body excluding gastrointestinal tract, 2 wk feeding study, absorption measured after 2 days fecal excretion	[Sullivan et al., 1984]
mice	Oyster incorporated Cd mixed with synthetic diet (high Zn)	0.4	$mg \cdot kg^{-1}$ food	0.25	n	Accumulation in whole-body excluding gastrointestinal tract, 2 wk feeding study,	[Sullivan et al., 1984]

						absorption measured after 2 days fecal excretion	
mice	$CdCl_2$ + synthetic food (high Zn)	0.4	$mg \cdot kg^{-1}$ food	0.22	n	Accumulation in whole-body excluding gastrointestinal tract, 2 wk feeding study, absorption measured after 2 days fecal excretion	[Sullivan et al., 1984]
mice	$CdCl_2$	11.2	$mg \cdot kg^{-1}$	0.70	o	Retention, 1 wk after administration	[Liu and Klaassen, 1996]
Geometric mean				**0.51**			

C_0 is exposure concentration

Influx via nutrition (n), injection (i) or oral administration (o)

Appendix 6

A6.1 Metal ion characteristics

Covalent indices ($\chi^2_m r$) were calculated using Pauling`s electronegativity values (χ_m) provided by Allred (1961) and effective ionic radii (r) (in Ångstrom units) corresponding to octahedral coordination from Shannon and Prewitt (1969, 1970). Electronegativity values for the correct oxidation state of the metal-ion were used, except for Cr (III) and Cu (II) as χ_m is provided for Cr (II) and Cu (I) only. For lead an ionic radius of 0.94 Å, corresponding to tetrahedral coordination, was used as the resulting covalent index is more in line with the known solution coordination chemistry of Pb^{2+} (Nieboer and Richardson, 1980). Electronegativity data, ionic radii and covalent indices compiled per metal included in this study are presented in Table A6 6.1.

Table A6 6.1: Metal ion characteristics: electronegativity values, ionic radii and covalent indices

Metal	**Ionic radius**	**Electronegativity**	**Covalent index**
	r (in Å)	χ_m	$\chi^2_m r$
Ag^+	1.15	1.93	4.28
Cd^{2+}	0.95	1.69	2.71
Co^{2+}	0.75*	1.88	2.65
Cr^{3+}	0.62	1.66**	1.69
Cs^+	1.7	0.79	1.06
Cu^{2+}	0.73	1.9***	3.29
Hg^{2+}	1.02	2	4.08
Ni^{2+}	0.69	1.91	2.52
Pb^{2+}	0.94****	1.87	3.29
Zn^{2+}	0.74	1.65	2.01

* Ionic radius for high spin state of Co^{2+}, radius for low spin state is 0.65 Å

** Electronegativity value for Cr^{2+} instead of Cr^{3+}

*** Electronegativity value for Cu^+ instead of Cu^{2+}

**** Ionic radius of Pb^{2+} for tetrahedral coordination instead of octahedral coordination (Nieboer and Richardson, 1980)

A6.2 Filtration rates

Absorption efficiencies for mollusks were preferentially calculated using filtration rates determined in the same study as the absorption rate constants. These

filtration rates were available for the following studies: Shi and Wang (2004a, 2004b) (*Ruditapes phillipinarum*, *Perna viridis*), Ng and Wang (2005) (*Perna viridis*), Baines et al., (2006) (*Mytilus edulis*), Wang and Fisher (1999) (*Macoma balthica*, *Mytilus edulis*) and Wang et al. (1996) (*Mytilus edulis*).
For other data, filtration rates from table A6 6.2 were used. The filtration rate used for *Daphnia magna* is also provided in this table.

Table A6 6.2. Species – specific filtration rates used

Species	**Filtration rate** $L \cdot kg^{-1}_{wet\ weight} \cdot d^{-1}$		**References**
Perna viridis	$2.9 \cdot 10^4$	Geometric mean (n = 39), this study	Shi and Wang 2004a, Ng and Wang, 2005
Mytilus edulis	$1.8 \cdot 10^4$		Wang et al., 1997
Ruditapes phillipinarum	$6.9 \cdot 10^3$	Geometric mean (n = 2), this study	Shi and Wang, 2004b
Dreissena polymorpha	$2.5 \cdot 10^4$		Roditi et al., 1996
Macoma balthica	$9.0 \cdot 10^3$		Wang and Fisher, 1999
Daphnia magna	$3.3 \cdot 10^4$	Geometric mean (n = 4), this study	Egloff and Palmer, 1971; Ferrando and Andreu, 1993; Fernandez-Casalderrey et al., 1994; Flickinger et al., 1982

*A*6.3 Absorption rate constants and exposure concentration in water

In our approach absorption rate constants determined at different exposure concentrations were combined to calculate mean species–specific absorption rate constants. This is only allowed if absorption rate constants are independent of the exposure concentration in water. We found that metal influx from the dissolved phase is generally linearly related to the dissolved metal concentration in water under laboratory conditions (Figure A6 6.1, Table A6 6.3). Absorption rate constants determined at different exposure concentrations can therefore be combined.

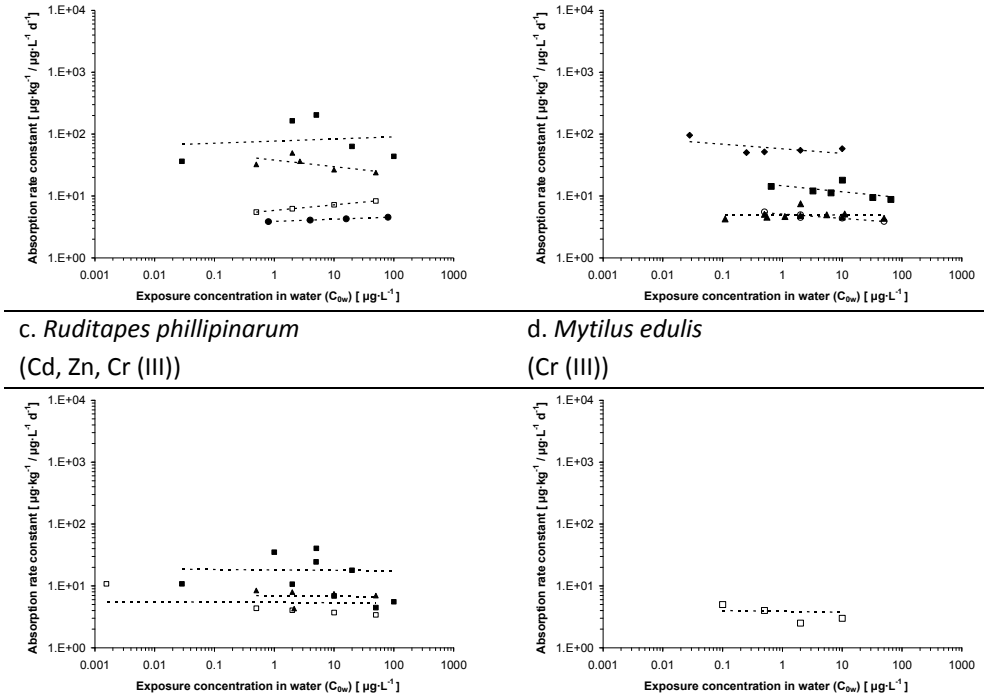


Figure A6 6.1. Metal absorption rate constants ($k_{X,w,in}$ in $L \cdot kg^{-1} \cdot d^{-1}$) vs. exposure concentrations in water (C_{0w} in $\mu g \cdot L^{-1}$). Absorption rate constants are plotted against exposure concentration, if rate constants are determined at three or more exposure concentrations.

◆ Silver (Ag), ▲ Cadmium (Cd), □ Chromium (III) (Cr), ■ Zinc (Zn), ◇ Mercury (Hg), △ Lead (Pb), ● Cesium (Cs), × Copper (Cu), ○ Cobalt (Co)

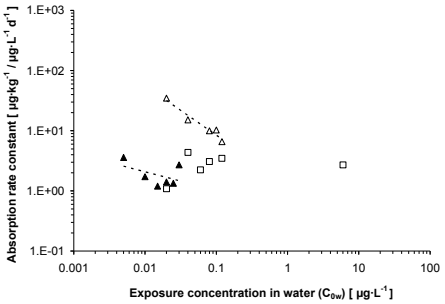
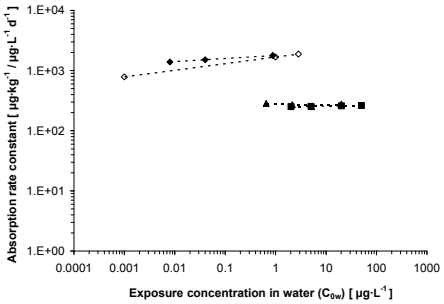
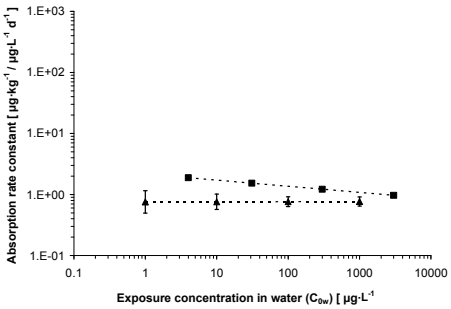
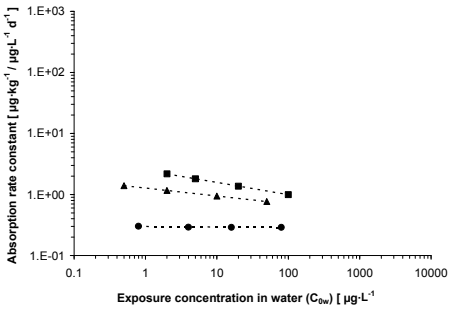
e. *Gammarus oceanicus* (Pb, Cd, Cr (III))	f. *Daphnia magna* (Cd, Zn, Ag, Hg)
	
g. *Acanthopagrus schlegeli* (Cd, Zn)	h. *Lutjanus argentimaculatus* (Cd, Zn, Cs)
	

Figure A6 6.1 (*continued*). Metal absorption rate constants ($k_{X,w,in}$ in $L \cdot kg^{-1} \cdot d^{-1}$) vs. exposure concentrations in water (C_{0w} in $\mu g \cdot L^{-1}$). Absorption rate constants are plotted against exposure concentration, if rate constants are determined at three or more exposure concentrations.

◆ Silver (Ag), ▲ Cadmium (Cd), □ Chromium (III) (Cr), ■ Zinc (Zn), ◇ Mercury (Hg), △ Lead (Pb), ● Cesium (Cs), × Copper (Cu), ○ Cobalt (Co)

Table A6 6.3 Regression analysis of metal absorption rate constants ($k_{X,w,in}$ in $L \cdot kg^{-1} \cdot d^{-1}$) with exposure concentrations (C_{0w} in $\mu g \cdot L^{-1}$).

