The CBR approach in measuring toxicity of narcotic chemicals

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Abstract

Over the past years, the risk assessment of organic chemicals such as Persistent, bioaccumulative and toxic compounds (PBTs) and Persistent organic pollutants (POPs) has received growing attention because of their proprieties: persistence, bioavailability and bioaccumulation. In order to predict the toxicity levels of organic chemicals, it is important to know their chemical structure and the modes of actions. Quantitative structure-activity relationships models (QSARs) were used to classify narcotic chemicals. However, one of the problems in ecological risk assessment of organic chemicals is the determination of bioavailability from the ecosystem to organisms and mostly, from organism to the target (organ or cells). It was shown that the bioavailability is related to biological and physical properties of the target sites, such as lipid and protein contents of membranes. The Critical Body Residue Approach (CBR) or Lethal Body Burden (LBB) was developed in order to estimate critical effect levels of narcotic chemicals in the organism and thereby, to make an estimation of the true level of risk.

Making real estimations means also developing models that are as similar to the organism as possible, in terms of biological structures and partition dynamics. The more the model reflects the organism, the more precise and accurate the estimations will be.

Undoubtedly, future researches have to be done. Models should be improved and the characterization of compartments and proteins need particular attention because of the high variability as was already mentioned. It would be useful to improve already existing models and develop new ones in order to have a wider representation of important tissues. Proteins and their own proprieties should be integrated in these models and described using specific parameters as was already done with lipids.

1. Introduction

Over the past 30 years there has been an increasing concern about the potential effects of environmental pollutants on the ecosystem. Especially aquatic toxicology has received particular attention.

One major group of organic substances of concern is called PBTs (Persistent, Bioaccumulative, Toxic substances). PBTs are persistent because they remain unchanged for a relatively long time in the environment. Moreover, these chemicals are not easily metabolized or excreted and therefore tend to accumulate in organisms (Devillers, J. et al. 1996). These chemicals also hold toxic properties and hence are harmful to wildlife and the ecosystem.

In addition, there is another group of chemicals called Persistent Organic Pollutants (POPs) that have the same characteristics as PBT's, but they are also capable of long-range atmospheric transport (Vallack, H.W. et al. 1998).

Examples of PBTs and POPs are chlorinated and brominated aromatic compounds such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins and-furans (PCDD/Fs) and organochlorine pesticides (i.e DDT and chlordane). These chemicals are mainly synthesized for industrial or agricultural purposes (Jones, K.C., et al. 1998). Exposure to these chemicals might affect the neurologic, endocrine and immune systems, or might lead to genotoxicity (Vasseur, P. et al. 2006).

Bioavailability and bioaccumulation depend greatly on chemical and physical proprieties of these compounds and their environment. For example, the hydrophobic character of POP's leads to their presence mainly in soil rather than the aqueous face. Moreover, because of their high hydrophobicity, they accumulate in fatty tissues and cell membranes, but the diffusion rate to the soil or tissues might be slow.

One of the problems in ecological risk assessment of organic chemicals is the determination of bioavailability from the ecosystem to organisms and from organism to the target (organ or cells).

As it has already been mentioned, the bioavailability is strictly correlated to biological, physical, and chemical proprieties of chemicals and their environment. When extrapolating data from measurable concentrations in the environment or test systems to an actual toxic effect, these factors play an important role. When a model is designed for the prediction of a toxic effect based only on the matrix concentration of a chemical without taking its properties into account the risk assessment becomes highly inaccurate. In order to better predict the toxic effect, the correlation between matrix concentration and target site concentration needs to be modeled. To better understand the correlation between matrix concentration and toxicity at the target site, both the properties of the chemical and the properties of the different matrices should be taken into account.

As a solution for the problem the Body Residue (BR) approach was proposed. This is an approach that models the concentration of a toxicant in a body in relation to its environment. This new approach used the dose at the target site to characterize toxicant potency as a better dose-metric parameter instead of threshold concentrations in various environmental media such as water or soil. The use of a body residue as a measure of the toxic concentration developed into the Critical body Residue Approach (CBR) or

Critical Body Burden (CBB). The CBR was defined by McCarty et al. (1987) as the concentration expressed in molarity per body weight, which exerts a specific toxic effect like death or growth reduction. The applicability of the CBR approach has several advantages: the toxicity is not related to the matrix concentration or exposure pathways. Moreover, bioavailability and exposure concentrations are combined and, finally, parameters such as time and space are included in the CBR concept (Barron, M. G. et al. 1996).

