

Apprehending the Scientific Gaps Hampering Adequate  
Assessment of the Environmental Fate, Bioavailability and  
Resulting Risk of Ionizable Organic Compounds

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# 1. Introduction

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The majority of both naturally occurring and man-made chemicals eventually ends up in the environment. The average human being consumes and uses a plethora of different chemicals – e.g. food additives, colorants, detergents, preservatives, pharmaceuticals, and perfumes – most of these even on a daily basis. It is inevitable that these chemicals end up in the environment, which can be illustrated with a very simple mass-balance example. Oversimplified, it could be stated that the total intake of substances, minus any weight gains, is equal to the amount of substances released into the environment, either by exhalation or excretion in urine, feces, sweat and other secretions. Some of these excreted substances will have undergone some extent of modification through metabolism, but the above is an appropriate and convenient example of the considerable strain everyday life puts on the environment.

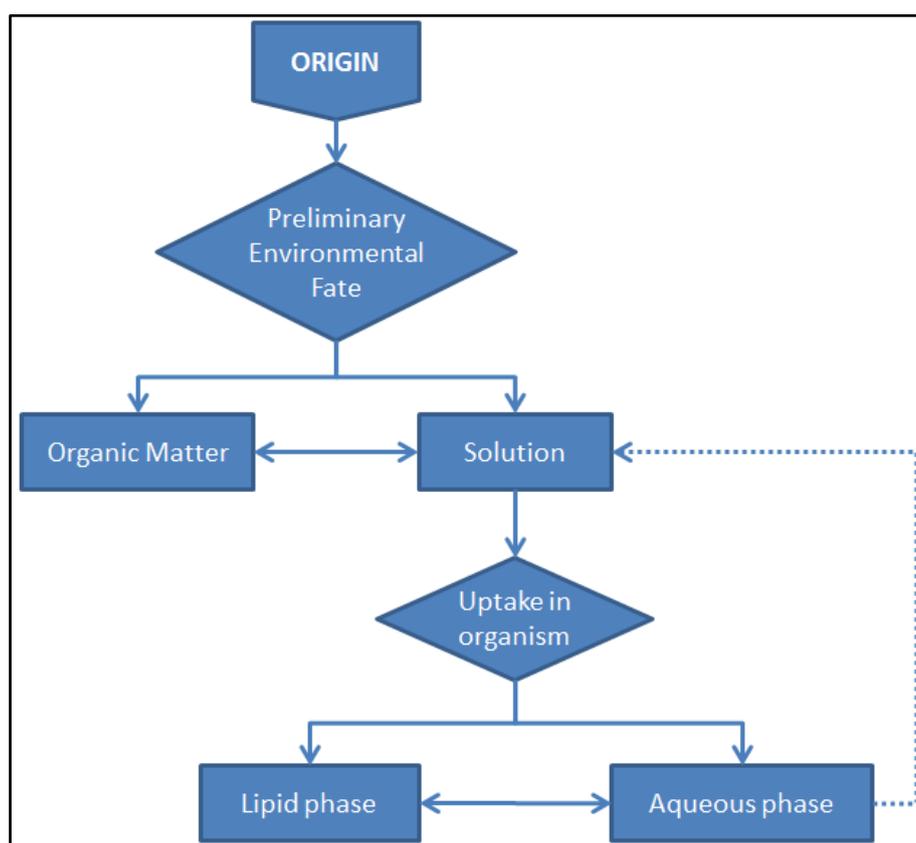
This strain of everyday life on the environment is further catalyzed by the ever increasing world population and industrialization of developing countries. Scientific progress has led to the ongoing discovery and synthesis of new chemicals; the CAS registry of the American Chemical Society has more than 67.5 million compounds listed, with multiple compounds added every minute. Because of the vast amount of new chemical entities – and numerous possible metabolites and breakdown products for each of them – it becomes increasingly difficult to predict and prevent possible negative environmental consequences. Although there are international regulations that make an effort towards adequate risk assessment, these regulations remain focused on only the most straightforward piece of the puzzle: neutral compounds.

Even though neutral compounds are ubiquitous and it would be unwise to ignore or downplay their significance, it could and should be argued that ionizable compounds are equally important with respect to environmental risk assessment. In this thesis, the current EU risk assessment procedure will first be described briefly, along with some critique on the assumptions made by regulators when dealing with ionizable pollutants. Thereafter, different sources of environmental contamination will be listed along with a multitude of possible compounds emanating from these sources, emphasizing categories containing cationic organic compounds. Furthermore, recommendations to update the existing risk assessment procedure will be presented.

These recommendations for a better suited risk assessment approach will focus on two aspects, here deemed *environmental bioavailability* (EBA) and *pharmacological bioavailability* (PBA). EBA deals with all processes influencing the accessibility of a given compound in the environment; EBA, in a sense, is what shapes the external exposure. TBA is one step further into the organism, dealing with the internal dose and therefore more focused on classical pharmacology, comprising biological systems and chemical properties that determine the fate of a molecule once an organism is exposed internally. Reading this thesis, it will become clear that both EBA and TBA depend on very different factors, when comparing neutral compounds with anionic and cationic species. These findings will be briefly revisited in the conclusion, along with a concise overview of the different aspects mentioned to refine risk assessment.

## 2. Current Risk Assessment Procedure

To evaluate the risk a chemical entity might pose upon release into the environment, the first step is to determine the environmental fate of this substance. The flowchart in figure X.1 gives an overview of how environmental risk assessment is currently organized. Please note that this flowchart is simplified, as is the underlying theoretical background used in environmental risk assessment. Although simplification is necessary to avoid overtly complicated models – which can hamper adequate performance – each degree of simplification is inseparably linked to the introduction of additional experimental error.



**Figure 2.1:** Flowchart showing the processes that influence the environmental fate of any substance released into the environment.

Figure 2.1 shows how risk assessment can be split up in two different determinants, which are the *environmental fate* and the actual *uptake into an organism*. After release into the environment at the point of origin, the preliminary environmental fate (PEF) determines what happens. Currently, regulation considers neutral compounds as being able to remain in solution as well as to sorb to organic matter. Predictions for PEF are based almost entirely upon octanol-water partitioning and the resulting  $K_{O/W}$  partition coefficient. Since scientific evidence supports the notion that the affinity for organic matter in soil is related to the affinity for octanol, models used in risk assessment usually calculate  $K_{OC}$  – the organic carbon partition coefficient – from  $K_{O/W}$  (IHCP). The standard error for such models is usually between 0.3 and 0.6 log units (Sabljic A.).

The top half of figure 2.1 indicates that, after release into the environment, there is partitioning between organic matter and the aqueous phase. Ongoing exchange between these phases means

that organic matter with sorbed pollutants can function as a long term reservoir, releasing additional pollutant in the aqueous phase if the dissolved concentration decreases. At present, risk assessment is based on the presumption that uptake into an organism is only possible for the fraction dissolved in the aqueous phase. However, it should be noted that soil particles are usually covered with an unstirred water layer, in which the concentration can be significantly higher than in the bulk aqueous phase, leading to a much higher exposure for soil dwelling organisms than expectations based upon the bulk concentration.

From the flowchart, it becomes apparent that exposure is linked to the concentration in solution, which is estimated based on  $K_{O/W}$ . Uptake, in turn, is bifurcated between fat and fluids, defined in figure 2.1 as *lipid phase* and *aqueous phase*. Since risk assessment regulation is dedicated to neutral molecules, partitioning between these two compartments is also modeled using the established relationship between  $K_{O/W}$  and  $IC_{50}$  or  $LC_{50}$  – the (nominal) concentration at which half of the exposed population is lost. These types of models are available for long-term prototype organisms such as the water flea and fathead minnow (Verhaar H.J.M). The standard error for these models is typically between 0.3 and 0.4 log units (Verhaar H.J.M).

So, in conclusion, risk assessment is based largely on  $K_{O/W}$  and chemical properties that can be derived from it. Lipophilicity governs the amount of substance partitioning into organic matter, while it also influences compartmentalization after exposure of an organism – and current risk assessment legislation relies heavily on these factors to determine environmental risk and toxicity. Finally, it should be noted that aqueous organisms are presumed to reach equilibrium with their surroundings, which translates into repartitioning from the aqueous phase back into solution (dotted line in figure 2.1). In the following sections, it will become clear that ionizable compounds should have a separate place in risk assessment, as it will be shown that assumptions based on only neutral species and  $K_{O/W}$  create a skewed picture.

## 3. Point of Origin

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Toxicologically relevant substances can enter the environment through different routes. Important sources are mostly anthropogenic, but some compounds also have significant natural sources (Albelda, Frias et al.) – such as cadmium (Zhou, Wang et al.), and surfactants (Wegner and Hamburger). Mycotoxins produced by fungi form another group of toxic chemicals introduced into the environment through natural causes; notable examples include aflatoxins and ergot alkaloids (Schenzel, Goss et al.). This thesis, however, will primarily focus on pollution from anthropogenic sources, as natural sources are not normally addressed in the field of environmental risk assessment. The point of origin is especially significant for ionizable compounds, as chapter 4 will show that a lot of ionizable compounds are discharged through the sewers, leading to widespread environmental contamination.

The four prime points of origin of chemicals in the environment are sewers, industry, landfills, and accidents. Sewage and landfills mainly contain potential pollutants consumed or produced by typical citizens, such as detergents and pharmaceuticals {brownawell 1; lapworth 1}, and heavy metals {albelda 1; zhou 1; US 1}. Pollution from industry is a different chapter entirely, as the concentration and properties of compounds can differ considerably. Noteworthy chemicals released from industrial sources include herbicides (Albelda, Frias et al.), detergents {1-3 from brownawell 1}, dyes {1-3 from brownawell 1}, and building blocks for chemical synthesis, such as aniline (Li, Lee et al.). Accidents can release an amalgam of environmental toxicants, depending largely on the nature of the accident. An accidental spill will usually release a very high concentration of a limited number of chemicals, while a large fire will release a plethora of compounds into the atmosphere – like PAHs – while runoff from extinguishing efforts can reach the aquatic environment.