Species	**Metal**	**Log ($k_{x,w,in}$)**	**r^2**	**SE**	**p**	**n**
Perna viridis	Zn	$0.03 \cdot Log(C_{0w}) + 1.89$	0.02	0.39	0.83	5
Perna viridis	Cd	$-0.11 \cdot Log(C_{0w}) + 1.58$	0.42	0.11	0.24	5
Macoma balthica	Ag	$-0.07 \cdot Log(C_{0w}) + 1.76$	0.38	0.10	0.27	5
Macoma balthica	Cd	$0.003 \cdot Log(C_{0w}) + 0.69$	0	0.07	0.94	10
Macoma balthica	Zn	$-0.10 \cdot Log(C_{0w}) + 1.17$	0.36	0.10	0.21	6
Macoma balthica	Co	$-0.07 \cdot Log(C_{0w}) + 0.71$	0.89	0.022	0.02	5
Gammarus oceanicus	Pb	$-0.82 \cdot Log(C_{0w}) + 0.10$	0.95	0.07	0.005	5
Gammarus oceanicus	Cd	$-0.31 \cdot Log(C_{0w}) - 0.30$	0.19	0.19	0.34	6
Lutjanus argentimaculatus	Cs	$-0.009 \cdot Log(C_{0w}) - 0.52$	0.64	0.0051	0.13	4

A6.4 Empirical absorption rate constants and elimination rate constants compiled

In Table A6 6.4 species – specific geometric mean absorption rate constants per metal are given. 95% Confidence intervals represent the variability between organisms from one species. In Table A6 6.5 all elimination rate constants compiled per species are provided. 95% Confidence intervals represent the variability between organisms from one species.

Table A6 6.4: Species-specific and metal-specific absorption rate constants and absorption efficiencies

Metal	Species	Weight kg	$k_{X,w,in}$ $L \cdot kg^{-1} \cdot d^{-1}$	95%CI	5% CI	n	$p_{X,w,in}$	References
Ag	P. viridis	1,3E-03	1,2E+03	1,6E+03	8,5E+02	33	4,11 %	Shi and Wang, 2004a; Ng and Wang, 2004b; Shi et al., 2003
Hg	P. viridis	1,3E-03	7,7E+02	2,4E+03	2,5E+02	4	2,69 %	Shi and Wang, 2004a; Pan and Wang, 2004; Wang et al., 2004
Cu	P. viridis	1,3E-03	1,7E+02			1	0,56 %	Chan, 1988
Cd	P. viridis	1,3E-03	4,5E+01	6,2E+01	3,2E+01	25	0,15 %	Shi and Wang, 2004a; Ng and Wang, 2005; Chan, 1988; Blackmore and Wang, 2002; Wang, 2001; Blackmore and Wang, 2003; Chong and Wang, 2001; Wang, 2002
Cs	P. viridis	1,3E-03	4,2E+00	4,7E+00	3,7E+00	4	0,01 %	Wang et al., 2000; Ke et al., 2000
Cr (III)	P. viridis	1,3E-03	6,7E+00	8,9E+00	5,1E+00	5	0,02 %	Chong and Wang, 2001
Zn	P. viridis	1,3E-03	1,4E+02	2,1E+02	9,0E+01	24	0,46 %	Shi and Wang, 2004a; Ng and Wang, 2005; Blackmore and Wang, 2002; Wang, 2001; Blackmore and Wang, 2003; Chong and Wang, 2001
Pb	P. viridis	1,3E-03	2,0E+01			1	0,06 %	Chan, 1988
Ag	M. edulis	1,9E-04	2,7E+02	1,1E+03	6,8E+01	6	0,46 %	Baines et al., 2006; Wang and Fisher, 1999; Wang et al., 1996
Cd	M. edulis	1,9E-04	5,5E+01	1,3E+02	2,4E+01	9	0,16 %	Baines et al., 2006; Wang and Fisher, 1999; Wang et al., 1996; Wang and Fisher, 1997
Cr (III)	M. edulis	1,9E-04	3,5E+00	5,7E+00	2,1E+00	4	0,02 %	Wang et al., 1997
Zn	M. edulis	1,9E-04	1,4E+02	2,5E+02	7,3E+01	9	0,44 %	Baines et al., 2006; Wang and Fisher, 1999; Wang et al., 1996; Wang and Fisher, 1997
Co	M. edulis	1,9E-04	2,0E+01	3,1E+01	1,3E+01	8	0,07 %	Baines et al., 2006; Wang and Fisher, 1999; Wang and Fisher, 1997
Cs	M. edulis	1,9E-04	3,0E+00			1	0,02 %	Nolan and Dahlgaard, 1991
Ag	R. philippinarum	1,6E-03	2,3E+02			1	0,25 %	Ng and Wang, 2004
Zn	R. philippinarum	1,6E-03	1,0E+01	1,7E+01	6,0E+00	14	0,07 %	Shi and Wang, 2004b; Wang, 2001; Chong and Wang, 2001; Ng and Wang, 2004
Cr (III)	R. philippinarum	1,6E-03	4,7E+00	8,5E+00	2,6E+00	5	0,01 %	Wang, 2001; Chong and Wang, 2001
Hg	R. philippinarum	1,6E-03	2,4E+02	2,4E+02	2,4E+02	1	2,12 %	Ng and Wang, 2004
Cd	R. philippinarum	1,6E-03	7,6E+00	8,7E+00	6,6E+00	14	0,06 %	Shi and Wang, 2004b; Wang, 2001; Chong and Wang, 2001; Ng and Wang, 2004
Cr (III)	D. polymorpha	5,9E-03	1,4E+02			1	0,47 %	Roditi and Fisher, 1999; Roditi et al., 2000
Ag	D. polymorpha	5,9E-03	5,5E+02			1	1,87 %	Roditi and Fisher, 1999; Roditi et al., 2000
Hg	D. polymorpha	5,9E-03	3,5E+02			1	1,17 %	Roditi and Fisher, 1999; Roditi et al., 2000
Cd	D. polymorpha	5,9E-03	3,0E+02			1	1,02 %	Roditi and Fisher, 1999; Roditi et al., 2000
Cd	M. balthica	1,4E-03	4,9E+00	5,5E+00	4,4E+00	10	0,05 %	Wang and Fisher, 1999; Griscom and fisher, 2002; Griscom et al., 2002; Lee et al., 1998
Zn	M. balthica	1,4E-03	1,2E+01	1,6E+01	9,0E+00	6	0,13 %	Wang and Fisher, 1999; Lee et al., 1998
Ag	M. balthica	1,4E-03	6,0E+01	8,4E+01	4,3E+01	5	0,67 %	Wang and Fisher, 1999; Griscom and fisher, 2002; Griscom et al., 2002; Lee et al., 1998
Co	M. balthica	1,4E-03	4,6E+00	5,5E+00	3,9E+00	5	0,05 %	Wang and Fisher, 1999; Griscom and fisher, 2002; Griscom et al., 2002; Lee et al., 1998
Cu	C. marinus		2,1E+02	8,4E+02	5,0E+01	3		Clason et al., 2004a
Pb	C. marinus		4,5E+01	2,8E+02	7,1E+00	3		Clason et al., 2004a
Co	C. marinus		7,0E+00	8,4E+00	5,8E+00	2		Clason et al., 2004a
Ni	C. marinus		5,9E+00	1,8E+01	1,9E+00	3		Clason et al., 2004a
Cd	C. marinus		1,7E+01	2,1E+01	1,3E+01	3		Clason et al., 2004a
Pb	G. oceanicus		1,3E+01	2,8E+01	5,9E+00	5		Clason et al., 2004b
Cd	G. oceanicus		1,8E+00	2,9E+00	1,2E+00	6		Clason et al., 2004b
Ni	G. oceanicus		1,1E+00	1,1E+00	1,1E+00	1		Clason et al., 2004b
Cu	G. oceanicus		3,8E+01	3,8E+01	3,8E+01	1		Clason et al., 2004b
Cd	T. longicornis	1,0E-06	1,4E+02	1,6E+02	1,2E+02	4		Wang and Fisher, 1998
Co	T. longicornis	1,0E-06	1,2E+02	1,4E+02	1,0E+02	4		Wang and Fisher, 1998
Ag	T. longicornis	1,0E-06	2,1E+03	3,0E+04	1,5E+02	2		Wang and Fisher, 1998
Zn	T. longicornis	1,0E-06	6,5E+02	9,5E+02	4,4E+02	4		Wang and Fisher, 1998

Table A6 6.4: Species-specific and metal-specific absorption rate constants and absorption efficiencies

Cd	D. magna	1,4E-06	3,2E+02	4,4E+02	2,4E+02	9	0,83 %	Yu and Wang, 2002
Zn	D. magna	1,4E-06	2,1E+02	3,4E+02	1,3E+02	8	0,54 %	Yu and Wang, 2002
Ag	D. magna	1,4E-06	1,6E+03	2,8E+03	8,7E+02	6	3,98 %	Lam and Wang, 2006
Hg	D. magna	1,4E-06	1,4E+03	4,4E+03	4,2E+02	3	3,47 %	Tsui and Wang, 2004
Cd	A. schlegelii	3,8E-04	9,3E-01	1,2E+00	7,2E-01	26	0,06 %	Tsui and Wang, 2004; Zhang and Wang, 2005; Zhang and Wang, 2006
Zn	A. schlegelii	3,8E-04	1,7E+00	2,4E+00	1,2E+00	22	0,12 %	Zhang and Wang, 2005; Zhang and Wang, 2006
Ag	A. schlegelii	4,0E-04	8,3E+00	7,1E+01	9,6E-01	4	0,58 %	Long and Wang, 2005
Cs	L. argentimaculatus	3,7E-04	2,9E-01	3,0E-01	2,8E-01	4	0,02 %	Zhao and Wang, 2001
Cd	L. argentimaculatus	3,7E-04	1,0E+00	1,6E+00	6,9E-01	4	0,07 %	Xu and Wang, 2002
Zn	L. argentimaculatus	3,7E-04	1,5E+00	2,6E+00	8,9E-01	4	0,11 %	Xu and Wang, 2002
Cd	T. jarbua	1,4E-03	3,9E-01	3,0E+03	5,2E-05	2	0,04 %	Zhang and Wang, 2005
Zn	T. jarbua	1,4E-03	1,3E+00	2,9E+02	5,9E-03	2	0,13 %	Zhang and Wang, 2005
Cd	O. mykiss	1,8E-01	3,8E-01	2,6E+00	5,5E-02	5	0,12 %	Calamari et al., 1982
Ag	P. platessa	4,5E-02	7,4E+00			1	1,71 %	Pentreath, 1977
Hg	P. platessa	4,3E-02	4,3E+00			1	0,98 %	Pentreath, 1976
Zn	G. affinis	4,1E-04	7,4E-01	1,9E+00	2,8E-01	6	0,05 %	Newman and Mitz, 1988
Hg	L. microlophys	8,5E-04	8,8E+00	5,7E+01	1,4E+00	2	0,75 %	Pickhardt et al., 2006
Zn	O.mykiss	5,5E-03	2,0E+00	2,0E+00	2,0E+00		0,27 %	Alsop and Wood, 1999
Hg	G. affinis	4,1E-04	1,3E+01	1,7E+02	9,7E-01	2	0,91 %	Pickhardt et al., 2006
Cd	P. cantonensis	3,0E-03	5,0E-01	8,0E-01	3,2E-01	4	0,06 %	Ni et al., 2005
Zn	P. cantonensis	3,0E-03	1,2E+00	3,6E+00	3,9E-01	4	0,14 %	Ni et al., 2005