As a tool to predict CBRs, Quantitive structure activity relationships (QSARs) are often used. QSARs describe the physical-chemical properties of a toxicant and its expected mode of action based on chemical groups in the molecule. Using the physical-chemical properties of a toxicant like hydrophobicity, polarity and size, a prediction for its concentration in certain matrices in comparison to other matrices can be made. This is done by predicting a coefficient for the concentration differences between two or more matrices in equilibrium.

One of the major target sites for toxicant in organisms is the cell membrane. In this thesis the approaches to predict toxic effect of chemicals at the cell membrane will be discussed.

In the first part, the classification of chemicals based on physical- chemical structure (QSARs) and mode of actions will be discussed. Then, some information about the expected targets of these chemicals will be given: it will be shown that certain classes of chemicals are most likely to have a toxic effect at the cell membrane. Furthermore, the membrane structure (lipids and proteins) and functions will be described in order to understand properties of the cell membrane and therefore the nature of chemical interactions at the cell membrane. Finally, some models used to predict the toxic effect of chemicals at the cell membrane membranes will be discussed. It will be shown that very simple models sometimes might suffice as a good prediction for toxic effects.

2. Chemical Classification, CBR and the Toxic ratio concepts

2.1 Chemical classification and QSARs

Quantitative and qualitative knowledge of chemicals are two very important focal points of environmental risk assessment. The chemical structure and physical-chemical properties of chemicals represent useful information to predict partition between environmental compartments, but also the mechanisms of uptake and distribution in organisms.

Over the past 30 years, a number of experimental models were proposed in order to predict the distribution and concentration of organic chemicals in different environmental compartments.

It is known that the Toxicity (T) of a chemical is generally expressed as Lethal Concentration (LC_{50}), which is the concentration that produces lethality in 50% of the organisms in a population in a specific range of time. Whereas, the Highest No Observed Effect Concentration (NOEC) is also used for sub-lethal toxicity (Maki, A.W. et al. 1985). The lack of experimental data motivated many researches to develop a new reliable screening tool: Quantitative Structure Activity Relationships (QSARs). This new approach was based on the mathematical relationship between toxicity and physical-chemical properties of the organic compounds such as the octanol-water partition coefficient (K_{ow}) and the presence of functional groups in the molecular structure. However, chemicals have not only different physical-chemical structures, but they also exert different modes of actions. Within this context, QSAR models were implemented based on the study of McKim and Bradbury, which identified six different modes of actions of chemicals (McKim, J.M. et al. 1987). A mode of action is defined as a physiological modification that characterized a specific biological response. The mode of actions and the final toxic effect are caused by a specific biochemical mechanism. McKim and Bradbury distinguished the following major modes of action: non polar narcotic chemicals, polar narcotic chemicals, chemicals acting trough uncoupling of oxidative phosphorylation mechanisms, irritation of respiratory membrane, inhibition of acetylcholinesterase (AChE) and abnormal activity of the central nervous system (McKim, J.M. et al. 1987).

The QSAR models and their databases were therefore improved and Verhaar, H.J.M et al. proposed a new classification, which included both the mode of action and the physical-chemical structure of organic compound (Verhaar, H.J.M. et al.1992):

Four different chemical groups were identified:

1. *Inert chemicals*: they are compounds that do not react with specific receptors in an organism. Their mode of action is called *narcosis*, which in aquatic organism is defined as a nonspecific and reversible disturbance of the membrane functions (Verhaar, H.J.M. et al.1992).
2. *Less inert chemicals*: they are called polar narcotic compounds and are characterized by a higher toxicity than baseline toxicity. Their toxicity is due the hydrogen bond donor activity.

Reports explained the quantitative difference between class 1 and 2 using the concept of LC_{50} and Lethal Body Burden (LBB) (McCarty, L.S. et al. 1987), which is the chemical concentration in the organism at the time of death.

