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Experimental and computational study of membrane affinity for selected energetic compounds

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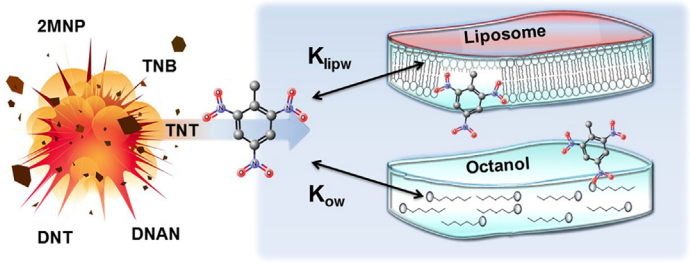
HIGHLIGHTS

- We performed experimental measurements and theoretical calculations of partitioning coefficients for energetic compounds.
- Selected energetic compounds possess moderate ability to penetrate into biological membrane.
- We made basic statistical analysis for available partitioning coefficients.

GRAPHICAL ABSTRACT



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ABSTRACT

The affinity of various energetic compounds for a biological membrane was investigated using experimental and computational techniques. We measured octanol–water ($\log(K_{ow})$) and liposome–water ($\log(K_{lipw})$) partition coefficients for the following chemicals: trinitrotoluene (TNT), 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT), 2,4-dinitroanisole (DNAN), 2methoxy-5-nitrophenol (2M5NP), 2,4,6-trinitrobenzene (TNB), and 2,4-dinitrophenol (2,4-DNP). In order to determine $\log(K_{lipw})$ experimentally, we used artificial solid supported lipid liposomes produced under trade mark TRANSIL. $\text{Log}(K_{ow})$ value were predicted with several program packages including the COSMOthermX software. $\text{Log}(K_{lipw})$ were estimated with COSMOmic as implemented in the COSMOthermX program package. In order to verify accuracy of our experimentally obtained results, we performed basic statistical analysis of data taken from the literature. We concluded that compounds considered in this study possess a moderate ability to penetrate into membranes. Comparison of both coefficients has shown that in general, the $\log(K_{ow})$ values are slightly smaller than $\log(K_{lipw})$.

1. Introduction

The penetration of chemicals through biological membranes is a key factor in the determination of the possible effects of substances such as toxicants and drugs interacting with biological systems

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(Endo et al., 2011). The simplest way to characterize the ability of a substance to bioaccumulate is to consider the partition coefficient between water and a lipid pool i.e. biological membrane. In its simplest form, bioconcentration has been characterized as the ratio of the equilibrium concentrations of a chemical (solute) that partitions between n-octanol (representing the lipid fraction) and water:

$$K_{ow} = \frac{[c_o]}{[c_w]} \quad (1)$$

where C_o and C_w are the molar equilibrium concentration of the solute in octanol and water at a specified temperature, correspondingly. General formula for partitioning coefficient was described by Nernst (1891).

However, to obtain an accurate estimate of this partition in a realistic lipid system, factors relating to membrane specifics such as type of lipid, (Kramer, 2001; Avdeef, 2012) chemical structure of the lipids that comprise the membrane, (Poulin and Theil, 2002; Rodgers and Rowland, 2006; Schmitt, 2008) external conditions, (Poulin and Theil, 2002; Rodgers and Rowland, 2006; Schmitt, 2008) etc.) need to be taking into account.

Despite the fact that the traditional definition of K_{ow} actually characterizes hydrophobicity of a substance, numerous studies have shown (Jonker and Van Der Heijden, 2007; Endo et al., 2011) that in many cases also bioconcentration can be described by this parameter quite efficiently. The exceptions are among highly hydrophobic ($\log(K_{ow})$ greater than 5–6) and ionic compound. Experimental measurement of K_{ow} is often times complicated and time consuming since partition equilibrium is difficult to determine. Given the difficulties in measuring partitioning of organic substances across a biological membrane, many researchers substitute octanol–water partition coefficients as a measure of the partitioning across lipid bilayer.

To improve the ease and repeatability of bioconcentration measurements, recently test membranes comprised of a class of artificial membrane vesicles made up from phospholipids (liposomes) were synthesized and made commercially available. Such model systems support the investigation of numerous biological processes (ligand–receptor interaction, (Plant et al., 1995; Pum et al., 1997; Yang et al., 2001, 2003) viral attack, (Ono and Freed, 2001; Xu et al., 2002) cellular signaling events (Kasahara and Sanai, 2001; Qi et al., 2001; Stoddart et al., 2002)) including measuring partition between water and liposome. Similar to Eq. (1) such partition will be defined as

$$K_{lipw} = \frac{[c_{lip}]}{[c_w]} \quad (2)$$

where C_{lip} and C_w are the molar equilibrium concentration of the solute in liposome and water respectively.