Table A6 6.5: Species-specific and metal-specific elimination rate constants and weight-corrected elimination rates

Metal	Species	weight kg	$k_{x,ex}$ d^{-1}	95%CI	5% CI	n	Weight-corrected $k_{x,ex}$ $kg^{x} \cdot d^{-1}$	95%CI	5% CI	n	References
Ag	P. viridis	1,3E-03	1,9E-02	2,0E-02	1,0E-02	16	3,8E-03	7,0E-03	2,0E-03	16	Ng and Wang, 2005; Shi et al., 2003
Cd	P. viridis	1,3E-03	1,3E-02	1,4E-02	8,4E-03	6	2,3E-03	3,8E-03	1,4E-03	6	Ng and Wang, 2005; Chong and Wang, 2001
Cs	P. viridis	1,3E-03	1,5E-01			1	3,0E-02			1	Wang et al., 2000; Ke et al., 2000
Cr (III)	P. viridis	1,3E-03	2,9E-02			1	6,1E-03			1	Chong and Wang, 2001
Zn	P. viridis	1,3E-03	3,1E-02	3,3E-02	1,9E-02	6	5,7E-03	8,9E-03	3,7E-03	6	Ng and Wang, 2005; Blackmore and Wang, 2002; Chong and Wang, 2001
Ag	M. edulis	1,9E-04	4,5E-02	4,6E-02	3,1E-02	5	4,7E-03	7,2E-03	3,1E-03	5	Baines et al., 2006; Wang et al., 1996
Cd	M. edulis	1,9E-04	6,9E-02	6,9E-02	2,4E-02	8	6,0E-03	1,8E-02	2,0E-03	8	Baines et al., 2006; Wang et al., 1996; Wang and Fisher, 1997
Cr (III)	M. edulis	1,9E-04	1,1E-02	1,1E-02	8,7E-03	3	2,3E-03	3,0E-03	1,8E-03	3	Wang et al., 1997
Zn	M. edulis	1,9E-04	5,4E-02	5,4E-02	2,3E-02	8	4,7E-03	9,7E-03	2,3E-03	8	Baines et al., 2006; Wang et al., 1996; Wang and Fisher, 1997
Co	M. edulis	1,9E-04	8,7E-02	8,8E-02	2,6E-02	7	7,6E-03	2,0E-02	2,9E-03	7	Baines et al., 2005; Wang et al., 1997
Zn	R. phillipinarum	1,6E-03	1,7E-02	1,9E-02	5,1E-03	3	3,6E-03	1,1E-02	1,2E-03	3	Shi and Wang, 2004b; Chong and Wang, 2001
Cr (III)	R. phillipinarum	1,6E-03	2,3E-02			1	5,0E-03			1	Chong and Wang, 2001
Cd	R. phillipinarum	1,6E-03	2,0E-02	2,2E-02	4,5E-03	3	4,2E-03	1,7E-02	1,0E-03	3	Shi and Wang, 2004b; Chong and Wang, 2001
Ag	D. polymorpha	5,9E-03	8,8E-02			1	9,0E-03			1	Roditi and Fisher, 1999
Cd	D. polymorpha	5,9E-03	1,1E-02			1	1,1E-03			1	Roditi and Fisher, 1999
Cd	M. balthica	1,4E-03	2,0E-02	2,2E-02	4,3E-03	2	4,0E-03	2,3E-02	6,8E-04	2	Griscom et al., 2002; Lee et al., 1998
Zn	M. balthica	1,4E-03	1,2E-02			1	2,3E-03			1	Lee et al., 1998
Ag	M. balthica	1,4E-03	9,6E-03			1	1,9E-03			1	Griscom et al., 2002
Co	M. balthica	1,4E-03	2,6E-02			1	5,2E-03			1	Griscom et al., 2002
Cu	C. marinus		3,4E-01	8,5E-01	1,4E-01	3					Clason et al., 2004a
Pb	C. marinus		1,3E-01								Clason et al., 2004a
Co	C. marinus		1,8E-01			2					Clason et al., 2004a
Ni	C. marinus		1,5E-01	3,0E-01	7,5E-02	3					Clason et al., 2004a
Cd	C. marinus		2,9E-01	3,5E-01	2,3E-01	3					Clason et al., 2004a
Pb	G. oceanicus		3,6E-01	6,1E-01	2,2E-01	5					Clason et al., 2004b
Cd	G. oceanicus		1,7E-01	2,6E-01	1,1E-01	6					Clason et al., 2004b
Ni	G. oceanicus		4,7E-01			1					Clason et al., 2004b
Cu	G. oceanicus		1,2E+00			1					Clason et al., 2004b
Cd	T. longicornis	1,0E-06	2,2E-01	7,1E-01	6,7E-02	3	6,9E-03	2,2E-02	2,1E-03	3	Wang and Fisher, 1998
Co	T. longicornis	1,0E-06	2,7E-01	4,6E-01	1,6E-01	3	8,7E-03	1,4E-02	5,2E-03	3	Wang and Fisher, 1998
Ag	T. longicornis	1,0E-06	2,2E-01	4,6E-01	1,0E-01	2	6,8E-03	1,4E-02	3,2E-03	3	Wang and Fisher, 1998
Zn	T. longicornis	1,0E-06	7,0E-02	1,7E-01	2,9E-02	3	2,2E-03	5,3E-03	9,2E-04	3	Wang and Fisher, 1998
Cd	D. magna	1,4E-06	6,5E-02	1,4E-01	3,1E-02	3	2,2E-03	4,7E-03	1,0E-03	3	Guan and Wang, 2004
Zn	D. magna	1,4E-06	2,9E-01	3,0E-01	2,7E-01	3	9,8E-03	1,0E-02	9,3E-03	3	Guan and Wang, 2004
Ag	D. magna	1,4E-06	2,8E-01	3,2E-01	2,5E-01	4	9,6E-03	1,1E-02	8,6E-03	4	Lam and Wang, 2006
Hg	D. magna	1,4E-06	5,1E-02	7,6E-02	3,5E-02	3	1,8E-03	2,6E-03	1,2E-03	3	Tsui and Wang, 2004
Cd	A. schlegelii	3,8E-04	8,9E-02			1	1,3E-02			1	Zhang and Wang, 2007
Zn	A. schlegelii	3,8E-04	1,6E-02			1	2,2E-03			1	Zhang and Wang, 2007
Cs	L. argentimaculatus	3,7E-04	2,1E-02			1	3,0E-03			1	Zhao et al., 2001
Cd	L. argentimaculatus	3,7E-04	2,5E-02			1	3,5E-03			1	Xu and Wang, 2002
Zn	L. argentimaculatus	3,7E-04	1,5E-02			1	2,1E-03			1	Xu and Wang, 2002
Cd	T. jarbua	1,4E-03	3,0E-02	5,1E-02	1,7E-02	3	4,7E-03	8,0E-03	2,7E-03	3	Long and Wang, 2005
Ag	T. jarbua	6,0E-04	3,4E-02	4,7E-02	2,5E-02	5	5,3E-03	7,4E-03	3,8E-03	3	Long and Wang, 2005
Cd	O. mykiss	1,8E-01	1,1E-02	4,0E-02	3,0E-03	5	7,0E-03	2,6E-02	1,9E-03	5	Calamari et al., 1982
Ag	P. platessa	4,5E-02	2,3E-02				1,0E-02			1	Pentreath, 1977
Hg	P. platessa	4,3E-02	3,7E-03				1,7E-03			1	Pentreath, 1976
Hg	G. affinis	5,9E-04	1,7E-02	8,9E-02	3,1E-03	5	1,7E-02	8,9E-02	3,1E-03	5	Pickhardt et al., 2006
Hg	L. microlophys	8,5E-04	1,2E-02	2,9E-01	5,1E-04	2	2,1E-03	5,0E-02	8,6E-05	2	Pickhardt et al., 2006

Appendix 7

A7.1 Physiological partition coefficients

Blood–air partition coefficients (K_{BA}) were predicted based on chemical affinity for different blood components including polar lipids ($p_{pl,bl}$), neutral lipids ($p_{nl,bl}$), proteins ($p_{p,bl}$) and water content ($p_{H2O,bl}$). These physiological parameters were collected for various mammalian species, including humans, rats, dogs, mice and pigs. The water percentage of blood was obtained from Altman (1961). To our knowledge the polar lipid fraction and neutral lipid fraction of whole blood have not been reported in literature. These fractions were therefore estimated from the polar lipids fraction and neutral lipids fractions in erythrocytes and plasma, following the approach of Poulin and Krishnan (1996) (Eqn. 7.1).

$$p_{nl,bl} = p_{nl,plasma} \cdot p_{plasma,bl} + p_{nl,erythrocytes} \cdot p_{erythrocytes,bl}$$ Equation 7.1

$p_{nl,bl}$	=	Neutral lipid content of blood	[g·mL^{-1}]
$p_{nl,plasma}$	=	Neutral lipid content of plasma	[g·mL^{-1}]
$p_{plasma,bl}$	=	Plasma fraction of blood	[mL·mL^{-1}]
$p_{nl,erythrocytes}$	=	Neutral lipid content of erythrocytes	[g·mL^{-1}]
$p_{erythrocytes,bl}$	=	Erythrocytes fractions of blood	[mL·mL^{-1}]

Table A7.1. Species-specific blood parameters: water content, neutral lipid content, polar content and protein fraction of whole blood