3. *Reactive chemicals*: this third group is characterized by compounds that react with biomolecules and may be further metabolized in more toxic compounds (Verhaar, H.J.M.et al. 1992).

4. *Specifically acting chemicals*: they are classified as chemicals that exert their toxicity through interaction with specific structures like receptors (Verhaar, H.J.M. et al. 1992).

2.1 Critical body residue (CBR)

Chemicals present in the aquatic environment exert toxicity after uptake across external membranes or ingestion. However, the adverse effect is caused when the toxic concentration exceeds the critical threshold concentration at the target site. To assess critical toxic concentrations at the target site, the Critical Body Residue approach has been proposed. The CBR can also be called, Internal Lethal Concentration (ILC), or Lethal Body Burden (de Bruijn, J. et al.1991). In all these cases, the Critical Body Residue (CBR) is defined as the concentration of the toxicant, which is bioaccumulated in an aquatic organism and causes 50% mortality of the test organisms (Barron M.G. et al. 1997). The concentration of the toxicant is based on the wet weight of the organisms (mg/kg, mmol/kg) or lipid wet weight of the organisms (mg/kg, mmol/kg).

McCarty et al. proposed the CBR approach to estimated critical body residue of hydrophobic chemicals, which causes 50% mortality in a fish population in a specific range of time as equation 1 shows:

$$CBR \text{ or } LBB = BCF \times LC_{50} \qquad (1)$$ *(McCarty, L.S. et al. 1987)*

Where CBR (critical body residue) or lethal body burden (LBB) represents the concentration of a chemical able to cause 50% of mortality in a specific range of time and it is usually expressed in g/Kg. BCF is time-dependent Bioconcentration Factor of the chemical expressed in L/Kg. LC_{50} is the concentration of the chemical in water that cause 50% of mortality in a specific range of time expressed in g/L.

Toxicity and the bioconcentration of a narcotic chemical are a function of the hydrophobicity and the log bioconcentration factor (equation 2):

$$Log\ BCF = log\ K_{ow} - 1.3 \qquad (2)$$ *(Barron, G.M. et al 1992)*

Assuming that the log acute and chronic toxicity is equal to the concentration of a chemical in the aquatic organism (C_w) the CBR can be estimated using Equation 3:

$$CBR = BFC * C_w \qquad (3)$$ *(Barron, M.G. et al. 1992)*

Over the past years, several studies on guppies, goldfish and fathead minnows have been carried out in order to classify chemicals according to the CBR thresholds.

Data from experiments showed that for baseline toxicants the acute ILC_{50} was found constant between 2 to 8 mmol/ Kg (McCarty, L.S. et al.1991). For narcotic chemicals, the ILC_{50} measured was 2 mmol/Kg, while for sub lethal toxicity it was 0.02 mmol/L.

The variation of the ILC_{50} values depends on several factors. First of all, the exposure time is one of the most important factors, especially for acute (≤ 96 hours) and chronic (> 96 hours) exposures. Some studies showed that ILC_{50} values might decrease over time suggesting that the chemical might change its mode of action because of the formations of more toxic metabolites (De Wolf, W. et al. 1997, Chaisuksant, Y. et al. 1997). On the other hand, Van Wezel et al. (1995) have found no differences in the CBR over time (van Wezel, A.P. *et al*. 1995) and only one case in which the CBR increased with time (van Wezel, A.P. *et al*. 1995) It is important to point out that the measurements were done in steady-state conditions.

The lipid content is another very important factor. It represents one of the main targets in the membrane for narcotic chemicals (Meador, V. et al. 1993, van Wezel, A.P. *et al*. 1995). Organisms with higher lipid content take longer time to exhibit toxicity. This was proved for non-specific acting narcotics and chemicals that interact with a receptor. However, it should be taken in consideration that there is always a high variability related to different organisms and tissues, especially in terms of lipid and protein content. These differences might lead to inaccuracy of the data. In order to reduce the variability and increase the accuracy, the lipid normalization of the data was proposed (Meador, V. et al. 2006). With this context, McCarty et al. (1991) suggested also that the ILC_{50} values measured in bigger organisms were smaller than small organisms. Besides the higher lipid content, the time necessary to reach the concentration able to lead to an adverse effect is longer in bigger animals (McCarty, L.S. et al. 1991, Pawlisx, A,V. et al. 1993).

