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Short Communication

The Role of Hydrophobicity in Bio-Accessibility of Environmental Pollutants Among Different Organisms

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Abstract

This study concerns the relationship between hydrophobicity and bio-accessibility of environmental pollutants among the protozoan *Tetrahymena pyriformis*, the water flea *Daphnia magna*, and the fish *Poecilia reticulata*. The toxicological data of 55 chemicals in terms of 50% effect concentration was selected toward these three biological objects along with their hydrophobic potential (octanol-water partition coefficients (log K_{ow})). Overall, a significant correlation was achieved among all test systems, with the highest between *Tetrahymena pyriformis* and *Poecilia reticulata* (R = 0.93). The acute toxicity results revealed substantial differences in the sensitivity of the three test systems, but at a certain level of hydrophobicity (log K_{ow} values 0.5 to 2.5), where all environmental pollutants have the utmost ability to reach biological compartments as cytosole and target sites within the membranes, to interfere with normal cell functioning by effecting normal enzymatic activity and directly to biological macromolecules.

Keywords: hydrophobicity, toxicity, protozoan, water flea, fish

Introduction

In the modern industrial world, organic chemicals are in extensive use and our environment is regularly exposed to such chemicals through industry, agriculture, and other human activities [1-3]. However, limited information is available about their potential risk to our environment, especially in third world countries because of time and monetary constraints. Ultimately, different efforts have been made to look into other avenues, i.e., physico-chemical (log P, molecular weight, solubility, and Henry's Law Constant) properties, different biological barriers (cell wall, cytosol organelles, and enzymes) and modelling of existing data to save resources in terms of money, labour, and biological objects to address the toxic potential of pollutants. Among different physico-chemical properties (surface adsorption forces = hydrophobicity, solubility, hydrogen bonding, polarity,

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polarizability, and lone-pair electrons) of organic pollutants, hydrophobicity plays a substantial role in the bio-accessibility of these chemicals [4-7]. It has been extensively identified that, according to their mode of action, there are two main classes of organic chemicals in the aquatic environment: chemicals of non-specific mode of action and those with a specific mode of action [8-10]. Chemicals with non-specific toxicity interact with the lipids of bio-membranes and therefore their affect is based on their hydrophobicity. The hydrophobic potential of the environmental pollutants can always be calculated from octanol-water partition coefficients (log K_{ow}) [11-13]. On the other hand, chemicals with a specific mode of action have specific target-oriented effects (based on the specific receptors), but the non-specific parts (because of their hydrophobic potential) also have a contributory effect [9]. In higher hydrophobic chemicals the concentration of the chemicals remains low in an aqueous environment and their specific toxic effects will not be apparent [14]; however, the fractional non-specific toxic effect from hydrophobicity will remain and will act as an additive [15-16]. Marine bacterium *Vibrio fischeri*, protozoa *Tetrahymena pyriformis*, water flea *Daphnia magna*, green algae, and fish are the most commonly used laboratory species [17-23]. A large amount of toxicity data obtained by reliable and robust methods is available for these test systems [24-29]. However, it still remains unclear whether the linear relationship between hydrophobicity and toxicity exists for all environmental pollutants [30], therefore the area still needs to be unfolded using different criteria (molecular descriptors, cell wall affinity, structure complexity, and Henry's Law Constant). The aim of this study was to identify toxicological differences while keeping in view the different hydrophobicity levels among *Tetrahymena pyriformis*, *Daphnia magna*, and *Poecilia reticulata*. The sensitivity of the three bio-indicators with reference to their hydrophobicity was analysed to obtain a range of hydrophobicity with clear differences in their pertinent sensitivities toward different chemicals.

Materials and Methods

For this work bibliographical research was carried out and the toxicity data of 55 organic compounds, including narcotic compounds, was obtained from literature toward three different test systems using organisms including *Tetrahymena pyriformis*, *Daphnia magna,* and *Poecilia reticulate*. The molecular descriptor (log K_{ow} values = octanol/water partition coefficient) has been calculated using EPI Suite (USEPA, 2009). The 96-h guppy (*P. reticulata*) mortality (LC_{50}) and the 40-h ciliate (*T. pyriformis*) population growth impairment (IGC_{50}) data (secured from TETRA - TAX) for these toxicants were collated in an appendix by [24] as the source of the data. The protocol details for *T. pyriformis* population growth impairment are given in [29]. The presented toxiciare value for *Daphnia magna*, the medium lethal concentration (LC_{50} Immobilization) for essays of 48-h were collated in an appendix by [28] as the source of the data. All the comparisons were made using Sigma plot 11 software.