Species	$p_{H2O,bl}$ $ml \cdot ml^{-1}$	**References**	$p_{nl,bl}$ $g \cdot mL^{-1*}$	$p_{pl,bl}$ $g \cdot mL^{-1*}$	$p_{p,bl}^{**}$ $g \cdot mL^{-1*}$	**References**
Human	80.6%	Altman, 1961	0.0033	0.0024	0.043	Haddad et al., 2000
Rat	81.6%	Altman, 1961	0.0023	0.0023	0.039	Davies and Morris, 1993, Nelson, 1967, Nelson, 1972
Rabbit	81.7%	Altman, 1961	0.0023	0.0019	0.038	Davies and Morris, 1993, Nelson, 1967, Nelson, 1972
Dog	80.1%	Altman, 1961	0.0034	0.0028	0.052	Davies and Morris, 1993, Nelson, 1967, Nelson, 1972
Pig	79.1%	Altman, 1961				Davies and Morris, 1993, Nelson, 1967, Nelson, 1972

*The density of blood is approximately 1 $g \cdot mL^{-1}$ (1.06 $g \cdot mL^{-1}$)

**Total plasma proteins (incl. albumin), this fraction excludes hemoglobin

Table A7.2: Typical values for blood parameters of mammals: water content ($p_{H2O,bl}$), neutral lipid fraction ($p_{nl,bl}$), polar lipid fraction ($p_{pl,bl}$) and protein fraction ($p_{p,bl}$) of blood, based on mean values for hematocrit, plasma volume and lipid fractions in erythrocytes and plasma

Parameter	Value	Unit	Max.	Min.	SD	n	Species	References
$p_{H2O,bl}$	$8.0 \cdot 10^{-1}$	$ml \cdot ml^{-1}$	0.823	0.772	0.014	12	Human (children, adult), cat, cattle (cow, bull), dog, goat, horse, rabbit, rat, sheep, swine	Altman, 1961
$p_{nl,bl}$	$2.3 \cdot 10^{-3}$	$g \cdot mL^{-1}$						Nelson, 1972, Davies and Morris, 1993
$p_{pl,bl}$	$2.0 \cdot 10^{-3}$	$g \cdot mL^{-1}$						Nelson, 1972, Davies and Morris, 1993
$p_{p,bl}$	$4.3 \cdot 10^{-2}$	$g \cdot mL^{-1}$	$3.6 \cdot 10^{-2}$	$5.4 \cdot 10^{-2}$	0.007	6	Human, dog, rabbit, rat, mouse, monkey	Davies and Morris, 1993
Hematocrit	0.422	$mL \cdot mL^{-1}$	0.46	0.36	0.036	6	Human, dog, rabbit, rat, mouse, monkey	Davies and Morris, 1993
Plasma volume	0.598	$mL \cdot mL^{-1}$	0.67	0.57	0.036	6	Human, dog, rabbit, rat, mouse, monkey	Davies and Morris, 1993
$p_{pl,plasma}$	$1.1 \cdot 10^{-3}$	$g \cdot mL^{-1}$	$2.7 \cdot 10^{-3}$	$2.2 \cdot 10^{-4}$	$5.5 \cdot 10^{-4}$	10	Cat, cow, dog, goat, horse, rabbit, rat, sheep, pig, guinea pig	Nelson, 1967
$p_{nl,plasma}$	$2.9 \cdot 10^{-3}$	$g \cdot mL^{-1}$	$5.2 \cdot 10^{-3}$	$1.0 \cdot 10^{-3}$	$1.3 \cdot 10^{-3}$	10	Cat, cow, dog, goat, horse, rabbit, rat, sheep, pig, guinea pig	Nelson, 1967
$p_{pl,erythrocytes}$	$3.1 \cdot 10^{-3}$	$g \cdot mL^{-1}$	$3.7 \cdot 10^{-3}$	$2.6 \cdot 10^{-3}$	$3.4 \cdot 10^{-4}$	10	Cat, cow, dog, goat, horse, rabbit, rat, sheep, pig, guinea pig	Nelson, 1972
$p_{nl,erythrocytes}$	$1.3 \cdot 10^{-3}$	$g \cdot mL^{-1}$	$1.6 \cdot 10^{-3}$	$1.0 \cdot 10^{-3}$	$1.9 \cdot 10^{-4}$	10	Cat, cow, dog, goat, horse, rabbit, rat, sheep, pig, guinea pig	Nelson, 1972

Table A7.3: Hematocrit (erythrocyte volume percentage in blood) and plasma (volume %) in blood

Species	**Hematocrit** Volume %	**Plasma** Volume %	**Reference**
Human	44%	57.7%	Davies and Morris (1993)
Rat	46%	57.8%	Davies and Morris (1993)
Mouse	45%	58.8%	Davies and Morris (1993)
Rabbit	36%	66.7%	Davies and Morris (1993)
Dog	42%	57.2%	Davies and Morris (1993)
Pig	*Not available*	*Not available*	

Table A7.4: Polar lipid content and neutral lipid content of plasma and erythrocytes

Species	$\rho_{pl,plasma}$ $g \cdot mL^{-1}$	$\rho_{nl,plasma}$* $g \cdot mL^{-1}$	$\rho_{pl,erythrocytes}$ $g \cdot mL^{-1}$	$\rho_{nl,erythrocytes}$ $g \cdot mL^{-1}$	**Reference**
Human					Nelson, 1967; Nelson, 1972
Rat	0.001	0.003	0.003	0.001	Nelson, 1967; Nelson, 1972
Mouse	*Not available*	*Not available*	*Not available*	*Not available*	Nelson, 1967; Nelson, 1972
Rabbit	0.001	0.003	0.003	0.001	Nelson, 1967; Nelson, 1972
Dog	0.003	0.005	0.003	0.001	Nelson, 1967; Nelson, 1972
Pig	0.002	0.005	0.003	0.001	Nelson, 1967; Nelson, 1972

- Neutral lipid content in plasma is assumed to equal the total lipid content minus the polar lipids content of plasma.

A7.2 Diffusion coefficient

The (pure) water diffusion coefficient (d_w) for neutral organic chemicals is calculated according Schwarzenbach (1993) (Eqn. 7.2)

$$d_w = \frac{2.7 \cdot 10^{-8}}{M^{0.71}}$$ Equation 7.2

dw	=	pure water diffusion coefficient for neutral organic substances	[$m^2 \cdot s^{-1}$]
M	=	molecular weight of the chemical	[$g \cdot mol^{-1}$]

A7.3 Temperature correction of vapor pressure and water solubility

Generic physical–chemical properties (measured at T = 25 ºC) were corrected for temperature (T = 37ºC) following MacLeod et al. (2007) and Beyer et al. (2002). According these authors temperature –dependency can be expressed as a change in internal energy (ΔU ($kJ \cdot mol^{-1}$).

$$\Delta U_A = -3.82 \cdot Ln\left(P_L^0\right) + 67.5$$ Equation 7.3

ΔU_A	=	Heat of phase transition	[$kJ \cdot mol^{-1}$]
P_L^0	=	Vapor pressure at 25 ºC	[Pa]

$$Ln\left(P_L^T\right) = Ln\left(P_L^0\right) + \frac{\Delta H_{Vp}}{R} \cdot \left(\frac{1}{T_0} - \frac{1}{T_T}\right)$$ Equation 7.4

P_L^T	=	Vapor pressure at temperature T	[Pa]
ΔH_{Vp}	=	Enthalpy of vaporization	[$J \cdot mol^{-1}$]
R	=	Ideal gas constant (8.314)	[$J \cdot mol \cdot K^{-1}$]
T_0	=	298 Kelvin (25ºC)	
T_t	=	310 Kelvin (37ºC)	

$$\Delta H_{Vp} = \Delta U_A + R \cdot T$$ Equation 7.5

ΔU_w (20 $kJ \cdot mol^{-1}$) and ΔU_o (0 $kJ \cdot mol^{-1}$) are assumed to be constant as vapor pressure generally exhibits stronger temperature dependence than water solubility or octanol solubility. Volatile chemicals have a low ΔU_A and a relatively small change in K_{AW} is predicted.

A7.4 Elimination rate constants

Following uptake via air, chemicals can be eliminated via excretion with urine (Eqn. 7.6), egestion with feces (Eqn. 7.7), exhalation with air and growth dilution (Eqn. 7.8) (Hendriks et al., 2001, Hendriks et al., 2005, Veltman et al., 2008). The total elimination rate can be predicted as the sum of these individual loss rates. The different rate constants are a function of the octanol water-partition coefficient of the chemical and the size and trophic level of the species i.e. they depend on resistances that substances encounter in the water and lipid layers of organisms and on metabolic flows that carry substances into and out of these organisms. Elimination via excretion and elimination via egestion can be predicted considering two types of transport resistances, i.e. diffusion resistance through water and lipid layers (ρ_{H2O} and ρ_{CH2}) of the membrane and a flow delay imposed by either the drinking rate or the food ingestion rate. The adjacent coefficients and exponents needed to determine the various rate constants have been calibrated on hundreds of rate constants from laboratory studies and are extensively described in Hendriks et al. (2001). Chemical elimination via excretion with urine can thus be predicted as:

$$k_{X,w,ex} = \frac{1}{K_{TW}} \cdot \frac{w^{-\kappa}}{\rho_{H_2O,w} + \frac{\rho_{CH_2}}{K_{ow}} + \frac{1}{\gamma_0}}$$ Equation 7.6

The chemical egestion rate constant is predicted as a function of the diffusion resistance in water layers and membrane lipid layers, and the flow delay imposed by the food ingestion rate. Similar to excretion with urine, the diffusion resistance in membrane lipid layers is inversely related to the K_{ow} of the chemical. The food ingestion rate of species depends on their assimilation efficiency of food (p_{b_as}). Here we modeled egestion rate constants for herbivorous mammals, i.e. assimilation efficiency is representative for herbivores (p_{b_as} = 47%)

$$k_{X,n,ex} = \frac{1}{K_{TW}} \cdot \frac{w^{-\kappa}}{\rho_{H_2O,n} + \frac{\rho_{CH_2}}{K_{ow} \cdot qT} + \frac{1}{K_{TW,plant} \cdot (1 - p_{b\_as}) \cdot (\gamma_{b\_pr} + \gamma_{b\_re} \cdot q_{wc} \cdot q_{ap}) \cdot \frac{qT}{p_{b\_as}}}}$$ Equation 7.7

Chemical “loss” via biomass dilution is independent of chemical properties and can be estimated solely as a function of species-weight (Eqn. 7.8). Biomass dilution includes individual growth as well as reproduction and replacement of tissues.

$$k_r = qT \cdot \gamma_{b\_pr} \cdot w^{-\kappa}$$ Equation 7.8

$k_{X,w,ex}$	=	Chemical excretion rate constant	[d^{-1}]
$k_{X,n,ex}$	=	Chemical egestion rate constant	[d^{-1}]
k_r	=	Growth dilution rate	[d^{-1}]
$\rho_{H2O,w}$	=	Water layer resistance from/to water ($2.8 \cdot 10^{-3}$)	[$d \cdot kg^{-\kappa}$]
$\rho_{H2O,n}$	=	Water layer resistance from/to food ($1.1 \cdot 10^{-5}$)	[$d \cdot kg^{-\kappa}$]
ρ_{CH2}	=	Lipid layer resistance ($6.8 \cdot 10^{1}$)	[$d \cdot kg^{-\kappa}$]
q_T	=	Temperature correction coefficient (correction of differences in metabolic flows between cold-blooded species and warm-blooded species) (4.56)	[/]
q_{wc}	=	Warm-blooded to cold-blooded respiration coefficient (6.0)	
q_{ap}	=	Animal to plant respiration coefficient (6.0)	
p_{b_as}	=	Food assimilation efficiency for herbivorous mammals (typical value) (47%)	
γ_{b_pr}	=	Average (re)production rate coefficient ($7.5 \cdot 10^{-4}$)	
γ_{b_re}	=	Average respiration coefficient ($7.5 \cdot 10^{-4}$)	
K_{TW}	=	Tissue-water partition coefficient	
$K_{TW,plant}$	=	Tissue-water partition coefficient for plants	

The tissue-water partition coefficient is estimated according Hendriks et al. (2005) and includes substance affinity for neutral lipids, polar lipids and proteins and solubility in water (Eqn. 7.9). To estimate the K_{TW} for plants, chemical affinity for lignin and carbohydrates are included as well, as these are important plant components (Hendriks et al., 2005). The fractions of different tissue components represent typical values for mammals and plants (Hendriks et al., 2005).