Chemicals exert their toxicity on the specific target sites with different modes of action, which are defined as a set of several physiological and behavioral signals that characterize the adverse effect in the organism. The endpoints in these experiments are more vaguely defined and therefore the researchers themselves might add to the variability.

A list of the main modes of action is mentioned in section 2.1. Chemicals may exert their toxicity with more than one mechanism and which one is most important depends on the concentration at the target site and the affinity to the target site. For example, McCarty et al. (1993) showed that chemicals such as PAHs, polychlorinated biphenyls at concentration of 2-8 μmol/g act as narcotic compounds but at lower concentrations, they exerted different responses. In addition, some chemicals might exert different mode of action for different species (McCarty, L.S. et al. 1993).

2.2 The toxic ratio as a descriptor of intrinsic toxicity

PBTs that are a wide group of chemicals that received particular attention because they are characterized by three important properties: Persistence (P), Bioconcentration (B), and Toxicity (T) (Maeder, V. et al. 2004). In order to estimate PBTs acute toxicity in aquatic organisms the traditional Lethal Concentration LC_{50} was used in combination with QSARs. In this case, the LC_{50} describes both the transfer of a chemical from the water to the organism and the toxicity related to different modes of action.

If the LC_{50} descriptor is used in PBTs assessment, the three parameters P, B and T are considered dependent from each other. For example, chemicals characterized by high T, tend also to have high B. To calculate the Internal Concentrations in the new PBTs assessment approach, the Toxic Ratio is used as a descriptor (Maeder, V. et al. 2004). As It was explained by Maeder et al., the two dimensions B and T are independent from each other. For example, even if the B dimension is high, the relative T might be very low because the toxicity is exerted by narcosis. In this case, the T dimension gives information relative to the real potency of the chemical at the target site that is might not depend on the ability of the chemical to bioaccumulate. In addition, the T parameter might be used also in case of hydrophilic chemicals, which do not easily bioaccumulate (very low BCF) but they lead to adverse effects. The P dimension is finally correlated to the B because the more a chemical is persistent, the higher the chance that a chemical is bioaccumulative (Maeder, V. et al. 2004).

In conclusion, the use of TR as a descriptor to measure Internal concentration of PBTs is very useful because the three dimensions are included and each of them gives specific information. However, its use is restricted only for PBT assessment because the LC_{50} and BFC data are widely available (Maeder, V. et al. 2004).

3. Targets: Membranes, Lipids and Proteins

In the previous chapter, the toxicity of chemicals was correlated to their physical and chemical properties, taken in account their modes of action. The modes of actions are defined as a set of physiological and behavioral modifications at the target site that cause the adverse response. However, the toxic responses are a consequence of the interaction between chemicals and biomolecules (i.e: receptors) at the specific target sites.

Therefore, it is important to identify the main target sites in an organism, which are membranes (specifically lipids and proteins) and DNA.

In this chapter, the attention will be focused on membrane structure, lipids and proteins and the consequence of chemical accumulation at the target site.

3.1 Membrane structure

The biological membrane is a fundamental component of the cell because it is an impermeable barrier between the external and internal environment. The general structure consists of a complex matrix of two phospholipids bilayers, which are the support for membrane proteins. Phospholipids are structured in order to have a hydrophilic head (consisting mainly of a phosphate and polar groups) and two hydrophobic chains, which influence the fluidity, stability and the dynamic nature of the membrane. Biomolecules that have both hydrophilic and hydrophobic groups are defined as amphiphiles. About 30-50% of the membrane consists of several types of proteins, which are classified in intrinsic (i.e. receptors or carriers) and extrinsic (i.e. enzymes), and for their functions (Fletcher, A., 2010, van Meer, G. et al. 2004). Interactions between lipids and proteins are regulated by hydrophobic and electrostatic forces. These forces stabilize the phospholipidic bilayer and the (trans-)membrane proteins, the Van der Waals forces play an important role in the protein-protein interaction.

Another common membrane characteristic is the presence of proteins organized in a specific order to regulate the systems of electron transfer, energy accumulation (ATP) and intracellular transport processes.