### 3.1 Sewage

Pollutants from sewage can reach the environment through a number of pathways. The most direct pathway is residual contamination of effluent, where after sewage treatment fractions remain of substances that are – at present – technically or financially impossible to be fully removed. Numerous studies dealing with trace level detection have been performed (Lapworth, Baran et al.); table 3.1 gives a confined overview of the diversity of detected compounds and their average levels. Additionally, table 3.1 serves as an example of the broad range of chemicals that are released into sewage.

Sewage sludge forms a second source of environmental contamination that can be traced back to the sewers. Sewage sludge is the semi-solid material remaining after modern industrial wastewater treatment processing. Due to its high nutritious value, sewage sludge is widely used as a fertilizer, a practice where liquid or dried sludge is applied to agricultural soil. This procedure is virtually a direct injection of contaminants into soil, since there is ample evidence that pollution remains in sewage sludge. An extensive analysis carried out by the US Environmental Protection Agency (EPA) found significant amounts of heavy metals and bioactive compounds, such as barium, manganese, pyrene, fluoranthene, brominated flame retardants (BFRs), pharmaceuticals (summed mean concentration  $\geq 82$  mg/kg), and steroid hormones {US 1}.

The third pathway is technically a combination of the two sources mentioned previously. This pathway originates from the use of septic tanks, which are sometimes used quite extensively in rural areas and developing countries. Septic tanks can malfunction in different ways, potentially leading to leaching of pollutants or leaking of contaminated fluids. Research has shown that septic tanks are even a greater source of groundwater pollution with organic compounds than industrial and municipal waste water combined (Lapworth, Baran et al.), although the impact of this pollution is typically more localized than with industrially treated waste water.

## 3.2 Industry

Pollution from industrial sources comprises a large array of classes of compounds, originating at very diverse sites. Moreover, certain branches can be included or excluded, based on the exact definition of which sources 'industrial' encompasses. The definition adopted in this thesis includes all sources directly linked to manufacturing, resource extraction and commercial business. Healthcare and traffic emissions can also be seen as industrial sources.

An important industrial source of environmental contamination is the agricultural sector (Lapworth, Baran et al.). Animals are held in ways that regular treatment with antibiotics has essentially become mandatory, while the use of growth hormones to enhance growth and meat production is still customary in multiple countries. Besides animal husbandry, crop farming also has a significant impact on pollution. Millions of tons of herbicides, fungicides and pesticides are used globally each year (Albelda, Frias et al.), of which significant quantities end up in soil and surface water through rain, while a large portion of the remainder enters the environment after consumption, through sewage. Other noteworthy industrial sources are the mining industry (Zhou, Wang et al. ; Lapworth, Baran et al.), other resource extraction industries {1-3 brownawell 1}, factories and chemical plants (Albelda, Frias et al.), traffic (Albelda, Frias et al.), and hospitals – one of the few sites where radioactive metal ions can enter the environment (Albelda, Frias et al.).

## 3.3 Landfill

Landfills are a notorious source for heavy metal pollution (Albelda, Frias et al.), where soil contamination is the main concern (Zhou, Wang et al.). Inadequately fortified landfill sites might also leach contaminants into groundwater or surface water. However, environmental pollution from landfill sites is not limited to heavy metals. In fact, landfill sites consistently come up as the most significant contributor to organic contaminants in groundwater; their impact on a representative selection of different organic contaminants is approximately a factor 10 higher than from agricultural sources or industrial and municipal wastewater combined (Lapworth, Baran et al.).

## 3.4 Accident

Due to the random nature of accidents, it is not possible to define the exact pollutants released. Three good examples of accidents leading to release of chemicals into the environment are spilling, flooding, and fire. Spilling can happen when handling and transporting containers, or when larger stationary containers are damaged. Depending on the contents of the container, a spill can have a very high concentration of chemicals, which leads to severe contamination at the point of origin. This contamination can be directed at soil, surface water and groundwater. The same holds true for

flooding, where soil near rivers or other bodies of water is the most likely target for contamination due to a flood.

The picture is more complex when looking at pollution originating from a fire. During a large fire, elevated temperatures lead to the partial combustion of all kinds of materials, with thousands of possible products – a lot of which can be toxic, for instance carcinogenic PAHs. Furthermore, volatile chemicals present near the fire will evaporate and be released into the atmosphere. Extinguishing efforts can lead to contamination of surrounding soil and surface water, while modern industrial fire extinguishing equipment contains compounds that are themselves environmental pollutants {18 matsuda 1}.

**Table 3.1:** Some environmental contaminants. The value listed under impact gives an idea of the amounts present in the environment. Values in g/L are concentrations recorded in sewage, values in kg/yr are based on quantities detected in large rivers, values in tons are based on estimates of annual use. pK<sub>a</sub> values were obtained from PubChem (#) if available and calculated using SPARC (@) if not available. Impact figures were obtained from the following sources: A = {emccda}; B = (Hicks); C = (ter Laak, van der Aa et al.); D = (Lapworth, Baran et al.); E = (Lajeunesse, Smyth et al.); F = (Metcalfe, Beddows et al.); G = (2011); H = (Ternes); I = (Dinham); J = {EPA}.

Substance	Impact	pK <sub>a</sub>	Ionized @ pH ...		
			pH = 4	pH = 6	pH = 8
<i>Pharmaceuticals</i>					
Triclosan	509 ng/L <sup>D</sup>	7.9 (acid) <sup>#</sup>	<0.1 %	1.2 %	55.7 %
Sulfamethazine	120-616 ng/L <sup>D</sup>	7.65 (acid) <sup>#</sup>	<0.1 %	2.2 %	69.1 %
Sulfamethoxazole	1073 kg/yr <sup>C</sup>	5.7 (acid) <sup>#</sup>	2.0 %	66.6 %	99.5 %
Ibuprofen	1512 kg/yr <sup>C</sup>	multiple <sup>#</sup>	>88.8 %	>86.3 %	99.8 %
Atorvastatin	19-44 ng/L <sup>F</sup>	4.46 (acid) <sup>#</sup>	25.7 %	97.2 %	>99.9 %
Diclofenac	4102 kg/yr <sup>C</sup>	4.15 (acid) <sup>#</sup>	41.5 %	>98.6 %	>99.9 %
Clofibric acid	30 ng/L <sup>H</sup>	3.16 (acid) <sup>@</sup>	87.4 %	>99.9 %	>99.9 %
Salicylic acid	418 ng/L <sup>D</sup>	2.98 (acid) <sup>#</sup>	91.3 %	>99.9 %	>99.9 %
Sertraline	351 µg/kg <sup>E</sup>	8.77 (base) <sup>@</sup>	>99.9 %	99.80%	85.50%
Metoprolol	2132 kg/yr <sup>C</sup>	9.09 (base) <sup>#</sup>	>99.9 %	99.90%	92.50%
Fluoxetine	38-99 ng/L <sup>F</sup>	9.53 (base) <sup>@</sup>	>99.9 %	>99.9 %	97.10%
Atenolol	1299 kg/yr <sup>C</sup>	9.60 (base) <sup>#</sup>	>99.9 %	>99.9 %	97.50%
Citalopram	657 µg/kg <sup>E</sup>	9.64 (base) <sup>@</sup>	>99.9 %	>99.9 %	97.80%
<i>Life-style</i>					
Cocaine	300-600 tons <sup>G</sup>	7.15 (base) <sup>@</sup>	99.9 %	93.4 %	12.4 %
MDMA	50-150 tons <sup>A</sup>	9.9 (base) <sup>@</sup>	>99.9 %	>99.9 %	98.8 %
Amphetamine	200-600 tons <sup>A</sup>	10.13 (base) <sup>#</sup>	>99.9 %	>99.9 %	99.3 %
Caffeine	9,770 ng/L <sup>D</sup>	N/A	Neutral	Neutral	Neutral
<i>Industrial</i>					
Glyphosate	80,000 tons <sup>J</sup>	multiple <sup>#</sup>	100 %	100 %	100 %
Carbendazim	12,000 tons <sup>B</sup>	4.2 (base) <sup>#</sup>	61.3 %	1.6 %	<0.1 %
Paraquat	25,000 tons <sup>I</sup>	N/A	QAC; 2 <sup>+</sup>	QAC; 2 <sup>+</sup>	QAC; 2 <sup>+</sup>
<i>Hormones</i>					
Estrone	9 ng/L <sup>D</sup>	N/A	Neutral	Neutral	Neutral
17β-Estradiol	31 ng/L <sup>D</sup>	N/A	Neutral	Neutral	Neutral

## 4. Substances of Interest

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Emphasis will lie on the environmental fate and bioavailability of charged compounds, primarily positively charged molecules such as amines and quaternary ammonium compounds. This selection was based on the virtual absence of charged species from risk assessment regulations (IHCP); it thus seems evident there is a knowledge gap hampering adequate risk estimation {kah & brown, adsorption of ionizable pesticides in soils}. This knowledge gap is also apparent in regulation literature itself, where large deviations between modeled and actual values for  $K_{OC}$  are indicated (IHCP). Relevant citations include an *overestimation* of 0.55 log units for organic acids (anionic), an *underestimation* of 1.0-1.5 log units for alkyl ureas (cationic), and an *underestimation* of 1-2 log units for aliphatic amines and PAHs with amino groups (both cationic) (Sabljic A.). These additional errors come on top of the error margins of 0.3-0.6 log units in  $K_{OC}$  calculation and 0.3-0.4 log units for  $IC_{50}$  modeling (chapter 2).