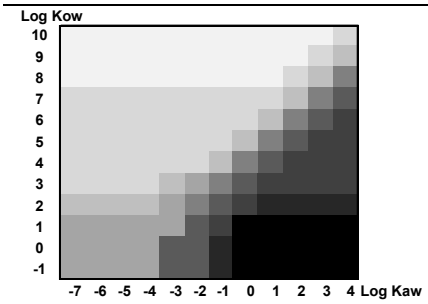
$$K_{TW} = p_{nl,t} \cdot K_{ow} + p_{pl,t} \cdot K_{ow}^{0.94} + p_{p,t} \cdot K_{ow}^{0.63} + p_{H2O,t} \cdot K_{ow}^{0}$$ Equation 7.9

Figure 3 shows the influence of physico-chemical properties on the substance elimination rate constants. The total elimination rate, i.e. the sum of exhalation, excretion, egestion and growth dilution, is a function of the octanol-water partition coefficient (K_{ow}) and the air-water partition coefficient (K_{aw}). The exhalation rate constant depends on both the chemical octanol-water partition coefficient (K_{ow}) and the air-water partition coefficient (K_{aw}). An optimum elimination via exchange with air occurs for substances with a low $\log K_{ow}$ (i.e. $\text{Log}K_{ow}<2$) and a relatively high $\log K_{aw}$ ($\log K_{aw} >0$). The elimination via excretion with urine, egestion with feces and growth dilution is independent of the air-water partition coefficient (Figure A7.3c). There is a substantial contribution of exchange via air to the total elimination rate

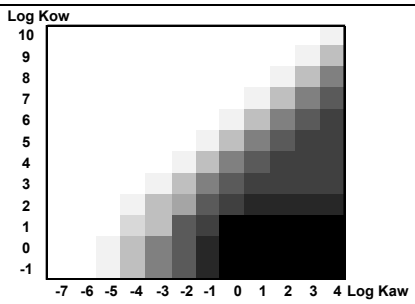
constant for substances with a relatively high K_{aw} ($logK_{aw}$ = -3) (Figure A7.3d). Moreover, figure A7.3d shows that for volatile organic compounds elimination is dominated by exchange with air (>90%). Therefore, other elimination pathways can be ignored when calculating a bioaccumulation factor for exposure to air pollutants. There may, however, be a substantial contribution of metabolization to the total loss rate.

Figure A7.3. Influence of physico-chemical properties, i.e. octanol-water partition coefficient (K_{ow} at 25ºC) and the air-water partition coefficient (K_{aw} at 37ºC) on the different elimination rate constants. Elimination rate constants are calculated for rats (weight = $2.4 \cdot 10^{-1}$ kg)

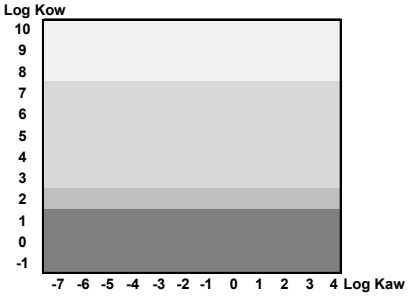
a. Total elimination rate constant (in d^{-1}) (exhalation + excretion + egestion + growth dilution) ($k_{X,a,ex} + k_{X,w,ex} + k_{X,n,ex} + k_g$)



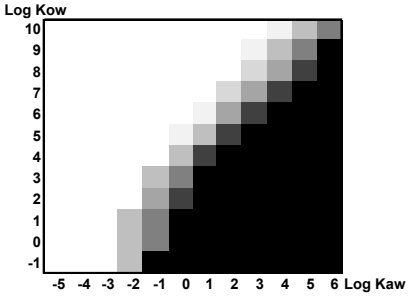
b. Exhalation rate constant ($k_{X,a,ex}$ in d^{-1})



c. Total elimination rate constant excl. exhalation (in d^{-1}) (excretion + egestion + growth dilution) ($k_{X,a,ex} + k_{X,w,ex} + k_{X,n,ex} + k_g$)



d. Percentage of elimination via exhalation compared to total elimination rate (exhalation+egestion+excretion+growth dilution)



Elimination rate constant ($k_{X,ex}$ in d^{-1}): ■ > $1 \cdot 10^{2}$, ■ $5 \cdot 10^{1}$ – $1 \cdot 10^{2}$, ■ $1 \cdot 10^{1}$ - $5 \cdot 10^{1}$, ■ 1 - $1 \cdot 10^{1}$, ■ $5 \cdot 10^{-1}$ – 1, ■ $1 \cdot 10^{-1}$ - $5 \cdot 10^{-1}$, ■ $5 \cdot 10^{-2}$ - $1 \cdot 10^{-1}$, ■ $1 \cdot 10^{-2}$ -$5 \cdot 10^{-2}$, ■ $1 \cdot 10^{-2}$ - $5 \cdot 10^{-3}$, □ <$5 \cdot 10^{-3}$

Fig. 3d. Percentage of elimination via exhalation ($k_{X,a,ex}$ in %): ■ > 90%, ■ 70% - 90%, ■ 50% - 70%, ■ 30% - 50%, ■ 10% - 30%, ■ 5% - 10%, ■ 1% - 5%, □ <1%

Appendix 8

The chemical absorption rate constant for fish is generally estimated according Equation 1.8. The absorption rate constant depends on the resistances encountered in the aqueous and lipid layer, and a flow delay imposed by the ventilation rate. The flow delay imposed by blood perfusion can be included in the equation, analogue to uptake via inhalation, following (Hayton and Barron, 1990):

$$k_{X,w,in} = \frac{1}{\text{Aqueous layer resistance} + \text{Lipid layer resistance} + \text{Flow delay}_{ventilation} + \text{Flow delay}_{perfusion}}$$

The individual resistances and flow delays are estimated according Hendriks et al. (2001) and Veltman et al. (2009) (Chapter 7), for a 1 kg fish:

Aqueous layer resistance [kg·d·dm^3]: $\rho_{H2O,0} \cdot w^{\kappa}$

Lipid layer resistance [kg·d·dm^3]: $\left(\frac{\rho_{CH2}}{K_{ow}}\right) \cdot w^{\kappa}$

Flow delay ventilation [kg·d·dm^3]: $\left(\frac{1}{\gamma_w}\right) \cdot w^{\kappa}$

Flow delay perfusion [kg·d·dm^3]: $\left(\frac{1}{G_B \cdot K_{BW}}\right)$

Description	Parameter	Value	Unit	Reference
Water layer resistance from/to water	$\rho_{H2O,0}$	$2.8 \cdot 10^{-3}$	$d \cdot kg^{-\kappa}$	Hendriks et al., 2001
Lipid layer resistance for influx/efflux of organic substances in animals	ρ_{CH2}	$6.8 \cdot 10^{1}$	$d \cdot kg^{-\kappa}$	Hendriks et al., 2001
Species weight	w	1	kg	
Octanol-water partition coefficient	K_{ow}			
Allometric exponent	κ	0.25		Hendriks et al., 2001
Water absorption-excretion constant	γ_w	200	$kg^{\kappa} \cdot d^{-1}$	Hendriks et al., 2001
Perfusion rate (for a 1 kg fish)	G_B	50	$dm^3 \cdot kg^{-1} \cdot d^{-1}$	Nichols et al., 1990
Blood-water partition coefficient	K_{BW}			Veltman et al. (Ch. 7. Eqn. 7.5)

The blood-water partition coefficient for fish is estimated similar to the blood-air partition coefficient for mammals (Chapter 7, Eqn. 7.5):

$$K_{BW} = p_{nl,bl} \cdot K_{ow} + p_{p,bl} \cdot K_{ow}^{0.63} + p_{H2O,bl} \cdot K_{ow}^{0}$$

K_{BA} = Blood-air partition coefficient
K_{ow} = Octanol-water partition coefficient (25°C)
p_{nl} = Neutral lipid percentage of blood (bl) (0.86%)
p_p = Protein percentage of blood (bl) (13.02%)
p_{H2O} = Water percentage of blood (bl) (86.13%)

The fractions of the blood constituents, i.e. total lipid content and water, are obtained for a lake trout (*Salvelinus namaycush*) from Lien et al. (2001). Lien et al. (2001) did not distinguish between neutral and polar lipids, therefore it is assumed that the total lipid content represents neutral lipids only. Additionally, the remaining fraction, is assumed to represent the blood protein content.
The different resistances and flow delays are plotted against the octanol-water partition coefficient of the chemical in Figure A8.1. As the resistances and flow delays act in series, the uptake rate constant is limited by the largest resistance /

flow delay. It can be concluded from Figure A8.1 that the chemical uptake rate constant across gill epithelia is a function of the aqueous layer resistance and the flow delay imposed by ventilation for chemicals with a $logK_{ow}$ > 5.5. For substances with a $logK_{ow}$ < 5.5 the lipid layer resistance is the rate-limiting factor. This latter statement should, however, be taken with care, as the value of the lipid layer resistance has been obtained through calibration on total uptake rate constants (Hendriks et al., 2001). The flow delay imposed by blood flow may have been included in this "lipid layer resistance". If this is the case, the blood flow delay may be subtracted from the lipid layer resistance to obtain the "true" lipid layer resistance. This shows that the "flow delay blood" is substantially lower than the "true" lipid layer resistance, and thus for chemicals with a $logK_{ow}$ < 5.5 the chemical uptake rate constant is dependent on the lipid layer resistance.