The characteristic of the membrane, such as flexibility and dynamicity are strictly correlated to the physical-chemical properties and the complex variety in the lipid distribution (Boesze-Battaglia, K., et al. 1997). The first study of membranes was conducted by Singer and Nicholson in 1972, who built the ‘fluid mosaic model’ (Singer, S.J. et al. 1972).

The most abundant class of lipids is represented by phospholipids that contain two fatty acid tails attached to a glycerol group. The top region has a polar group that is connected by glycerol to two fatty acid tails that influence packing and movement in the lateral plane of the membrane.

The nomenclature of phospholipids is based on their head group, which consists on choline, ethanolamide, serine or inositol. Phosphatidylcholine, phosphatidylserine and phosphatidylinositol

provide for water and/or ions to bind to polar headgroups. On the other hand, phosphatidylethanolamines have a hydrophobic character that promotes surface-to-surface interactions without direct protein binding (Boesze-Battaglia, K., et al. 1997).

Cholesterol is the second most abundant lipid in membranes and it belongs to the class of sterols. The general core structure, which consists of four hydrocarbon rings with a hydrophobic tail and and 3-b-hydroxyl groups. Cholesterol reduces the movement of phospholipid chains and thereby rigidify the membrane. This characteristic is called the condensing effect (Boesze-Battaglia, K., et al. 1997; Xu, X. et al. 2000).

Sphingolipids are another important class of lipids of membranes. The general structure consists of three main components: a sphingoid base (sphingosine) backbone, which is attached to a fatty acid tail, and a head-group. The nomenclature of sphingolipids is made according to their polar head groups, which can be: hydroxy-group (shyngosine and ceramide), phosphate, fatty acids, phosphocoline (sphingomyelin), glucose, galactose (glycosphingolipids). Sphingolipids play an important role for adhesion sites for proteins from the extracellular tissue, communication between cells and activities of membrane receptors. Besides that, the lipid backbone affects the biophysical changes of the membrane like the permeability or the curvature (Snook, C.F. et al. 2006).

About 30% of the total membrane weight is from membrane proteins. There are two different broad classes of proteins: intrinsic or transmembrane and peripherial proteins.

The so-called *transmembrane* proteins are oriented in the lipid bilayer in such a way that they adapt to the amphipatic structure of the membrane, therefore they have both hydrophobic and hydrophilic sides. From the molecular point of view, they exist in two different forms: Alpha-helical (plasma membrane of eukaryotes) and Beta-barrels (mainly in Gram +/- bacteria) (Lee, A.G. et al. 2004).

Membranes are dynamic structures where integral proteins and phospholipids interact thought hydrophobic effects and hydrogen bonding, which are important for the thickness of the lipid structure and fluidity. Therefore, the phospholipidic head groups phosphatidycholines and phosphatidylethanolamine) affect proteins conformation influencing the protein activities (A.G Lee, 2004). Examples of (trans-) membrane proteins are receptors, channels (Na+ K- ATPase, Ca++), transporters, and respiratory complexes.

Peripheral membrane proteins are attached to integral membrane proteins or connected to the peripheral regions of the lipid bilayer. The reversible bonding to the bilayer regulates enzymatic activities.

Narcotic chemicals are defined as compounds able to exert a non- specific disturbance of the membrane functions. The disturbance of the membrane is caused by the interaction of the toxic compound with the protein-lipid system. This phenomena was well explained by van Wezel, A.P. et al. (1996) that study the effect of chlorinated benzenes on membrane burdens. The fluidity of phospholipids bilayer was measured using phospholipid vesicles as model membrane (L-a-dipalmitoylphosphatidylcholine). The study showed that the phospolipid bilayer, during the main phase transition at physiological temperature, is in the gel

phase and liquid-crystalline phase. The presence of narcotic chemicals in the membrane burden was able to cause the increase of the membrane permeability (van Wezel, A.P. et al. 1996). Similar results were obtained by Muller et al. (1999), which investigated the toxic effect in membrane burdens of alcohol ethoxylates (AEO), a non-ionic surfactants. The membrane toxicities were studied using energy-transducing membranes of the purple bacterium Rhodobacter sphaeroides. The results showed that the presence of AEO was the cause of increased permeability of the phospholipids bilayer (Muller, M.T et al. 1999).