In addition to the acknowledged difficulties charged compounds pose for environmental risk assessment, their relevance as substances of interest is further highlighted by the vast quantities released into the environment through the sewers and by other means (Ternes). Not only is 49% of REACH chemicals ionizable between pH 4 and pH 10; at least 14% of REACH chemicals is positively charged at environmentally relevant pH levels (Franco, Ferranti et al.). Basic compounds, the largest group of cationic organic compounds, are an adequate example to highlight the relevance of ionized molecules in risk assessment. Partitioning of the charged species can still make a significant contribution to total sorption at  $pH \geq pK_a + 2$ , where more than 99% of a basic compound will be in the neutral form (Li, Lee et al.). The sections below will provide some examples of environmental pollutants in different categories, underlining important cationic members of each category.

### 4.1 Pharmaceuticals & Consumer Products

Pharmaceuticals and consumer products primarily enter the environment through sewage or by disposal to landfills. Environmental contaminants from consumer products can be compounds from very different categories, such as food additives, detergents, fabric softeners, synthetic musks, and various chemicals added to product packaging such as plasticizers or dyes (Clarke and Smith ; Lapworth, Baran et al.). Detergents and fabric softeners are generally ionizable by nature, as their main active ingredients are surfactants; amphiphiles that usually contain a charged phosphate, sulfonate or quaternary ammonium group that constitutes the hydrophilic head (Ivankovic and Hrenovic), see section 4.3. Pharmaceuticals are still emerging as environmental contaminants, with higher numbers of compounds that reach detectable levels in groundwater and drinking water each year (Clarke and Smith). Noting that 40% of commonly prescribed drugs are organic cations at physiological pH (Neuhoff, Ungell et al.), it is evident that pharmaceuticals constitute a major category of interest.

### 4.2 Herbicides, Pesticides & Fungicides

Biocides are widely used in animal husbandry and crop farming, where the economic concerns to feed an ever increasing world population consistently seem to outweigh scientifically backed claims to restrain their use. The list of environmentally persistent herbicides and pesticides is long, thus it

would be senseless to reiterate all relevant compounds. However, some notable examples include carbendazim (a weak base), paraquat (a quaternary ammonium compound) and glyphosate (a zwitterion) {Jones 1}.

### 4.3 Cationic & Anionic Surfactants

Surfactants are the most widely discharged synthetic chemicals (Ishiguro, Tan et al.). Introduction into the environment can take place through wastewater discharge {Ishiguro 1}, remediation of groundwater or soil {Ishiguro 1}, additives in industrial resource extraction {1-3 from Brownawell 1}, or natural secretion {Wegner}. Furthermore, detectable levels of surfactants can remain in sewage sludge or effluent after wastewater treatment {17-19 from Ishiguro 1}.

Surfactants, by definition, contain lipophilic and hydrophilic moieties. The most common form is a hydrophilic headgroup attached to a lipophilic alkyl chain of variable length (Ying). Because organic matter in soil, sediment and solution also consists of hydrophilic and lipophilic moieties, mutual attraction between surfactants and organic matter is expected and observed experimentally {2+20+21 from Matsuda 1}. Furthermore, organic matter in the environment usually contains carboxylic acids, which after dissociation constitute suitable binding sites for positively charged headgroups of cationic surfactants {Matsuda 1}. In addition to this, contaminants already bound on dissociated carboxylic groups can be displaced by cationic surfactants, especially since their lipophilic portion further anchors them to the organic matter (Zachara, Ainsworth et al.).

#### 4.3.1 Cationic

Cationic surfactants contain ionizable amine groups or permanently charged ammonium moieties in their headgroup. Notable examples of cationic surfactants are quaternary ammonium (QAC), benzalkonium (BAC), cetylpyridinium (CPB or CPC) and alkyl methylammonium (TMA or DMA) compounds (Ying ; Ivankovic and Hrenovic). The majority of these compounds have a structure resembling the phospholipids that make up cellular membranes, and are therefore able to be taken up into the cell membrane, thereby disrupting or even destroying these vital membranes {23 Ishiguro 2}. Their amphiphilic nature also makes them hard to isolate, as they always take up residence along the interface of two phases.

#### 4.3.2 Anionic

Anionic surfactants closely resemble their cationic counterparts, except for the headgroup which is negatively charged. This negative charge can be provided by phosphate, carboxylate, sulfate or sulfonate groups; compounds with sulfur containing headgroups are most common. Some general examples include alkyl benzene sulfonate (LAS), alkane sulfonate (SAS), sodium dodecyl sulfate (SDS), and sodium lauryl sulfate (SLS) (Jensen ; Ying ; Ivankovic and Hrenovic). Another relevant category is the perfluorinated anionic surfactants. These compounds were marketed as dirt repellent coating material, but their environmental persistence proved problematic (Clarke and Smith); the golden standard for these compounds is PFOS.

### 4.4 Heavy Metals

Heavy metals and radionuclides are briefly mentioned here, primarily because they can compete with organic cations for binding sites and because they can influence the solubility and mobility of

organic matter. Their intrinsic relevance as toxic environmental contaminants will not be discussed in depth, as this deviates too far from the organic nature of the other substances of interest. Virtually all heavy metals are cationic, aside from complex ions with – for instance – oxygen.

Heavy metals enter the environment as leachate from landfill or directly from industrial sources, such as electroplating, metallurgy, tannery, and resource extraction (Zhou, Wang et al.). Zinc, copper and lead are common pollutants in runoff from roads, while nickel, chromium, manganese and cadmium are also linked to traffic density (Albelda, Frias et al.). Additionally, arsenic, iron, aluminum and manganese are commonly found in sediments and rock formations; since their presence is widespread, these metals can reach significant levels in groundwater (Albelda, Frias et al.). Cadmium is of particular interest from risk assessment perspective, as it has a relatively high toxicity and mobility (Zhou, Wang et al.), and its uptake into plants is known to be enhanced by the presence of organic carbon {107 from Jones 1}.

# 5. Preliminary Environmental Fate

Preliminary environmental fate (PEF) is the temporary or semi-permanent fate of any substance that enters the environment. PEF is an important indicator for the immediate and potential risk associated with pollution. One determinant of PEF is the point of origin; contamination originating in soil will first lead to sorption to soil, followed by eventual contamination of underlying groundwater or runoff into surface water. The flowchart in figure 5.1 gives an overview of the connection between different categories of PEF. These categories will all be discussed in-depth in the sections below, except for breakdown which is – of course – not a preliminary fate, but a permanent fate.

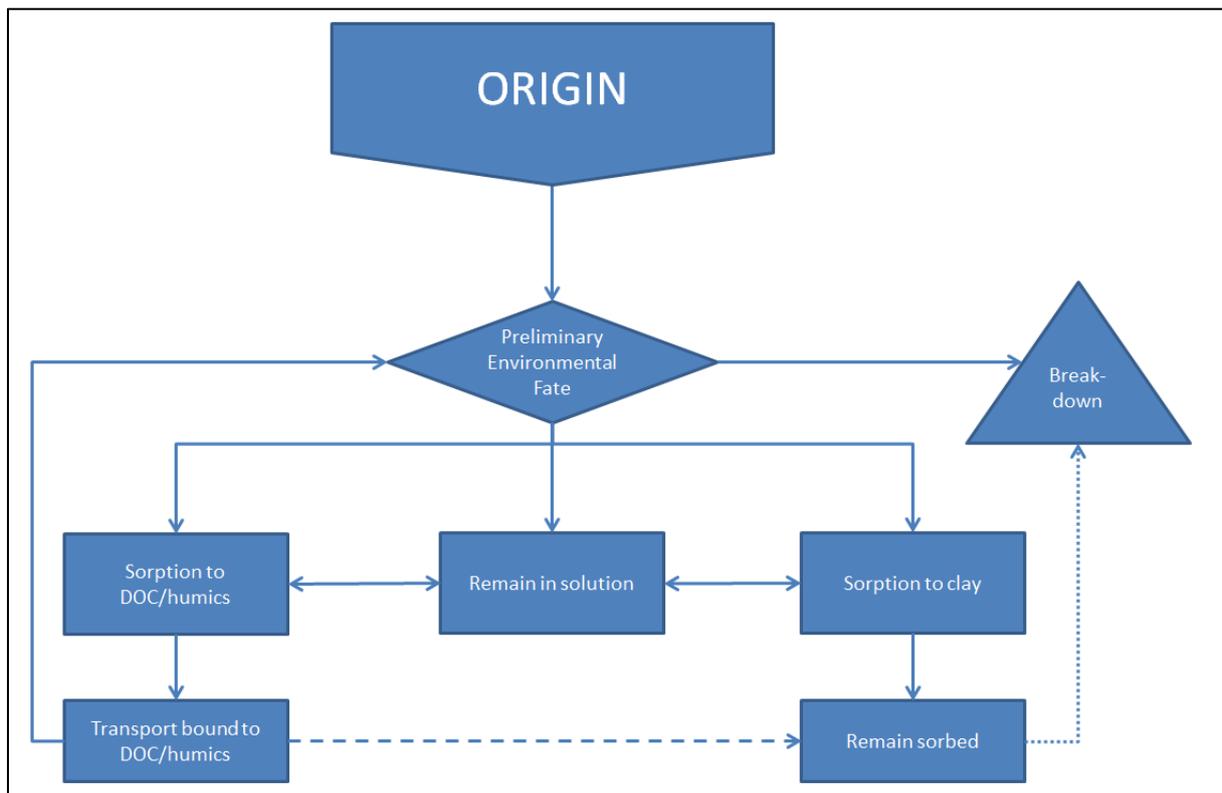


Figure 5.1: Flowchart to illustrate factors influencing environmental fate.