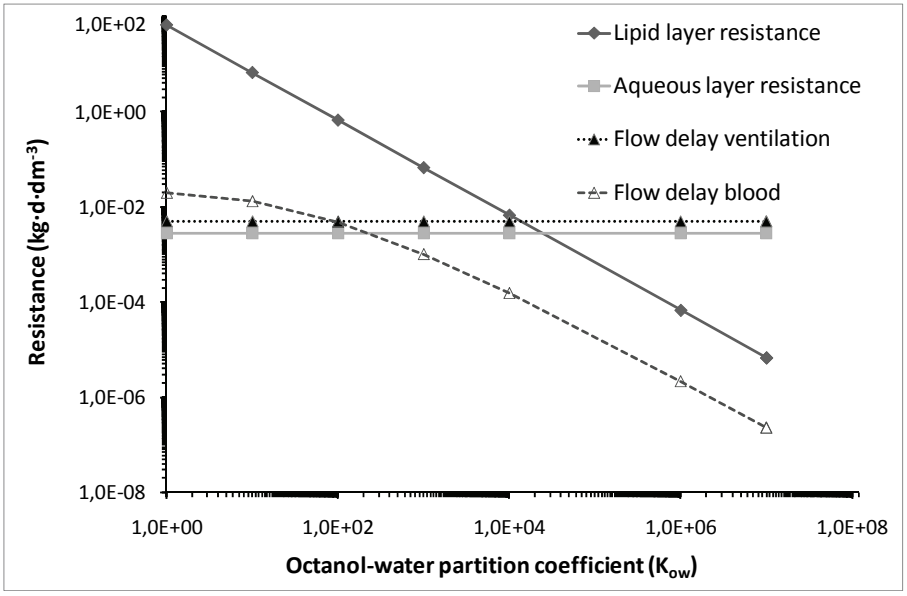


Figure A8.1. Flow delays (blood, ventilation) and resistances (lipid layer, aqueous layer) plotted against the octanol-water partition coefficient (K_{ow}).

Summary

Bioaccumulation has become an important criterion in chemical regulation worldwide. Yet, most of the thousands of substances and species that are of interest for environmental management have not been monitored at relevant locations and periods, because of financial, ethical and technical constraints. Environmental risk assessment therefore requires quantitative tools that can reliably estimate the bioaccumulation potential of various chemicals, including metals and non-organochlorines, for a range of species in different ecosystems. The last decades several mechanistic models have been developed that predict internal chemical concentrations based on chemical properties and species-characteristics. These models have been successfully validated for organochlorine accumulation in aquatic organisms and air-breathing mammals. Similar models for other substances, such as metals, and other ecosystems, as terrestrial environments, are largely absent. Although most models estimate accumulation based on substance properties and species characteristics, key physiological parameters are often obtained by species-specific calibration or parameterization, and these models are often not easily scaled to other species. Chemical risk assessment can benefit from a generic model-concept that is applicable to a wide range of organisms and various chemicals, without increasing model-complexity or input-parameter requirements.

The unique characteristics of the bioaccumulation model OMEGA (Optimal Modeling for Ecotoxicological Applications) are the combination of classical thermodynamic diffusion theory with biological allometry. In other words, default values for uptake and elimination rate constants are estimated as a function of well-known properties of species and chemicals, such as adult mass (w), body composition, trophic level and species type, and the octanol-water partition coefficient (K_{ow}) of the chemical. The model is applicable to both organic chemicals and metals. Metal uptake rate constants from water and food are assumed to be dependent on the exposure concentration and follow saturable uptake kinetics analogous to Michaelis-Menten kinetics for enzymes. The model has been previously validated for PCB and metal accumulation in species of the Rhine–Meuse delta. To test the generic applicability of the model-concept, it should be validated for other ecosystems, such as marine environments, and for different substances, including non-organochlorines. Additionally, a further refinement was necessary for the estimation of metal accumulation kinetics. While uptake and elimination rate constants of organic compounds have for long been linked to their octanol-water partition coefficient, no equivalent relationships existed for metals.

A mechanistic estimation of metal accumulation was therefore not yet possible. To advance science of metal bioaccumulation modeling, mechanistic estimation routines needed to be developed that relate metal uptake and elimination kinetics to a metal specific property. Finally, chemical inhalation and exhalation rate constants were not related to species-based allometric relationships and chemical specific properties. It was therefore considered useful to extend the model concept to exposure via air and develop quantitative relationships for chemical exchange via lung epithelia in mammals. The advantage for bioaccumulation modeling in general is the applicability of these relationships to a wide range of (terrestrial) organisms and various chemicals, without increasing model-complexity or input-parameter requirements. Additionally, these relationships may provide insight in species-specific differences in chemical uptake and elimination rate constants from air. The aim of this thesis was to validate and improve the generic applicability of elementary accumulation concepts, in particular those incorporated in the OMEGA model, to a wide range of species, chemicals and exposure routes.

To this end four model validation studies with independent field accumulation data were carried out. In **Chapter 2** results are described of the model validation for PCB and brominated flame retardant accumulation in various species of estuarine and marine ecosystems. It is concluded that the model can be applied to predict accumulation of PCBs and brominated flame retardants in estuarine and marine organisms, if biotransformation rates are negligible. In particular for brominated diphenyl ethers, substantial deviations between model predictions and field data are observed as a result of species- and congener-specific biotransformation rates.

In **Chapter 3** the model is validated for accumulation of organotins, as triphenyltin and tributyltin, in various estuarine organisms, such as mollusks, polychaeta, crustaceans and fish. Organotins are ionizable compounds that form complexes with ligands in the abiotic and biotic environment. As complexation to biotic ligands is also known for metals, elimination kinetics of organotins are modeled following two approaches: 1) comparable to nonpolar organic compounds and 2) comparable to metals. The results indicate that uptake of organotins mainly occurs via hydrophobic passive diffusion mechanisms, while elimination is more comparable to elimination kinetics of metals. In particular, elimination rate constants for triphenyltin are reasonably well predicted following metal elimination kinetics. Elimination of tributyltin is, however, substantially underestimated, probably due to biotransformation. It is concluded that the model can not be directly applied to predict accumulation of organotins. However, this research does illustrate how a mechanistic bioaccumulation model can be used to provide a more mechanistic understanding of field bioaccumulation data.

In **Chapter 4** and **5** it is shown that the model accurately predicts accumulation of cadmium in various terrestrial species, including earthworms, voles and shrews. Cadmium accumulation in earthworms is less than linearly related to exposure concentrations, which is correctly predicted by the model assuming saturable uptake kinetics. Additionally, these studies suggest that Cd accumulation may be predicted for other species as well, if elimination kinetics are similar to earthworms, voles and shrews, i.e. binding to metallothionein is the main detoxification mechanism and elimination occurs via "growth dilution" only.

These model validation studies also show some current limitations of the model. At present, accumulation of nonpolar organic compounds can not be accurately predicted if biotransformation rates are substantially contributing to the total elimination rate. Several improvements in estimating metal accumulation kinetics are necessary. Firstly, regulation of uptake and/or elimination rate constants should be included in the model concept to provide a more accurate description of accumulation kinetics of essential metals, such as Cu and Zn. Secondly, refinement of metal exchange via ingestion and egestion of food is necessary.

In **Chapter 6** and **7** two model development studies are described. It is shown that metal uptake via the gills in aquatic organisms and organic chemical exchange via the lungs in mammals, can be predicted analogous to the model-concept for organic chemical exchange via the gills in aquatic organisms. Hence, accumulation kinetics are related to a minimum number of well-known chemical properties and species characteristics. Metal uptake kinetics in aquatic organisms are successfully related to the metal-specific covalent index and the gill ventilation rate. Elimination kinetics show no metal-specific behavior and a single, metal-generic elimination rate constant is derived. This single, weight-corrected elimination rate constant probably does not completely reflect complex-metal elimination kinetics, as metal elimination represents an integral of loss from various different "storage" compartments. Yet, in absence of empirical data, the weight-corrected elimination rate constant is likely the best estimate of elimination.

In **Chapter 7**, accumulation kinetics of nonpolar, organic chemicals via air exchange in various mammals, are succesfully predicted based on two chemical properties, the octanol-water partition coefficient (K_{ow}) and the air-water partition coefficient (K_{aw}), and few species characteristics, as adult mass and body composition (lipid fraction, protein fraction, water fraction). The results show that 76% of the modeled inhalation and exhalation rate constants are within a factor of 2 from independent empirical values for humans, rats and mice. And 87% of the predicted blood-air partition coefficients are within a factor of 5 from empirical data. At

present, this model is, however, limited by the availability of measured biotransformation rates.

In **Chapter 8** the results of these studies are discussed and recommendations for further research are provided. Overall, it is concluded that the model can be used to predict accumulation of persistent nonpolar organic chemicals, including non-organochlorines and volatile organic chemicals, in species inhabiting terrestrial and aquatic (incl. marine) environments. The model is currently limited by the availability of biotransformation rates for a range of chemicals and species.

The model can also be applied to predict cadmium accumulation in earthworms and shrews, and possibly other terrestrial species, provided that the metal detoxification mechanisms are comparable to those of earthworms and shrews.

For metals, the model-concept is successfully extended to predict metal uptake via the gills in aquatic organisms. Further refinement is, however, necessary to improve model predictions of metal elimination, metal uptake via the diet, and accumulation of essential metals, such as Cu and Zn.