4. Descriptors and Models to predict CBR

In the previous sections, it has been explained that measuring concentrations of toxicants in the aquatic environment and predicting the concentration at the target site, is not as precise and accurate as measuring the Internal Lethal Concentration at the target site, the membrane. The structure and function of membranes have been briefly described to understand the compartments that have a higher affinity for hydrophobic chemicals.

The toxicity of chemicals called non-specific toxicity is caused by partitioning of the chemical into membranes and it represents the minimum level that a chemical has to reach in order to exert the toxicity. The partitioning equilibrium status greatly depend on 1) the chemicals, 2) the variability of the different components of the membrane and 3) the variability of these components in different organisms, as showed in Table 1:

Table 1: Chemical compositions of organisms

Organism (reference) [a]	%Lipid	%Protein	%Carbohydrate	Other
bacterium (1)				
Escherichia coli	10	60	5	25% DNA/RNA
phytoplankton (2, 3)	20 ± 10	50 ± 15	30	
lichen (4)				
Cladonia spp.	2	3	94	
vascular land plants				
"grasses" (5, 6, 7)	0.5 – 2	15 – 25		1 – 5% cutin
deciduous leaves (oak, maple) (8, 9)	3	15	42	26% lignin
pine needles (8)	28	8	47	17% lignin
"wood" (10)	4	1	66	29% lignin
apples (11)	5	0	95	
almonds (11)	56	21	22	
spinach (11)	0	50	50	
aquatic invertebrates				
zooplankton (2)	15 – 35	60 – 70	10	
copepod (8)	10	65	25	
amphipod (*Pontoporeia hoyi*) (12)	9 – 46			
shrimp (*Mysis relicta*) (12)	10 – 41			
oyster (8)	12	55	33	
zebra mussel (13)	8 – 12	50 – 60	30 – 40	
polychaete (*Abarenicola pacifica*) (14)	12			
chironomid larvae (15)	6 – 13	65 – 71	21 – 23	
terrestrial invertebrates				
earthworm (*Lumbricus rubellus*) (16)	5			
aquatic vertebrates				
fatty fish				
trout filet (11)	30	70	<3	
lake trout (17)	6 – 18			
salmon (11, 18)	11			
lean fish				
cod filet (18)	0.7			
pike filet (18)	0.7			
terrestrial vertebrates				
deer meat (11)	10	90	<1	
beef (roast) (11)	20	80	<3	
caribou muscle (19)	5 – 12			

[a] (1) Neidhardt et al. (1990); (2) Parsons and Takahashi (1973); (3) Shifrin and Chisholm (1981); (4) Ahti and Hepburn (1967); (5) Hinson et al. (2001); (6) Böhme et al. (1999); (7) Tolls and McLachlan (1994); (8) Hunt (1979); (9) Aber and Martin (1999); (10) Thompson (1996); (11) Rombauer et al. (1997); (12) Cavaletto and Gardner (1998); (13) Nagela et al. (1993); (14) Weston et al. (2000); (15) Beattie (1978); (16) Ma et al. (1998); (17) Thomann and Connolly (1984); (18) Ewald (1996); (19) Kelly and Gobas (2001).

These data are taken from Environmental Organic Chemistry-Chapter 10, p337, Table 10.1)

In order to investigate the partitioning equilibrium, surrogates of biological membrane and partitioning coefficient descriptors of organic phases have been studied. Examples of this are the (trioleine)-water partitioning and the octanol-water partitioning. The two coefficients were compared by Chiou, C.T. et al. 1985. The Trioleine (glyceryl trioleate) was proposed because the chemical structure of glyceryl trioleate is comparable to triglycerides in aquatic organisms. The results showed that although there is a structural similarity between trioleine and the membrane lipids, the use of log Kow was a better descriptor for estimations of the membrane partitioning of non-ionic chemicals then trioleine. The trioleine-water system showed problems to reach equilibrium, therefore it was not a good descriptor for chemicals that are unstable in water or quickly metabolized by the organism. Hence, it was not suitable in short time exposure studies (Chiou, C.T et al. 1985).