## 5.1 Remain in Solution

The fraction of a substance that simply remains in solution is most straightforward. This freely dissolved fraction will disperse through the available aqueous phase by diffusion, until a uniform concentration is reached. It is a generally accepted notion that only freely dissolved molecules are available for uptake into an organism, which makes the dissolved fraction the most important one from a risk assessment perspective. Aside from availability for uptake into organisms, the aqueous phase is also the major phase for degradation, as dissolved molecules have prolonged exposure to water, oxygen, radiation and reactive molecules in solution, in addition to more extensive uptake by microorganisms. It is, however, important to note that if there is any sorption to DOC or soil, the freely dissolved concentration is in continuous equilibrium with the fraction sorbed to DOC and soil – although this is not necessarily an equilibrium that develops rapidly.

## 5.2 Sorption to Organic Carbon

Organic carbon participating in sorption can be classified into two groups: dissolved organic carbon (DOC) and precipitated organic matter, as a constituent of soil or sediment – although EU regulations do not discriminate between these types of organic carbon (Pavan, Worth et al.). Organic carbon in soil and solution is largely made up of humic substances (HSs); 60-70% of soil organic matter consists of HSs {17 from Jones 1}, and roughly 40% of DOC is composed of HSs {18 from Jones 1}. It is estimated that the total amount of HSs on earth is in excess of 30 billion tons, making it the most abundant naturally occurring macromolecule {19 from Jones 1}. HSs are of particular interest in environmental risk assessment, due to the presence of a large variety of functional groups and their capacity for cation exchange (Ishiguro, Tan et al.).

HSs can be divided in three categories, based on their solubility in water. Fulvic acid (FA) is soluble in water regardless of pH {Jones 1}, humic acid (HA) is only soluble between pH 4 and pH 10 {matsuda 1}, and humins make up the remainder – substances that are insoluble in water under all conditions {Jones 1}. FAs are oligomeric acids with additional functional groups, while HAs usually are more structured particles, with internal chemical bonds (Ishiguro, Tan et al.). HAs can be far larger than FAs and generally contain a multitude of functional groups (Ishiguro, Tan et al.); they are therefore of prime interest in scientific experiments.

Since they contain acidic groups, HSs can bind positively charged compounds. Their binding capacity is further supplemented by the miscellaneous functional groups, supplemented with aromatic and lipophilic moieties {2-3 from Matsuda 1}. This heterogeneous molecular makeup provides potential binding sites for a wide range of compounds. Of particular interest is the binding of heavy metal ions to HA, which generally produces complexes with a lower solubility than the HA itself {Matsuda 1}. This phenomenon can lead to precipitation of HA rich in heavy metals, polluting sediment while reducing availability of heavy metals for the overlying water layer.

## 5.3 Sorption to Clay

EU regulations do not incorporate clay into risk assessment models. Moreover, the term 'clay' is used only once in document of 250 pages that details the use of models in environmental risk assessment (IHCP ; Pavan, Worth et al.), although this document does highlight the fact that non-polar compounds predominantly sorb to organic matter, and the assumption that clay is insignificant when looking at purely non-polar substances is largely correct (IHCP). However, as stated in section chapter 4, charged species should no longer be overlooked in contemporary risk assessment models, and it is primarily cationic organic compounds that can have strong interactions with clay.

Clay consists of phyllosilicates; thin sheets of metal containing silicates, stacked together to form a dense and firm, semi-solid material. Depending on the molecular composition, layers of clay can be neutral or have a negative charge. The negative charge is normally neutralized by  $K^+$  or  $Na^+$  ions, but these ions can be displaced by multivalent metal ions {Vermeer 1}, or organic cations {azejjel 1}. Especially in mixed soil, where clay and organic matter are packed together, organic cations can have strong interactions with negatively charged silicates on the clay surface. A convenient example of this are the commercially available organoclays; clays treated with surfactants to coat them with lipophilic alkyl chains, and which are then used to remediate soils or to remove organic contaminants from polluted water {zhao 1; Song 1}.

## 5.4 Transport While Sorbed

It is logical that DOC is very mobile, but precipitated HSs and – to a lesser extent – clay particles can also become mobile, as a result of currents and mechanical agitation. During transport and after settling in a fresh environment, sorbed substances will remain in equilibrium with the surrounding aqueous phase. This means that deposition of contaminated particles in clean water will lead to desorption of pollutants and concomitant pollution of the surrounding water. Especially HSs are known for this transport of contaminants to remote sites {Jones 1}.

## 5.5 Permanent and Semi-Permanent Sorption

Due to the high amount of functional groups present on a typical HS molecule, combined with the possible prolonged exposure to radiation and free radicals, it is possible for sorbed molecules to become covalently linked to the HS molecule. As permanent sorption effectively removes a contaminant from the cycle, this process is regarded as environmental breakdown. However, this only holds true if the sorption mechanism is indeed permanent.

Besides covalent linking leading to true permanent sorption, compounds with very high affinity or which sorb under favorable conditions can reach a state of semi-permanent sorption. The classic example for this is deep pore sorption, where some molecules diffuse deep into organic matter and sorb at hydrophobic sites with little interaction with the unstirred water layer surrounding the whole particle. Semi-permanent sorption can also be dependent on very high affinity of the sorbate molecule. Surfactants are a good example of high affinity sorbates {Matsuda 1}, as their positively charged headgroup can form a very strong ionic bond with a carboxylate {2/20/21 from Matsuda 1}, while the long alkyl chain is flexible enough to twist to the most favorable conformation {22-26 from Matsuda 1}.

# 6. Environmental Bioavailability

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Environmental Bioavailability (EBA) refers to the notion of bioavailability as adopted in environmental sciences, as opposed to the pharmacological definition of bioavailability discussed in the next chapter. In environmental sciences, a molecule is deemed bioavailable if it can readily be taken up into an organism. This is to separate the concentration available for uptake from the fraction not accessible by an organism. Bioavailability is an important concept, as it is an essential link between the hazard – intrinsic quality of a given toxicant – and the associated risk, which is the chance the hazard will express itself.

EBA is an important concept within the context of this thesis, as ionizable molecules have different and additional possibilities of interaction with organic matter and soil particles. Furthermore, ionized species can compete with neutral compounds for the limited number of binding sites available, while the binding of cationic surfactants to clay particles can provide additional options for interactions between neutral compounds and clay particles. Below, a number of examples or case studies will be presented to elucidate the major factors that influence and shape EBA. The chapter and with a list to highlight the most important statements presented in each section.

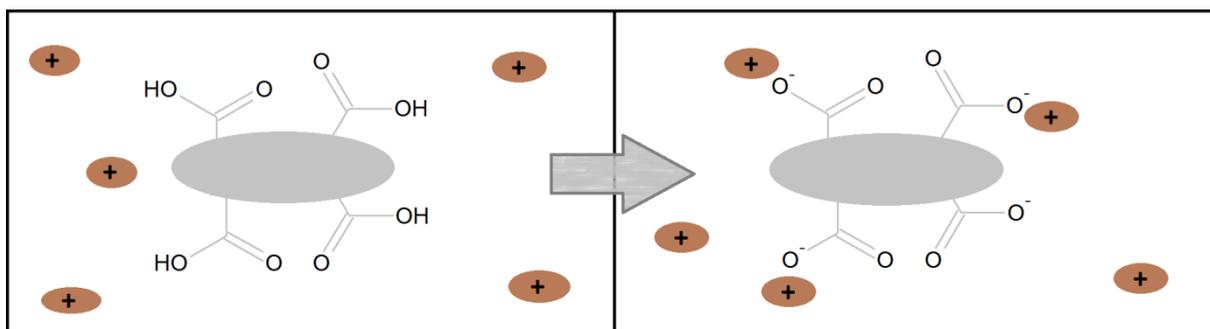
## 6.1 Baseline Sorption

Baseline sorption is used here to describe the effect of sorption on EBA on an 'as is' basis, so with no regard to the interplay of different factors contributing to and influencing sorption. Baseline sorption is generally higher for the charged species, as additional options for interactions are vacant for cations. As noted above, only the concentration of a compound that is accessible to the organism can be taken up. Therefore, toxicity of heavy metals is associated with the free cation as opposed to complexes {93 and 94 from Jones 1}, and biocides or other contaminants which are bound to soil are generally considered to be deactivated {Jones 1; Ishiguro 1; Matsuda 1}.

Since any amount of sorption removes molecules from solution, baseline sorption always has a negative effect on EBA, and risk assessment can favor substances with high binding to HSs and clay. On the other hand, however, HA and FA are usually very mobile in solution, and thus can function as a vector, enhancing the transport of contaminants {matsuda 1; ishiguro 1}, increasing their EBA at the site of deposition – although desorption is always based on equilibrium, which means the concentration at the site of deposition will never be higher than at the site of origin.

## 6.2 Influence of pH: Negative Charge on Humic Acid

A major factor in the reactivity of HSs is the negative surface charge, originating from the dissociation of carboxyl groups (Ishiguro, Tan et al.). As an arbitrary rule, carboxylates in HSs will begin to protonate around pH 6. The degree of protonation has a direct influence on the sorption of cationic molecules and inorganic cations, but can also affect sorption behavior of neutral compounds – which might be repelled by the presence of surface charge near carboxylates.