Samenvatting (Nederlands)

Achtergrond en doelstelling

Door de economische groei is de industriële productie, de consumptie en wereldwijde handel van goederen, enorm toegenomen na de Tweede Wereldoorlog. Dit heeft geleid tot de introductie van duizenden synthetische chemische stoffen in het milieu. De meeste van deze synthetische stoffen zijn essentieel voor het behouden van onze huidige levensstandaard en nuttig voor het effectief bestrijden van plagen. Echter, sommige stoffen bleken onverwachte, negatieve effecten op de gezondheid van de mens en het milieu te hebben. De bewustwording van mogelijke negatieve effecten van synthetische stoffen, als DDT (dichloro-difenyl-trichloroethane) en PCBs (polychloorbifenylen), begon in de jaren ’60 met het verschijnen van het boek Silent Spring van Rachel Carson. In de jaren 70 stapelde het bewijs zich op dat persistente (slecht-afbreekbare) gechloreerde koolwaterstoffen, als PCBs en DDT, veel voorkomende milieuverontreinigingen zijn. Wereldwijde monitoringsprogramma’s hebben laten zien dat PCBs en DDT voorkomen in zowel aquatische als terrestrische organismen, als algen, mossels, vissen, zeehonden en walvissen, en regenwormen, kleine zoogdieren en vogels. Ook zijn deze stoffen aangetoond in Arctische soorten als ijsberen. Dit illustreert dat persistente organische microverontreinigingen over lange afstanden getransporteerd kunnen worden en terecht komen in ecosystemen waar geen emissie-bronnen aanwezig zijn. De monitoringsprogramma’s laten eveneens zien dat de concentraties van stoffen in organismen vaak vele malen groter zijn dan de concentraties in het omringende abiotische milieu. Met andere woorden deze stoffen kunnen accumuleren in planten en dieren (bioaccumulatie). Bovendien kunnen deze stoffen biomagnificeren, wat betekent dat chemische concentraties toenemen in een voedselketen. Dit resulteert in zeer hoge concentraties van deze stoffen in top-predatoren en kan mogelijk negatieve effecten hebben op het voortbestaan van de populatie. Een notoir voorbeeld is het transport van PCBs in de water – zooplankton – vis – vogel voedsel keten wat zeer waarschijnlijk heeft geleid tot de drastische, wereldwijde reductie van verschillende vis-etende vogel populaties in de jaren 50 en 60 van de vorige eeuw. Vanaf de jaren ’70 is de productie en het gebruik van verschillende organische pesticiden, als DDT, verboden in Noord-Amerika, West-Europa en Japan, en het gebruik van PCBs is beperkt. Deze beleidsmaatregelen hebben geleid tot een verbetering van de water- en sediment-kwaliteit. Lessen uit het verleden hebben ons geleerd dat slechts geringe

concentraties van chemische stoffen in het abiotische milieu kunnen resulteren in concentraties in dieren en planten, die hoog genoeg zijn om tot negatieve effecten te leiden. Bovendien worden de stoffen die verboden zijn vervangen door andere stoffen met vergelijkbare eigenschappen. Voor een adequate risicobeoordeling van chemische stoffen is het daarom van belang om de mate van bioaccumulatie van een stof te bepalen.
Bioaccumulatie is tegenwoordig een belangrijk criterium in de regulatie van chemische stoffen wereldwijd. In de nieuwe Europese wetgeving voor chemische stoffen wordt bioaccumulatie, tesamen met persistentie en toxiciteit, als criterium gebruikt voor het prioriteren van stoffen voor beleidsmaatregelen. Ook wordt bioaccumulatie gebruikt voor het afleiden van milieukwaliteitsnormen voor water, sediment en bodem. De risicobeoordeling van chemische stoffen wordt echter sterk belemmerd door het gebrek aan relevante data. Gemeten bioaccumulatie–data, in lab of veld studies, zijn schaars in vergelijking tot de hoeveelheid commercieel beschikbare stoffen. Bovendien zijn de meeste empirische studies gericht op de ongeveer 100 bekende persistente organische microverontreinigingen. Echter, verschillende niet-organische stoffen zijn ook geclassificeerd als mogelijk schadelijk vanwege hun bioaccumulatie capaciteit. Deze lijst omvat organometalen als tributyltin en methyl-kwik, maar ook verscheidene zware metalen. Ook worden er continu nieuwe stoffen geproduceerd en uitgestoten in het milieu. Recentelijk is er bezorgdheid onstaan over de wereldwijde verspreiding en bioaccumulatie van nieuwe persistente organische microverontreinigingen als broomdiphenyl ethers (gebromeerde vlamvertragers) en perfluoro-octaansulfonaat. Alhoewel bioaccumulatie van stoffen relatief grondig is onderzocht in verschillende vissoorten, zijn metingen voor andere soorten, als terrestrische zoogdieren, nauwelijks aanwezig. Het merendeel van de duizenden stoffen en soorten die van belang zijn voor chemische risicobeoordeling zal niet gemeten kunnen worden op alle locaties en in alle perioden, vanwege praktische, financiele en ethische beperkingen. Het is daarom van belang om betrouwbare kwantitatieve methoden te ontwikkelen om de bioaccumulatie van stoffen te schatten. Deze modellen moeten bij voorkeur generiek toepasbaar zijn voor verschillende stoffen en meerdere soorten, dat wil zeggen dat bioaccumulatie moet worden geschat op basis van een aantal belangrijke stof- en soort-eigenschappen.

Bioaccumulatie treedt op wanneer de snelheid waarmee een organisme een chemische stof uitscheidt lager is dan de totale opname snelheid, als gevolg van “opslag” in bepaalde lichaamscompartimenten en een gebrek aan efficiente

afbraak mechanismen. Bioaccumulatie is een dynamisch proces en is het resultaat van opname van een chemische stof via meerdere blootstellingsroutes en afgifte via verschillende mechanismen. Over het algemeen bestaan er drie mogelijke opname routes: 1) drinken en / of ventileren van water, 2) eten van voedsel en 3) inhalatie van lucht. Er kunnen zes verschillende afgifte routes worden onderscheiden: 1) excretie met urine en afgifte via de kieuwen, 2) egestie met fecaliën, 3) exhalatie met lucht, 4) biotransformatie (afbraak van de stof in het lichaam), 5) productie (groei van het organisme) en reproductie (geboorte, leggen van eieren, lactatie), 6) afsterven van weefsel (huid, veren, cellen etc.). De mate waarin een stof kan ophopen in een organisme is afhankelijk van verschillende stof- en soort-specifieke eigenschappen. Een belangrijke stof-eigenschap is de octanol-water partitie coefficient (K_{ow}) voor organische stoffen. De K_{ow} is een parameter voor de hydrofobiciteit ("watervrezenheid") van de stof. Stoffen met een hoge K_{ow} proberen uit de water-fase te ontsnappen en hebben een sterke affiniteit voor lichaamsvet. Een stof met een hoge K_{ow} heeft daarom een hoge bioaccumulatie capaciteit. Tot nu toe was er geen metaal specifieke eigenschap bekend die de bioaccumulatie van metalen in dieren en planten uitdrukt.

Belangrijke soort-specifieke eigenschappen zijn: het (volwassen) gewicht, het type organisme (koud-bloedig, warmbloedig, ademhalend via longen of kieuwen), de positie in de voedselketen (trofisch niveau) en de lichaamsopbouw (vet-fractie, eiwit-fractie en water-fractie). Het gewicht van een organisme bepaald grotendeels het basale metabolisme. De benodigde hoeveelheid eten en drinken per dag, en de benodigde hoeveelheid zuurstof per dag zijn gerelateerd aan het gewicht van de soort volgens allometrische relaties. Over het algemeen geldt dat de voedsel-, water-, en zuurstof- inname snelheid per kilogram lichaamsgewicht afneemt van lichte naar zwaardere soorten.

Organismen kunnen worden verdeeld in verschillende typen: warm-bloedig of koud-bloedig en ademhalend via de longen of via de kieuwen. Warm-bloedige dieren hebben extra energie nodig voor het constant houden van hun lichaamstemperatuur en eten daardoor meer dan koud-bloedige dieren van hetzelfde gewicht. Tevens bezitten warm-bloedige dieren vaak een extra vetlaag ter isolate voor het constant houden van hun lichaamstemperatuur. Hierdoor is de accumulatie van chemische stoffen vaak hoger in warmbloedigen dan in koudbloedigen van hetzelfde gewicht. Organismen kunnen verder worden gekarakteriseerd door de wijze waarop ze ademhalen, via de longen of via de kieuwen. Het respiratie mechanisme heeft ondermeer gevolgen voor de eliminatiesnelheid van chemische stoffen. Over het algemeen worden stoffen

makkelijker verwijderd via de kieuwen dan via de longen. Dit heeft tot gevolg dat stoffen tot hogere concentraties ophopen in zoogdieren dan in vissen.
De positie in de voedselketen, het trofisch niveau, bepaald de mate van blootstelling aan chemische stoffen in voedsel. Aangezien concentraties van persistente apolaire organische stoffen toenemen in de voedselketen krijgen carnivoren een hogere dosis chemische stoffen binnen via eten dan herbivoren. De lichaamsopbouw is een belangrijke parameter voor de bioaccumulatie van stoffen. Apolaire organische verbindingen hebben een hoge affiniteit voor vet, en des te vetter het organisme des te hoger de bioaccumulatie van chemische stoffen. Metalen bevinden zich voornamelijk in eiwitten of minerale delen (botten, pantser, schelpen etc.) van het lichaam. Er is echter geen één op één relatie gedefinieerd voor de accumulatie van metalen en bepaalde lichaams fracties. Andere belangrijke soort-eigenschappen zijn de soort-specifieke biotransformatie capaciteit voor organische stoffen en de soort-specifieke ontgiftigingsmechanismen voor metalen.

Op het moment bestaan er verschillende bioaccumulatie modellen voor apolaire organische stoffen. Een veel gebruikt concept is het één-compartiments model. Hierin wordt het organisme voorgesteld als bestaande uit één compartiment, dat is opgedeeld in verschillende homogene fracties, zoals vet, water en eiwit. Alle opname- en eliminatie snelheidsconstanten voor dit compartiment worden beschreven op basis van eerste orde kinetiek. Dat wil zeggen dat deze snelheidsconstanten onafhankelijk van de blootstellingsconcentratie zijn. Opname- en eliminatie snelheden worden geschat op basis van stof-specifieke eigenschappen als de octanol-water partitie coefficient (K_{ow}) en soort-specifieke eigenschappen als de vetfractie, de ventilatie snelheid (voor vissen) en de voedsel opname snelheid.. Deze modellen zijn succesvol in het schatten van accumulatie van gechloreerde organische stoffen, als PCBs, in voornamelijk aquatische organismen. Soortgelijke modellen zijn echter nauwelijks beschikbaar voor andere ecosystemen, als de terrestrische omgeving, en andere stoffen, als niet-gechloreerde organische stoffen en metalen. De meeste van deze modellen schatten accumulatie weliswaar op basis van stof-eigenschappen en soort-eigenschappen, maar de fysiologische parameters worden vaak bepaald door middel van soort-specifieke calibratie of parameterisatie. Daardoor kunnen deze modellen niet direct worden toegepast op andere soorten.
De unieke eigenschappen van het bioaccumulatie model OMEGA (“Optimal Modeling for Ecotoxicological Applications”) zijn de combinatie van de klassieke, stof-gerelateerde thermodynamische diffusie theorie met soort-gerelateerde allometrische relaties. Dit wil zeggen dat default waarden voor chemische

concentraties in een organisme worden geschat op basis van een minimum aan bekende stof- en soort-eigenschappen. Fysisch-chemische parameters zijn de octanol-water partitie coefficient (K_{ow}) voor organische contaminanten en een algemene weefsel-water partitie coefficient voor metalen. Belangrijke soort eigenschappen zijn het gewicht, lichaamsopbouw (vet-fractie, eiwit-fractie en water-fractie) en trofisch niveau. Het voordeel van dit model-concept is dat het generiek toepasbaar is voor verschillende soorten en meerdere stoffen. Biotransformatie kan op het moment niet worden geschat op basis van stof- en soort-specifieke eigenschappen, maar dit proces kan makkelijk worden toegevoegd aan het model als gemeten afbraak snelheden beschikbaar zijn.
OMEGA is gevalideerd met veld bioaccumulatie data van PCBs in verschillende organismen voorkomend in de Rijn-Maas delta. Het is van belang om het model verder te valideren met veld data voor andere ecosystemen (zoals mariene milieus), voor andere stoffen (vnl. niet gechloreerde organische stoffen en metalen) en andere soorten (zoals zoutwater organismen). Ook zijn er twee onderdelen geïdentificeerd waarvoor verdere model-verfijning en -ontwikkeling nodig is. Ten eerste, wordt in de oorspronkelijke versie van OMEGA de accumulatie van metalen geschat met behulp van een algemene weefsel-water partitie coefficient in plaats van een metaal-specifieke eigenschap. Daarnaast zijn er nog geen model vergelijkingen gespecificeerd voor uitwisseling van chemische stoffen via de longen. Het doel van dit proefschrift is om de generieke toepasbaarheid van het OMEGA-concept te valideren door vergelijking van model resulaten en meetgegevens en waar nodig te verbeteren, voor verschillende soorten, stoffen en blootstellingsroutes.