The difference in membrane composition increases the need to develop models that could represent the reality as much as possible.

It is known that hydrophobic organic chemicals tend to accumulate mainly in the storage lipid compartments, which consists of storage and neutral lipids. If these compartments are included in the model, the estimation of the CBR or LBB would be easier. Under this assumption, a simple three-compartment model was developed by Van Wezel, A.P et al. (1995) (Fig.1) and improved by Vaes, W.H.J. et al. (1997). The models described consisted of 95% aqueous phase and 5% lipids phase. The lipid phase included 75% of storage lipids (high affinity with hydrophobic chemicals) and 25% of polar lipids. In the first study, the LBB of three chlorophenols was estimated in fathead minnow (Pimephales promelas) using the n-octanol as a descriptor for polar lipids (membrane water partitioning) and n-hexane (K_{nw}) for non polar lipids (storage water partitioning). In the second study, Vaes et al. used the L-α-dimyristoyl phosphatidylcholine (DMPC) water partition coefficient (K_{DMPC}) and K_{ow} to describe polar lipids and neutral lipids, respectively. The results of the two studies showed that the quantitative difference between polar and non polar compounds is explained simply by the different partitioning to the lipid compartment of the membrane. Thus, the use of more complicated models has the advantage to improve the accuracy and precision in the estimation of the critical effect concentration of narcotic chemicals. On the other hand, the use of the right descriptor is fundamental for the correct interpretation of the data.

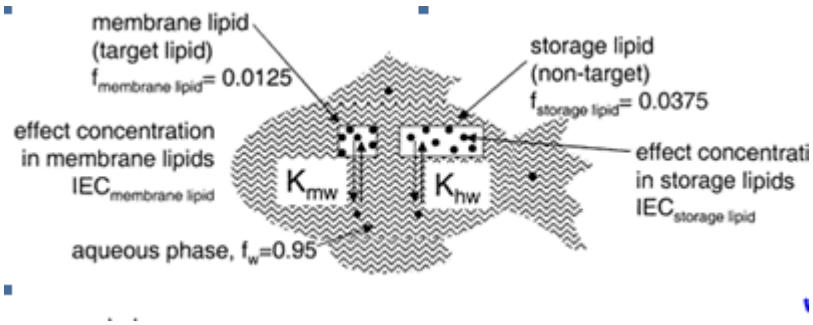
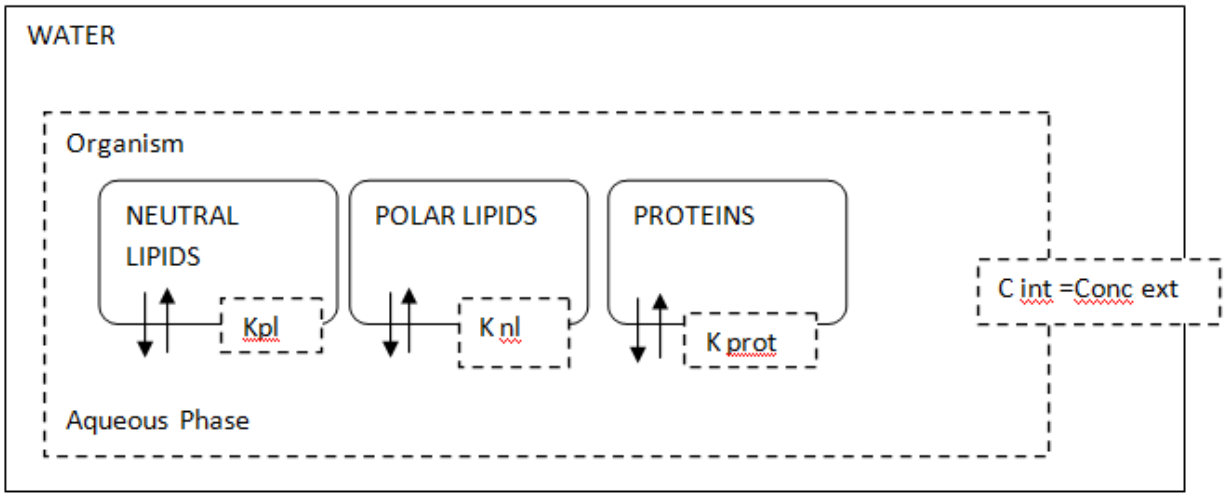