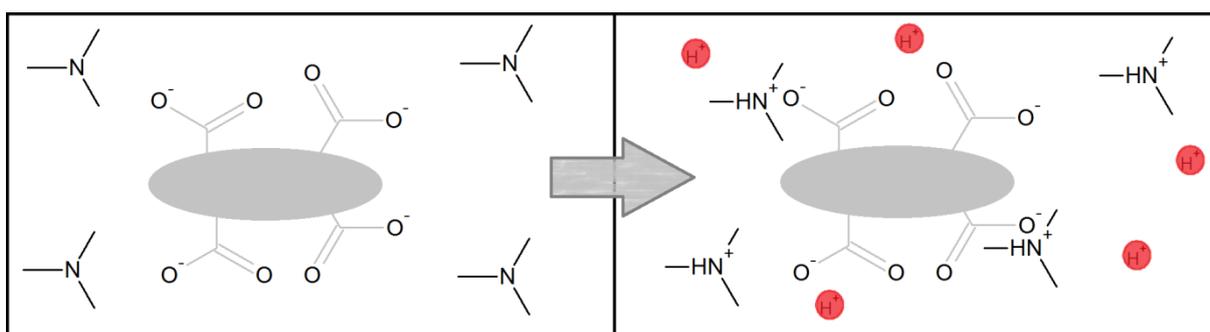


**Figure 6.1:** Illustrating the effect of pH on sorption to HA. Dissociation of the carboxyl groups creates negatively charged sites, suitable for binding of cationic molecules (brown).

An example of the effect of HSs deprotonation on sorption of inorganic cations is the sorption of  $\text{Cd}^{2+}$  to HA. The negative charge on the HA increases with increasing pH {30 from Vermeer 1}. This creates a flux of positively charged  $\text{Cd}^{2+}$  ions to the negatively charged surroundings of the HA, which leads to a higher concentration of  $\text{Cd}^{2+}$  in the vicinity of the HA, while the number of available binding sites for  $\text{Cd}^{2+}$  sorption is also increased {Vermeer 1}. From pH 6 to pH 9, sorption of  $\text{Cd}^{2+}$  to HA increased with approximately 0.8 log units {Vermeer 1}. The binding of paraquat, an herbicide with two quaternary ammonium groups, is an example of increased sorption of an organic cation as a result of changes in pH; sorption of paraquat to HA was a factor 1.5 higher at pH 10 than at pH 7 {Jones 1}.

### 6.3 Influence of pH: Positive Charge on Organic Base

The role of pH in the sorption of organic bases is very simple and straightforward when looking only at the changes in the organic compound. If the pH in the aqueous phase is at maximum 2 units above the  $\text{pK}_a$  of a basic compound (B), a relevant percentage of molecules will associate with  $\text{H}^+$  ions in solution, giving rise to  $\text{BH}^+$ . The fraction of  $\text{BH}^+$  will increase with decreasing pH; bases with  $\text{pK}_a$  of 7 or higher are most relevant in this perspective, as a significant fraction of any such compound will bear a positive charge within the environmentally relevant pH range.



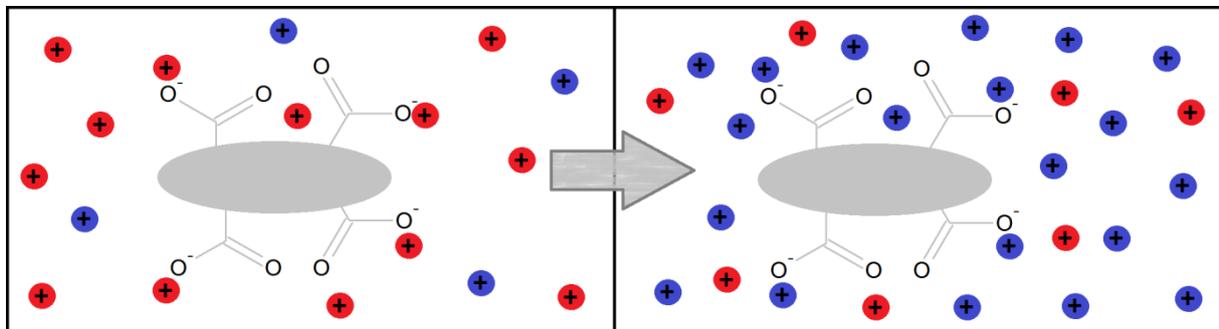
**Figure 6.2:** Illustrating the effect of pH on sorption of basic compounds. The increased concentration of  $\text{H}^+$  at lower pH leads to association of basic molecules with  $\text{H}^+$  ions, giving rise to positively charged molecules that bind to negatively charged carboxylates.

Bearing in mind the negative charge on clay particles and dissociated carboxyl groups in HSs, it is evident there will be significant attraction between  $\text{BH}^+$  and negatively charged sites. Depending on the affinity of the neutral molecule, the cationic species can still have an insignificant affinity for clay and HSs. However, the affinity of the positively charged  $\text{BH}^+$  moiety is generally much higher than of the neutral compound, and literature provides numerous examples of substances whose affinity significantly increases with decreasing pH {fabrega 1; 9 and 15 from fabrega 1}. Naturally, this

phenomenon is also true in the opposite direction, meaning that an increase in pH will lead to dissociation and desorption of organic bases. In soils with high pH, sorption usually is governed by the neutral species, and is more closely related to hydrophobicity {9, 11-14 from fabrega 1}.

## 6.4 Competition: Heavy Metals & Acidic Protons

If there are more ions present in solution than there are binding sites available, the ions that are present will compete for the limited number of binding sites. Competition of  $H^+$  with other cations can also be explained as pH-dependency of binding constants. The significance of  $H^+$  as a competitor for binding sites partly lies in the high pH difference between surface water and the gastrointestinal tract. The stomach typically has a pH of 2, which amounts to a concentration  $H^+$  of 10 mM. The EBA of sorbed compounds can be greatly enhanced upon ingestion of soil particles or dissolved HSs, as  $H^+$  displaces sorbed molecules; a suitable example of this is the sorption of  $Cd^{2+}$  to a hematite/HA complex, which decreases by roughly 2 log units when pH is lowered from 9 to 4 {Vermeer 1}.



**Figure 6.3:** Illustrating competition of two cationic compounds (blue and red) for binding sites. At the left, red is in the majority and fills up the binding sites. At the right, more blue is added and red is subsequently removed from binding sites.

## 6.5 Competition: Organic Cations & Multivalent Cations

The interchange of inorganic metal ions and organic cations is a form of competition for a limited number of binding sites. Negatively charged sites on HSs and clay particles are commonly filled with ubiquitous ions like  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$  or  $Mg^{2+}$ , but multivalent heavy metal ions such as  $Cd^{2+}$  or  $Cu^{2+}$  can also sorb to vacant sites. In fact, divalent metal ions usually have stronger interactions with humic substances than, for instance,  $Na^+$  {matsuda 1}. Moreover, HA and FA are known to actively complex with heavy metals, reducing their free concentration and toxicity {104 and 105 from Jones 1}.

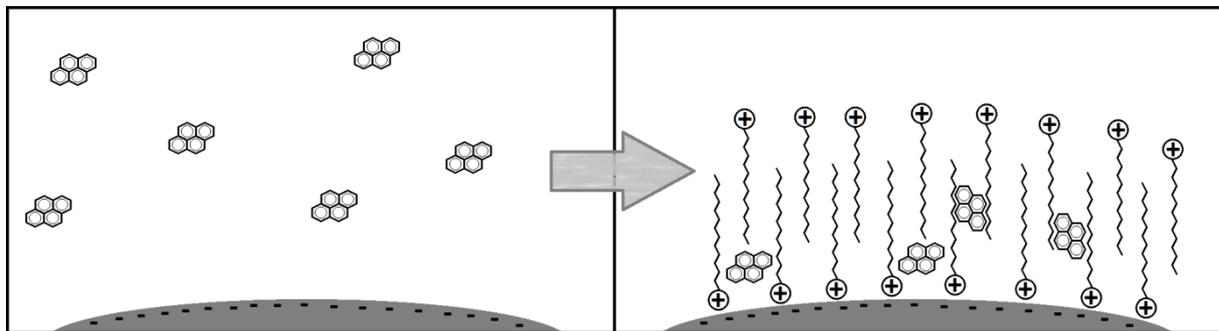
In comparison with heavy metal cations, it is logical that organic cations can have a much higher affinity for certain binding sites, since the organic backbone allows for additional interactions with functional groups and aliphatic moieties on the HSs {1 and 21 from fabrega 1}. Therefore, if an organic contaminant enters an aqueous phase where significant amounts of metal ions are sorbed to HSs, organic cations can displace these adsorbed metal ions and effectively raise the toxicity of heavy metals in the system {5 and 6 from brownawell 1; 20 from matsuda 1}. Interestingly, this competition phenomenon can coexist with increased sorption through cosorption, as seen for glyphosate with  $Cu^{2+}$  and  $Cd^{2+}$  (Zhou, Wang et al.).

Competition can also take place in the opposite direction, where the presence of inorganic cations interferes with the binding of organic molecules. This alternative phenomenon is mostly seen for surfactants, where binding is significantly decreased in the presence of high concentrations of metal

salts. Dodecyl trimethylammonium (C12TAB) and decyl trimethylammonium (C10TAB) are fitting examples, where the presence of 0.5 mM  $\text{Cd}^{2+}$ ,  $\text{Zn}^{2+}$  or  $\text{Pb}^{2+}$  leads to a factor 2 (C12TAB) and factor 3 (C10TAB) decrease in sorption coefficients, measured at pH 8 and with 0.01 M  $\text{Na}^+$  {matsuda 1}.

## 6.6 Cosorption: Organoclays & Pesticides

In contrast to competing with inorganic cations, organic cations can also allow or enhance the sorption of negatively charged or neutral compounds. The most accommodating example of this might be organoclays, industrially produced mixtures of clay minerals and cationic surfactants. Organoclays can be considered as clay particles coated by a bilayer of surfactant molecules, made up of cationic surfactants, which bind to the negatively charged groups on the clay particles. The second frame in figure 6.4 gives a schematic illustration of the bilayer created on the surface of organoclays.