There exist several experimental techniques to measure K_{lipw} (Castellana and Cremer, 2006). One of these techniques is based on the solid supported lipid membranes application, which is commercially available under the trade mark TRANSIL (Loidl-Stahlhofen et al., 2001a, 2001b). In these systems, porous silica beads are covered by a unilamellar liposomal membrane that is noncovalently bounded to the bead. The main advantage of these systems is the very short time (c.a. 2 min) needed to reach equilibrium. This is in contrast to other liposomal-based approaches where in some cases up to 12 h is required to reach equilibrium (Loidl-Stahlhofen et al., 2001a). Experimental measurements of K_{ow} are often similarly time-consuming.

The existence of two sets of parameters that characterize related chemical behavior, hydrophobicity (K_{ow}) and bioconcentration (K_{lipw}), raises the question on the comparison and relative importance of them. On one hand it has been stated that K_{lipw} is a more accurate descriptor to estimate membrane affinity of chemicals (Jonker and Van Der Heijden, 2007; van der Heijden and Jonker, 2009). Several publications indicate not only the difference between K_{ow} and K_{lipw} but also the absence of the correlation between the corresponding values (Jabusch and Swackhamer, 2005; Jonker and Van Der Heijden, 2007). It is also expected that penetration of neutral compounds from water solution into membrane is higher than from water to octanol ($K_{lipw} > K_{ow}$) (Gobas et al., 1988; Dulfer and Govers, 1995; Endo et al., 2011).

Conversely, it is also noted that K_{ow} is still a fundamental parameter that is used in most bioaccumulation models. Several publications show that even if a difference between K_{ow} and K_{lipw} exists, their values have the same trend (Endo et al., 2011). In addition K_{ow} values are available for a large number of chemicals, while the currently available amount of K_{lipw} values is significantly less.

A third approach to estimate both K_{ow} and K_{lipw} values is based on computational methods. Currently among the most efficient tools for such predictions are Quantitative Structure – Activity (Property) Relationships (QSAR/QSPR) techniques. These approaches have been successfully used for over forty years (Jonker and Van Der Heijden, 2007) especially in the environmental science and pharmaceutical industries to predict K_{ow} values. Another group of computational techniques is based on combination of quantum-mechanical and statistical approaches (Klamt, 1995; Gurtovenko et al., 2004; Patra et al., 2004; Klamt et al., 2008; Sapay and Tieleman, 2011). This approach allows both K_{ow} and K_{lipw} to be estimated. In addition, quantum-chemistry based techniques do not need a training set of compounds since they directly calculate K_{ow} and K_{lipw}. Both approaches have been shown to predict these values with near experimental accuracy, and are also cost effective. Another computational way to predict K_{ow} and K_{lipw} is based on classical molecular dynamics. However, this approach is very time consuming because one needs to get very long trajectories (hundred nanoseconds or even microsecond) (Lange et al., 2006; Zhang and Voth, 2011; Paloncýová et al., 2014). Therefore, selected computational studies can be used to supplement more extensive and costly experimental evaluations (Endo et al., 2011).

In this work, measurements and verifications of the K_{lipw} values for the following chemicals was undertaken: trinitrotoluene (TNT), 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitro-toluene (2,6-DNT), 2,4-dinitroanisole (DNAN), 2-methoxy-5-nitrophenol (2M5NP), 2,4,6-trinitrobenzene (TNB), and 2,4-dinitrophenol (2,4-DNP). These compounds are energetic compounds that may be potential environmental contaminants. Since experimentally obtained values of K_{ow} and K_{lip} cover the interval in several order of magnitude, in practice the log (K_{ow}) and $\log(K_{lip})$ are used. While there are numerous publications on predictions of the $\log(K_{ow})$ values for these compounds (Hansch and Leo, 1979; Jenkins, 1989; Hansch et al., 1995; Boddu and Redner, 2010), to the best of our knowledge, neither experimental nor computational values of $\log(K_{lipw})$ have been published. Thus, this study also includes comparison and analysis of $\log(K_{lipw})$ obtained experimentally and computationally.

2. Materials and methods

2.1. Experimental approach

TNT and 2,4-DNT are from ChemService (Westchester, PA, USA), 2,6-DNT from SRI International (Menlo Park, CA, USA), TNB, DNAN, 2M5NP, from Sigma–Aldrich (St. Louis, MO, USA). 2,4-DNP is from Fischer Scientific (Pitsburgh, PA, USA). Compound stock solutions