Figure 1. Three-compartment model for fish (These data are taken by Escher et al. 2002)

It is clear that lipids are not the only variable component of the membrane. The proteins represents between 20 and 30% of the total membrane weight. In chapter 3 it was explained that proteins are macromolecules that play an important role in the maintenance of the membrane integrity and functionality. They are characterized by hydrophilic, but also hydrophobic proprieties (i.e. trans membrane proteins). However, the models described before take in account only the water and lipids compartments excluding proteins. Thus, a more precise estimation of the CBR would include protein as a compartment as well. A clear example is proposed by Legierse et al. (1996), which applied a multi-compartment model. The bioaccumulation of chlorobenzenes and organophosphorus pesticide chlorthion was studied in pond snail. The organisms tested contained 0.4% polar lipid, 0.5% apolar lipid, 2.8% proteins and the rest 96% water. The compartments used in the model are represented in Figure 2.

Fig. 2. Multi-compartments model developed by Legierse et al. (1996)



In this model, each compartment contributes to the total concentration of the chemical in the organism as Equation 4 shows:

$$C\ org = \left(Cnl\ \frac{Mnl}{Morg}\right) + \left(Cpl\ \frac{Mpl}{Morg}\right) + \left(Cprot\ \frac{Mprot}{Morg}\right) + \left(Cint\ \frac{Ma}{Morg}\right) \qquad (4)$$

(4)(Legierse K.C.H.M et al al.1996)

Here, C org, Cnl, Cpl, Cint, Cprot are the concentrations in the organism, neutral lipids, polar lipids, aqueous environment and proteins, respectively. While, Morg, Mnp, Mlp,M prot. mean the wet weight of the whole organism, neutral lipids, polar lipids and proteins, respectively Legierse, K.C.H.M et al. (1996). Moreover, K_{ow} was chosen as a descriptor for the neutral lipid water partitioning coefficient (Chiou, C.T et al. 1985), while K_{DMPC} represented the polar lipid water partitioning coefficient. Besides, the cytosolar protein partition coefficient (K_{prot}) was used as a partition coefficient for proteins. The result showed that

the polar compound chlorothion was significantly present in the protein phase, confirming that protein added a significant contribution to the total accumulation of the chemicals (Legierse, K.C.H.M. et al. 1996).

5. Summary and Conclusions

One of the main problems in aquatic risk assessment is the bioavailability and bioaccumulation potential of narcotic chemicals in the environment, the organism, and specifically, the target site.

The Critical Body Residue approach developed by McCarty, L.S. et al (1987) turned out to be a valid tool to measure the critical concentrations of chemicals at the target site, in this case the membrane.

Chapter two showed that the prediction of critical effect concentrations included first of all, a detailed knowledge of the physical and chemical proprieties of narcotics. Narcotic chemicals act by disturbing the normal functionality of the membrane. In order to better understand the effect of chemicals, chapter three provides a description of the structure and functions of the target site. All this information contributes to the understanding of the concept of CBR and why it has been used in aquatic risk assessment. The estimation of CBR in biological matrix takes in account the theory of the compartments model. Because of the fact that the organism consists of different anatomical parts, the use of two, three and four compartments gives a better estimations of the critical body residue. As described in chapter four, the use of multi- compartment models was shown to be convenient for many reasons. They play an important role both as such and in the overall system. On the other hand, there are few disadvantages of using multi-compartment models for CBR estimations. Firstly, each compartment has to be described by the right coefficient. Second the distribution of the chemical over two or more compartments that are not in equilibrium with each other make the CBR estimation quite difficult, because diffusion rate constants have to be taken into account.

The first conclusion that can be drawn is that although the complexity of the multi-compartment models in terms of partitioning equilibrium and structural differences of the matrix, they are for most of the cases the best choice. The second conclusion is that targets such as proteins are considered fundamental for reliable CBR estimations.

The variability in structure and functions of both the narcotic chemicals and the target they act on, requires dynamic models that take into account different mechanisms of action and different concentration equilibriums. To develop these models future research is needed because of the lack of data on PBT chemicals to proteins.

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