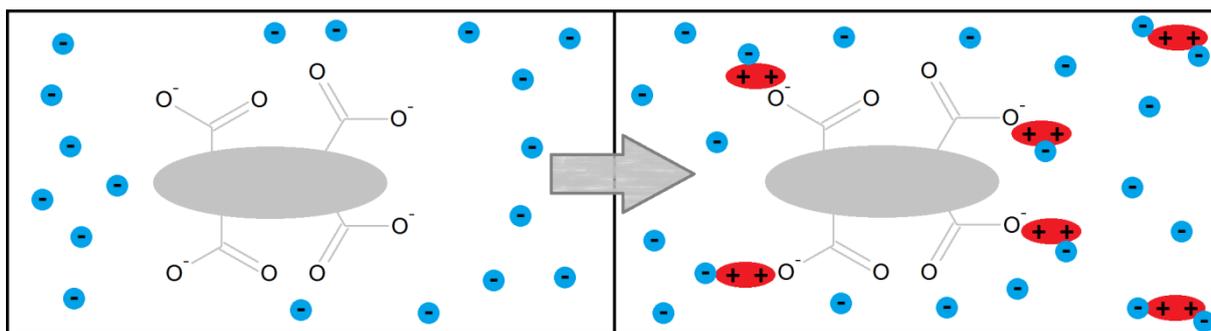


**Figure 6.4:** Illustrating the cosorption of cationic surfactants and lipophilic compounds to a clay particle. The aromatic compound has no sorption to the negatively charged clay particle (left). Addition of cationic surfactants creates a lipophilic shell around the clay particle, to which the aromatic compound sorbs with high affinity (right).

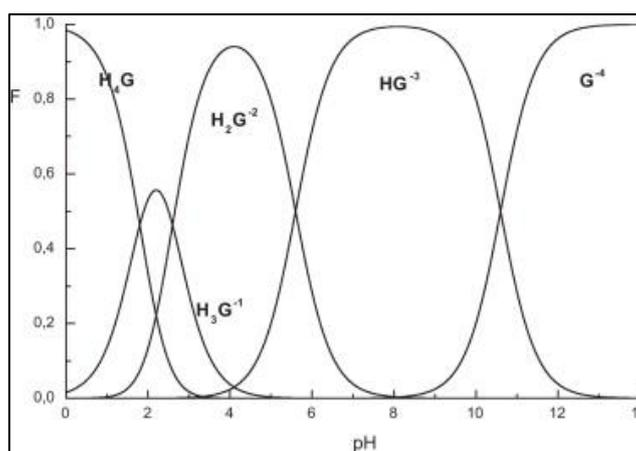
The adsorbed surfactants significantly increase the affinity for hydrophobic compounds of these so-called organoclays {6-9 from brownawell 1}. Distribution coefficients for terbutryn (base,  $\text{pK}_a$  4.30) and the more hydrophilic dicamba (acid,  $\text{pK}_a$  1.87) increased with a factor 55 to 128 and a factor 1.4 to 8.6, respectively, when compared with natural clay, measured at pH 7 and 0.01 M  $\text{CaCl}_2$  {azejjel 1}. However, the size of the surfactant molecule has a large influence on this, as smaller QACs generally show a decrease in sorption capacity as opposed to larger QACs {12 + 16 from Zhao 1; wang 1}. Finally, industrially prepared organoclays are not a proper example of what might actually happen in the environment, where low concentrations of various surfactant molecules are likely to be present. Therefore, it is unfeasible to model the extent to which this particular type of cosorption will emerge in an environmental setting.

## 6.7 Cosorption: Glyphosate & Heavy Metal Counterions

The process where multiple different ions sorb to a sorbent together is known as cosorption. As both HSs and clay particles have a negative charge, this process is most significant for anionic substances that can sorb together with inorganic cations. Glyphosate is a suitable albeit complicated example to illustrate the cosorption process. Figure 6.6 shows the chemical speciation of glyphosate as a function of pH. Glyphosate ions with a different degree of dissociation can be referenced as  $\text{H}_3\text{G}^-$ ,  $\text{H}_2\text{G}^{2-}$ ,  $\text{HG}^{3-}$ , etc. These ions can form complexes with  $\text{Cd}^{2+}$ , yielding – for instance – the neutral  $\text{Cd}^{2+}\text{H}_2\text{G}^{2-}$  complex (Zhou, Wang et al.). Since the total charge cannot be evenly distributed along the whole glyphosate molecule, there will exist some positively charged regions on the molecule. These positively charged regions can enhance sorption to negatively charged sorption sites.



**Figure 6.5:** Illustrating cosorption of an anionic compound with a divalent cation. The anionic compound does not sorb to the negatively charged HA; it is repelled by the negative charge (left). Divalent cations function as a bridge between the carboxylate on the HA and the anionic compound in solution, facilitating sorption of the anionic compound (right).



**Figure 6.6:** Speciation of glyphosate across the pH spectrum.

Although experimental results demonstrate significantly increased binding of glyphosate to different types of clay in the presence of 50  $\mu\text{mol/L}$   $\text{Cd}^{2+}$  (Zhou, Wang et al.), these results are valid only in solutions where  $\text{pH} \leq 6$ , as complexes become increasingly negatively charged above this pH {Yu and Li from Zhou 1; Morillo 2000 from Zhou 1}; recall that clay particles and organic matter in soil are almost exclusively negatively charged.  $\text{Cu}^{2+}$  is a second counterion that is known to increase sorption of glyphosate to clay and soil, although the effect of  $\text{Cu}^{2+}$  can be ambivalent in certain types of clay i.e. montmorillonite (Zhou, Wang et al.). In contrast, studies with goethite have found a 9% increase of glyphosate sorption under influence of  $\text{Cu}^{2+}$ , as well as increased  $\text{Cu}^{2+}$  adsorption in the presence of glyphosate – which means cosorption can be considered reciprocal in this case {Maqueda 2002 from Zhou 1}.

An important realization is that not only multivalent heavy metal ions can act as counterions for cosorption. In fact, even  $\text{NaCl}$  can have effects – both positive and negative – on sorption coefficients {brownawell 1}. Furthermore, the influence of counterions on sorption is not only limited to organic cations or anions, but aromatic systems can also interact with inorganic cations, due to the presence of  $\pi$ -electrons. These cation- $\pi$  interactions have been shown to significantly increase sorption to phospholipids; in the presence of  $\text{Cu}^{2+}$ , anthracene and pyrene reportedly have - respectively – a factor 5.2 and 2.5 increased distribution coefficient for biolipids (Qu, Wang et al.).

## 6.8 Highlights

- Any sorption process lowers EBA and consequently has a negative effect on toxicity.

- Deprotonation of HAs as a result of raising pH increases sorption of cationic substances.
- Protonation of bases increases sorption to negatively charged binding sites.
- H<sup>+</sup> ions can displace sorbed toxicants by competition, which is especially relevant in case of ingestion of sorbent particles, where bioavailability increases due to the pH in the stomach.
- Competition between heavy metal ions and organic cations can lead to decreased sorption of either ion; displacement of sorbed ions through competition can lead to increased toxicity.
- Cosorption is a complicated process, where presence of additional contaminants leads to lower EBA for both, as one contaminant sorbs to the other or they both sorb together.

# 7. Pharmacological Bioavailability

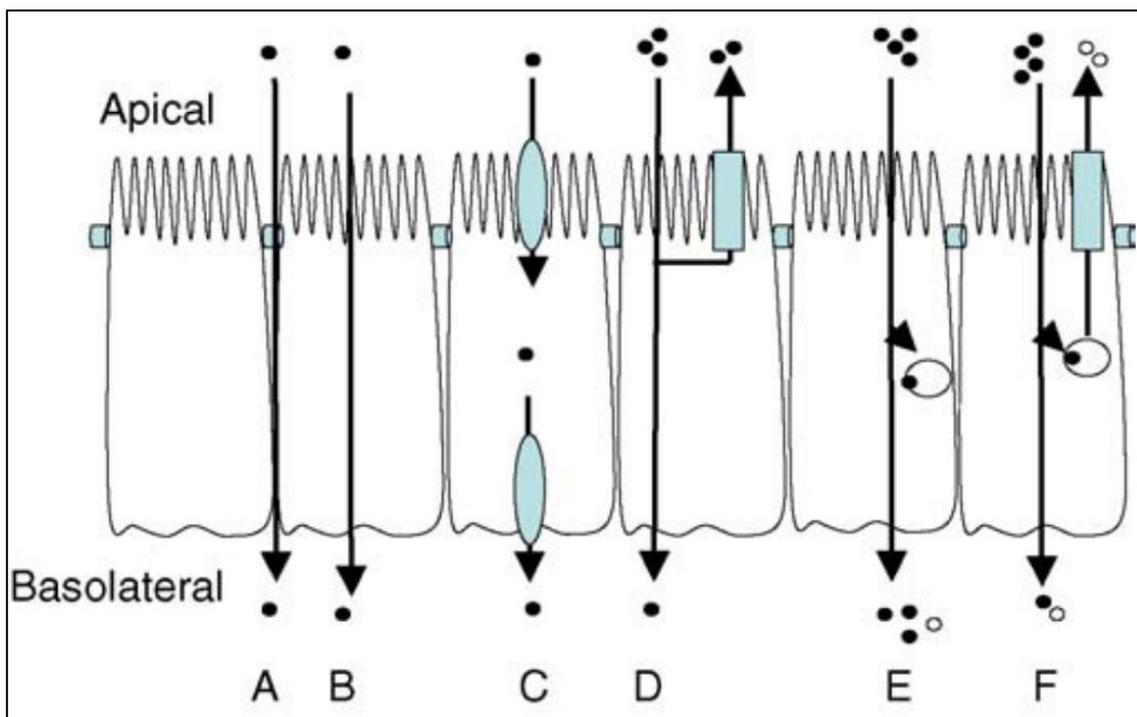
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Pharmacological bioavailability (PBA) is the percentage of a dose that is taken up into the organism. The following chapter will deal with PBA in the context of environmental toxicology; the presented facts and assumptions are not necessarily a reflection of clinical pharmacology. Although PBA seems to be an aspect that is highly dependent on the properties and species of an organism, PBA is considered to be a discrete property of a given substance, while it is acknowledged that PBA does differ between species. For risk assessment purposes, it is not always feasible or necessary to determine an exact PBA. However, for adequate risk assessment it is important to realize which factors influence PBA, and how these factors vary between neutral molecules and ionized species. The prime determinants of PBA will be discussed, along with a case study for each. But first off, it is interesting to briefly discuss how PBA is handled by regulators.