Model validatie

In de hoofdstukken 2 tot en met 5 zijn vier verschillende model validatie studies beschreven. In deze studies zijn door het model voorspelde concentraties van stoffen in organismen vergeleken met gemeten interne concentraties. Uit de resultaten gepresenteerd in hoofdstuk 2 blijkt dat het model de concentraties van PCBs en broomdifenyl ethers in verschillende zoutwater organismen, als mossels, garnalen en vissen, nauwkeurig voorspelt, mits biotransformatie geen belangrijke rol speelt. In het bijzonder voor broomdifenyl ethers worden relatief grote afwijkingen tussen model voorspellingen en veld data waargenomen, ten gevolge van soort- en stof-specifieke biotransformatie processen.
In hoofdstuk 3 worden de resultaten gepresenteerd van de modelvalidatie voor organotin accumulatie in zoutwater organismen van de Westerschelde. Organotins zijn ionizeerbare stoffen die complexen kunnen vormen met verschillende (negatief)

geladen liganden in het abiotische en biotische milieu. De speciatie van organotins heeft een belangrijke invloed op het lot van deze stof in het abiotische milieu en op de accumulatie kinetiek in biota. Ook voor metalen is bekend dat ze complexen vormen met biotische liganden, zoals eiwitten, wat resulteert in relatief lage eliminatie snelheden. Daarom zijn er twee model benaderingen gevolgd in de schatting van eliminatie snelheden voor organotins. Ten eerste is eliminatie gemodelleerd zoals voor organische stoffen en ten tweede is de eliminatie gemodelleerd zoals voor metalen. Model schattingen volgens deze twee methoden zijn vergeleken met gemeten eliminatie snelheden uit de literatuur. De resultaten laten zien dat organotins, als tributyltin en triphenyltin, voornamelijk worden opgenomen via passieve diffusie vergelijkbaar met opname mechanismen voor apolaire organische stoffen. Echter, de octanol-water partitie-coefficient blijkt een minder goede indicator voor deze opname te zijn. Eliminatie snelheden zijn daarentegen beter vergelijkbaar met model schattingen voor metaal-eliminatie. Geschatte eliminatie snelheden zijn goed voorspelbaar voor triphenyltin. Eliminatie wordt echter substantieel onderschat voor tributyltin, waarschijnlijk door een belangrijke bijdrage van biotransformatie aan de totale eliminatie. Deze studie suggereert dat verdere verfijning noodzakelijk is voor de beschrijving van opname en eliminatie kinetiek van organometaal verbindingen. Het laat echter ook zien hoe een mechanistisch model kan worden gebruikt om veld accumulatie gegevens beter te begrijpen.

In hoofstuk 4 en 5 worden model validatie studies beschreven voor metaal accumulatie in terrestrische organismen, zoals de regenworm, de rosse woelmuis en de spitsmuis. Deze studies laten zien dat OMEGA accumulatie van cadmium in regenwormen en muizen goed voorspelt. De velddata laten duidelijk zien dat cadmium concentraties in regenwormen afnemen met toenemende blootstellingsconcentraties. Deze trend wordt correct voorspeld door het model met de aanname dat metaal opname constanten afhankelijk zijn van de blootstellings concentratie en verzadigings kinetiek volgen. Ook suggereren de resultaten dat cadmium concentraties in andere organismen voorspelbaar zijn, mits de eliminatie kinetiek gelijk is aan die van regenwormen en muizen, dat wil zeggen dat eliminatie voornamelijk plaatsvindt door “groei verdunning”.

De vier model validatie studies laten ook een aantal huidige beperkingen van het OMEGA model zien. Voor apolaire organische stoffen, is het gebrek aan biotransformatie snelheden voor een groot aantal stoffen en meerdere soorten een beperkende factor in de toepasbaarheid van het model voor afbreekbare verbindingen. Voor metalen zijn er verschillende beperkingen aan te wijzen: ten eerste is het voor een meer accurate voorspelling van accumulatie van essentiële

metalen nodig om regulatie van opname- en eliminatie kinetiek toe te voegen aan het model. Ten tweede, is er verfijning nodig voor de model-beschrijving van metaal opname via voedsel. Verschillende studies hebben laten zien dat de metaal opname efficiëntie vanuit voedsel gerelateerd is aan de metaal-verdeling over verschillende compartimenten in het voedsel. Op het moment bestaan er echter geen kwantitatieve relaties die deze interne metaal verdeling in voedsel relateren aan de metaal assimilatie efficiëntie.

Model ontwikkeling

In hoofdstuk 6 en 7 worden twee nieuwe model ontwikkelingen in OMEGA beschreven. In deze hoofdstukken is aangetoond dat zowel metaal opname via de kieuwen in aquatische soorten, als accumulatie van stoffen via inademen en uitademen van lucht, kan worden voorspeld analoog aan het model-concept voor accumulatie van organische stoffen via de kieuwen in aquatische organismen. Dat wil zeggen dat accumulatie kinetiek gerelateerd is aan een minimum aantal bekende chemische eigenschappen en soort karakteristieken. Hoofdstuk 6 laat zien dat metaal opname snelheidsconstanten voor aquatische organismen kunnen worden geschat op basis van een metaal-specifieke parameter, de covalent index, en een soort-specifieke eigenschap, de ventilatie snelheid. Eliminatie snelheids constanten laten echter geen metaal-specifiek gedrag zien. De afgeleide, algemene metaal eliminatie snelheidsconstante is een duidelijke versimpeling van de complexe metaal-eliminatie kinetiek, aangezien verwijdering van metalen uit het lichaam het totale resultaat is van eliminatie van verschillende compartimenten. Echter, wanneer er geen gemeten eliminatie constanten bekend zijn, dan is deze algemene eliminatie snelheidsconstante wellicht de beste schatting die momenteel beschikbaar is.

In hoofdstuk 7 is de accumulatie kinetiek van apolaire organische stoffen via inademen en uitademen van lucht, succesvol geschat op basis van twee stof-eigenschappen, de octanol-water partitie coefficient (K_{ow}) en de lucht-water partitie coefficient (K_{aw}), en een aantal soort-eigenschappen, zoals gewicht, lichaamscompositie (vetfractie, eiwit fractie en water fractie). De resultaten laten zijn dat 76% van de gemodeleerde inhalatie en exhalatie snelheidsconstanten minder dan een factor 2 afwijken van empirsche waarden voor mensen, ratten en muizen. Tevens ligt 87% van de geschatte bloed-lucht partitie coefficienten binnen een factor vijf van gemeten data. Op het moment is het beperkt beschikbaar zijn van gemeten biotransformatie snelheidsconstanten een limiterende factor voor dit model.

In Hoofdstuk 8 zijn de resultaten van dit proefschrift bediscussieerd en er zijn aanbevelingen voor verder onderzoek gedaan. De algehele conclusie is dat de combinatie van soort-gebaseerde allometrische relaties met stof-gebaseerde thermodynamische diffusie theorie, zoals toegepast in OMEGA, kan worden gebruikt voor het schatten van accumulatie van persistente, apolaire organische stoffen, inclusief niet-gechloreerde organische stoffen en vluchtige organische stoffen, in zowel terrestrische als aquatische soorten. Een belangrijke limiterende factor voor het model is het beperkt beschikbaar zijn van gemeten of geschatte biotransformatie snelheidsconstanten. Het model-concept kan eveneens worden toegepast voor het schatten van cadmium accumulatie in regenwormen en muizen. De gebruikte methode kan worden geextrapoleerd naar andere terrestrische soorten, mits deze soorten een vergelijkbaar metaal-detoxificatie mechanisme hebben als regenwormen en muizen. Het model-concept blijkt ook succesvol in het schatten van de opname van metalen via de kieuwen in aquatische soorten. Verdere verfijning is echter nodig om de modelschattingen voor metaal eliminatie, metaal opname via voedsel, en accumulatie van essentiele metals, zoals koper en zink, beter te kunnen schatten.

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Curriculum Vitae

Karin Veltman was born on the 23rd of April 1978 in Delfzijl, The Netherlands. In 1996 she finished high school at the Ommelander College in Appingedam. After travelling one year in Australia and south-east Asia, she started studying chemistry at the University of Groningen (RuG) in 1997. In 2003 she graduated in chemistry with a specialization in environmental sciences. Her two master projects were on assessing the indoor exposure of humans to volatile organic chemicals and quantifying the average human exposure to multiple chemicals using a multimedia fate model. In July 2004 she started working as a junior researcher at the Department of Environmental Science of the Radboud University (RU) in Nijmegen. At first, she worked on a 6 month project, financed by RIKZ (Rijkswaterstaat), focussing on validation of a bioaccumulation model with field data from the Western Scheldt estuary. After this project, her position as a junior researcher at the Radboud University was extended two times more, and finally became a full PhD research on bioaccumulation modeling. The results of this PhD research have been published in 7 peer-reviewed journals. At the end of her PhD she made a 3 month research visit to Lawrence Berkeley National Laboratory (LBNL), Berkeley, California. During this stay, she focused on the development of mechanistic estimation routines for bioaccumulation of air pollutants. As of January 2008 she works as a post-doc at the Industrial Ecology Programme, Norwegian University of Science and Technology (NTNU), in Trondheim, Norway.

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