The prevalent consensus in environmental risk assessment legislation is that only neutral molecules can be taken up into an organism (bacteria, insect, worm, fish, or mammal) i.e. the PBA is governed fully by the neutral fraction (IHCP ; Pavan, Worth et al.). It is therefore quite alarming that there is a vast and increasing body of experimental evidence, which suggests that charged molecules have significant PBA. For organic bases, uptake rates start decreasing when pH approaches the pKa, but this decrease levels off as the pH gets lower and lower {5 from Erickson 1}, although the fraction of neutral chemical can drop by a factor of 100 over 2 pH units (Erickson, McKim et al.). A similar effect of pH on the uptake of organic acids is to be expected, but these are less relevant as the main scope of this thesis is organic cations. The significant PBA of ionized species is further established by uptake rates under experimental conditions, where the total uptake of substances exceeds what is possible if only the neutral species would be available for uptake (Erickson, McKim et al.).

In addition to the ample evidence ionized molecules can be absorbed into organisms, there is a second factor interfering with adequate risk assessment based on current models. As discussed in chapter 2, models used to predict uptake are largely based on  $K_{O/W}$ . Aside from the fact that  $K_{O/W}$  is – by definition – only relevant for the neutral fraction, there is another discrepancy when basing models on  $K_{O/W}$ . An actual organism contains not only storage lipids, but also membrane lipids and proteins. Even though the triglyceride composition of storage lipids has no significant influence on partitioning (Geisler, Endo et al.), partition coefficients for neutral organic compounds can be up to two log units higher for membrane lipids than for storage lipids (Endo, Escher et al.). It is likely that this difference would increase even further for ionized molecules, as their affinity for hydrophobic molecules such as triglycerides is very low.

Furthermore, different organs or compartments within an organism might have different affinities for a given compound, while some compounds will have specific toxic effects in explicit organs. This will become apparent in section 7.2; the section on OCTs provides additional background for the concept of organ specific toxicity, as some organs are rich in OCTs. Though it might be presumptuous to declare that all these factors must be incorporated into risk assessment, it should be evident by now that  $K_{O/W}$  is not suitable to predict toxicity of ionizable compounds, and in addition is not a perfect tool to model toxicity of neutral compounds with specific protein interactions or other direct mechanisms of toxicity either.



**Figure 7.1:** Mechanisms of intestinal absorption, also applicable to absorption at other sites. A = paracellular diffusion, only possible in cells with 'leaky' tight junctions. B = transcellular diffusion, based on passive diffusion and only possible for compounds that can cross the cell membrane. C = transporter-mediated transport, an example of this is transport by OCTs (see section 7.3). D = active efflux transport, serves to return unwanted chemicals to the apical compartment; competing ligands can inhibit efflux transport, leading to increased absorption (see section 7.3.1). E = intracellular metabolism prior to entering the basolateral compartment (not discussed in this thesis). F = specific metabolism with selective efflux of metabolites (not discussed in this thesis). (Reproduced from (Fearn and Hirst).

## 7.1 Tissue pH: Inhalation through Gills

Uptake of chemicals through the gills is a suitable example to illustrate how pH gradients influence PBA by influencing the neutral and ionized fractions and enhancing diffusion flux. Since there is also a considerable pH gradient along the gastrointestinal tract, this example is similarly applicable to absorption after ingestion. Since oral exposure is the most common route of exposure in mammals {1 from Broeders 2}, absorption through oral mucosa, in the stomach, and along the intestinal wall is an important aspect of PBA. Stomach pH can be as low as 1, while the pH in the small intestine can reach up to 8.

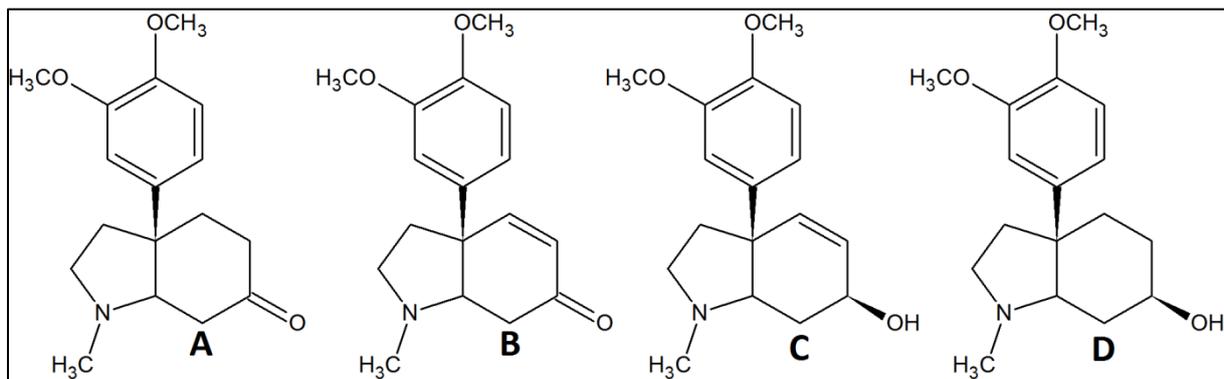
Since gills are typically exposed to the bulk concentration in the surrounding aqueous phase, measuring and modeling uptake through gills is quite fundamental and numerous studies have used fish gills for experimentation. A typical 1 kg rainbow trout excretes approximately 0.3 mM/h of ammonia (Erickson, McKim et al.), and 2 mM/h of carbon dioxide – which is largely metabolized to carbonic acid before release through the gills {11 + 12 from Erickson 1}. Release of these two chemicals alters local pH at the gill surface, which leads to a gradient from pH 8 at the point of inhalation to pH 7.1 at the end of the gills. This pH gradient influences the fraction of neutral and ionized molecules (Erickson, McKim et al.).

Separate diffusion gradients for neutral and charged species contribute to maintaining a high flux from the aqueous phase to the gill surface. This is because ionized and neutral molecules have separate diffusion gradients, which is further enhanced by the equilibrium between charged and

neutral fractions; if only the neutral molecule is taken up, ionized molecules will become neutral to restore the equilibrium. In addition to this, perfusion of the gills leads to sink conditions, as molecules that enter the bloodstream are rapidly transported to the bulk organism (Erickson, McKim et al.). Furthermore, there is also the possibility of paracellular transport {5 from Erickson 1}, and the possible uptake of ionized molecules contributes further to PBA {14-20 from Erickson 1}.

## 7.2 Tissue Type: Plant Alkaloids and Mucosal Tissues

Although perhaps only relevant in specific exposure scenarios where mucosal tissues endure prolonged contact with contaminants, it is interesting to appreciate the fact that different tissue types can have distinct permeabilities for certain molecules. The relevancy of this can be disputed somewhat, since exposure of mammals generally takes place orally {Broeders 2}, meaning that any given toxicant will typically encounter the same tissues from mouth to the large intestine. However, from a risk assessment perspective, this phenomenon can still be important when setting up experiments where tissue samples are used to determine permeability. In such experimentation, it should always be realized that permeability can be higher or lower in other types of tissue.



**Figure 7.2:** Mesembrine and three of its metabolites.

A representative example is formed by the four alkaloids mesembrine (A), mesembrenone (B), mesembrenol (C), and mesembranol (D) (figure 7.2). As can be seen in figure 7.2, these four compounds have practically the same molecular structure; there are differences only in the ketone or hydroxyl group (A+B vs. C+D) and the presence of a double bond in the ring system (B+C vs. A+D). Even though these molecular differences seem small and arbitrary, there is significant variation in the permeability of each compound between various mucosal tissues. Mucosal tissues of the cheek (buccal), bottom of the mouth (sublingual), and the intestines were tested with the four compounds in pure form and a mixture containing all four alkaloids.

When testing the alkaloids in their purified form, permeability was lowest for buccal mucosal tissue, and higher for sublingual and intestinal mucosal tissues (Shikanga, Hamman et al.). Mesembrine (A) had the highest permeability coefficient across intestinal mucosa, but for sublingual mucosa permeability was highest for mesembranol (D); mesembrenone (B) and mesembrenol (C) had comparable uptake in intestinal mucosa (Shikanga, Hamman et al.). These results not only support the notion that permeability and the resulting toxicity can be tissue or organ specific, but also indicate that the relative permeability of compounds can change depending on the type of mucosa. One explanation for this could be the presence of tissue specific transporters with high selectivity, which will be discussed in the next section.

## 7.3 Organic Cation Transporters

Organic cation transporters (OCTs), as the name already implies, deal with the transport of organic cations across the cell membrane. Transport can be in both directions, and substrates include endogenous as well as exogenous organic cations. In addition to OCTs, organic anion transporters also exist. However, anion transporters will not be further discussed here, as the scope of this thesis lies on positively charged compounds. Nevertheless, the properties and mechanisms which will be described for OCTs can almost uniformly be translated to their anionic counterparts {6 from Ciarimboli 2}.

OCTs are polyspecific transporters, meaning they can transport a broad spectrum of molecular structures (Ciarimboli). This polyspecificity makes it hard to predict whether a given molecule will be a substrate or not, especially since organic cations are frequently able to cross the cell membrane by other means, such as other transporters or paracellular diffusion, leading to additional complications when setting up experimental procedures to determine transport by OCTs. OCTs are known for their high expression in intestines, liver and kidneys {Koepsell}, thus forming a critical component of ADME (absorption, distribution, metabolism, excretion). The presence and significance of OCTs is also direct evidence that absorption of charged molecules is possible – again, in contrast with the presumption made by regulating authorities.

Examples of relevant substrates for OCTs, from an environmental risk assessment viewpoint, are NSAIDs {Khamdang}, cyperquat (Wu, Kekuda et al.), paraquat {Chen}, and numerous pharmaceuticals (Ciarimboli ; Ciarimboli). The importance of environmental contaminants as substrates for OCTs can be due to a number of different processes, discussed in more detail below. Contaminants can compete with endogenous substrates, interfering with homeostasis. Influx OCTs can actively take up contaminants, leading to much higher concentrations than would be expected based on passive diffusion. Furthermore, hormones and disease can influence expression rates of OCTs, which means estrogenic contaminants can influence OCT expression, while diseased or elderly individuals might be more susceptible to contaminants that are substrates for OCTs.

### 7.3.1 OCT Competition

The potential effects of OCT competition depend on the location of the OCT, direction of transport, and nature of the competing substrates. Different contaminants competing for an efflux transporter will lead to higher intracellular concentrations, which increases the likelihood of toxic effects (Ciarimboli). Contaminants competing with endogenous substrates will generally have negative effects, as transport of the endogenous molecule is hampered while the contaminant takes its place. Competition for renal influx transporters can have both negative and positive effects, partly depending on the linkage between influx and tubular excretion (Berkhin and Humphreys).

### 7.3.2 OCT Upregulation

Because OCTs have to deal with rapidly changing concentrations of substrates, especially in regions where they deal with excretion of waste molecules, they are prone to fast regulation if the need arises (Ciarimboli and Schlatter). Another interesting feature is their upregulation by male sex hormones i.e. testosterone, as experimentally established in rats (Ciarimboli and Schlatter). It is not likely that upregulation of OCTs by the means presented above will lead to an increased risk, although research into the exact effects of OCT upregulation is only in its infancy.

### 7.3.3 OCT Downregulation

Downregulation of OCTs is more probable to pose a threat, as renal excretion can be severely hampered. Experimentation in rats has shown that estrogenic compounds lower the expression of OCTs (Ciarimboli and Schlatter), an effect that might have additional significance, since table 3.1 in section 3.4 shows estrogenic compounds as emerging environmental contaminants. Downregulation of OCTs also occurs during disease, especially fever (Ciarimboli and Schlatter), and with aging (Wold, Joost et al. ; Goldstein, Pasino et al.). This would mean that subject already more susceptible to negative effects of toxicants have a higher risk, as the excretion of organic cations is decreased because of OCT downregulation.

### 7.4 Transcellular Transport: Chloroquine

Transcellular transport is essentially passive diffusion of a compound directly through the cell membrane, in contrast with active or assisted transport as mediated by, for instance, OCTs. This form of transport is possible since the cell membrane itself can function as a phase for the partitioning of chemicals. Chemicals that partition into the cell membrane are regularly able to diffuse through the membrane and exit intracellularly. A closely related phenomenon is the toxicological concept of narcosis, where chemical exert their toxic effect by partitioning into the cell membrane, thereby disrupting it and disturbing homeostasis. Compounds with high transcellular transport tend to have a high PBA, and in addition are able to pass the blood brain barrier, potentially giving rise to neurotoxic effects.

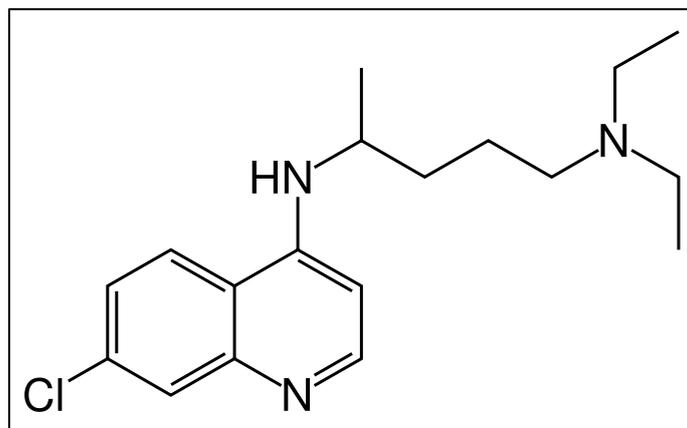


Figure 7.3: Molecular structure of chloroquine.

Despite their highly lipophilic nature, cell membranes are quite permeable to charged molecules {Erickson 2; 14-20 Erickson 1}. Chloroquine (figure 7.3) is an example of a compound that is charged at physiological pH, yet is still able to readily diffuse across biological membranes (Zhang, Zheng et al.). The  $pK_a$  of the tertiary amine is 9.86 and the  $pK_a$  of the quinoline is 7.65; at physiological pH of 7.4, a fraction of 99.7% is positively charged. While there is significant transport of chloroquine at physiological pH, this transport decreases with a factor 7 if the pH is lowered to 6.5 (Zhang, Zheng et al.) – a pH at which a large fraction of the quinoline becomes protonated (> 60%), and the molecule subsequently carries a divalent positive charge.

To make matters more complex, some contaminants can enhance transcellular diffusion or binding to membrane lipids. Some organic compounds can influence membrane permeability by increasing fluidity of the membrane {shikanga 1}, although specific experimentation is needed to distinguish

between this particular effect and changes in permeability by opening paracellular junctions and by the inhibition or activation of OCTs {smetanova 1; he}. Furthermore, inorganic cations can enhance binding of aromatic moieties (Zhou, Wang et al.), facilitating binding of organic compound containing an aromatic portion.

## 7.5 Highlights

- There is decent evidence for the significant uptake of ionized molecules into organisms.
- The presence of pH gradients in organisms can enhance uptake of ionizable compounds.
- Different tissue types can have distinct permeabilities for a given compound.
- Environmental organic cations can be substrates for OCTs, leading to toxic effects.
- Some cationic compounds can interfere with functioning of OCTs, leading to toxic effects.
- Ionized molecules are able to diffuse passively across biological membranes.
- Some contaminants can increase the permeability of biological membranes.

## 8. Conclusion

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The toxicity of any given compound is dependent on numerous factors. While risk assessment models reasonably predict the toxicity of neutral compounds, ionizable compounds still present a challenge, partly because their toxicity is dependent on additional factors. The toxicity of an ionizable compound in the environment is determined by the complex interplay of pH, soil composition, organic carbon content, presence of dissolved organic matter, the presence of other contaminants, the type of ions present and in what concentration, and other environmental conditions. To quantify all variables for every compound in each situation would be impossible, but recognizing and appreciating the core differences between ionized molecules and neutral molecules would already provide a significant improvement in the risk assessment of ionizable compounds.

Current models rely heavily on  $K_{O/W}$  as a determinant for both environmental fate and baseline toxicity.  $K_{O/W}$  is a sensible choice to predict the behavior of neutral molecules, but it would be wise to explore other avenues – such as partitioning into biolipids – when dealing with ionizable compounds, especially since  $K_{O/W}$  is hard to determine for ionized molecules. This can firmly be stated, since the preceding thesis has provided numerous examples illustrating ionized molecules are taken up into organisms, both by active uptake and by passive diffusion through cell membranes. Even though diffusion of charged molecules through lipid membranes is not predicted based on octanol-water partitioning, this diffusion is more than likely to pose a threat for the cellular machinery beyond – or inside – that lipid membrane.

In addition to ionized molecules being able to cross biological membranes, their behavior in the environment – like sorption to soil and sediment – is difficult to predict using models based on  $K_{O/W}$  as well. In sharp contrast with neutral compounds, the environmental behavior of ionizable compounds is subject to change based on pH. Lowering pH increases protonation of basic molecules and can decrease the neutral fraction of acids, while extremely low pH leads to protonation of humic acids and the subsequent ejection of positively charged molecules from their binding sites at carboxylate moieties. On top of this, inorganic ions can also influence sorption of ionized molecules, increasing the free concentration by competition or contributing to removal from the aqueous phase through cosorption. Since sorption is an important determinant for environmental risk, more insight into functional factors to model sorption of ionized molecules will be essential for adequate risk assessment.

So, in conclusion, the behavioral and physicochemical differences between ionized and neutral compounds are well documented. A significant portion of emerging contaminants are ionizable compounds, and 49% of REACH chemicals are ionizable at pH levels regularly occurring in the environment, underscoring the importance and size of this group of compounds. Distinguishing ionizable from non-ionizable molecules is elementary chemistry, so it would easily be possible to identify ionizable compounds and determine the environmental risk of such compounds in ways more suitable than  $K_{O/W}$  extrapolation. Specific models for their risk assessment need to be constructed based on existing and upcoming experimental data. Both the chemical industry and regulating authorities should recognize the importance of identifying missing pieces and finding them, instead of conveniently ignoring the ‘exceptions’ to the rule – which are legion.



## 9. References

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