Results and Discussion

The research performed resulted in three systemized toxicity test systems with relevance to their hydrophobicity. The toxicity database contains 55 organic chemicals with different hydrophobicity levels ranging from -0.24 to 3.93. Interspecies toxicity correlation was carried out for the toxicity values illustrated in Table 1 for all three test systems. Overall, a significant correlation was obtained among all test systems. The interspecies toxicity correlation was highest between *Tetrahymena pyriformis* and *Poecilia reticulata* (R = 0.93), while the correlation was slightly weaker between the toxicity toward *Tetrahymena pyriformis* and *Daphnia magna* (R = 0.73) and between the toxicity toward *Daphnia magna* and *Poecilia reticulata* (R = 0.74).

The aim of this study was to identify toxicological differences of different chemicals at hydrophobicity levels among *Tetrahymena pyriformis*, *Daphnia magna,* and *Poecilia reticulata*. The sensitivity of the three bio-indicators with their hydrophobicity data is illustrated in Table 1. The results obtained suggest that hydrophobic property of chemicals have a strong influence on chemical uptake by organisms as translated into different toxicity levels. The acute toxicity results reveal a substantial difference in the sensitivity of the three test systems, but at a certain level of hydrophobicity (log K_{ow} values 0.5 to 2.5, as shown in Fig. 1). In general, toxicological data suggests that all organisms' sensitivity to these organic chemicals lay on the same order of the magnitude. Analysing the acute toxicity bio-assays performed separately for these chemicals showed potentially that we cannot see any toxicological difference at very low (*Tetrahymena* vs. *Poecilia*) and very high (*Daphnia* vs. *Poecilia*) hydrophobicity levels. The hydrophobic effect is considered to be the major driving force for the intake of xenobiotics in aquatic species [31]. This results in the burial of the hydrophobic residues in the cellular membranes, causing the narcotic effect. However, the concentration of the chemicals in the aqueous cytosole is decreased, which results in less available to reaction targets such as protein sites in the hydrophilic environment of the cell contents of the aquatic species. The ability of a compound to chemically modify proteins and other endogenous macromolecules (DNA) depend on its hydrophobicity (log K_{ow}) and reactivity (the presence of reactive moieties) [32]. Hydrophobic chemicals on accumulation in membranes may therefore severely disturb the functioning of the membrane and also the different membrane-bound systems. The majority of all the industrial chemicals (about 60%) act as baseline toxicants, i.e., they interfere with the membrane structure

Table 1. Set of 55 organic chemicals with information about their hydrophobicity and toxicity toward the protozoan water flea and fish.

Sr. No.	Chemical	CAS	log K_{ow}	*Tetrahymena pyriformis*[a] (M)	*Daphnia magna*[b] (M)	*Poecilia reticulata*[c] (M)
1	Acetone	67-64-1	-0.24	-0.8	-0.62	-0.9
2	Ethanol	64-17-5	-0.14	-0.69	-0.59	-0.56
3	2-Methyl-2,4-pentanediol	107-41-5	0.58	-1.04	-1.22	-1.04
4	Isobutanol	78-83-1	0.77	-1.63	-1.82	-1.71
5	Aniline	62-53-3	1.08	-2.77	-5.33	-2.91
6	2,2,2-Trichloroethanol	115-20-8	1.21	-2.54	-3.00	-2.69
7	Phenol	108-95-2	1.51	-2.54	-3.44	-3.45
8	3-Methoxyphenol	150-19-6	1.59	-2.67	-3.48	-3.22
9	2-Methylaniline	95-53-4	1.62	-2.45	-5.31	-3.12
10	3-Methylaniline	108-44-1	1.62	-2.58	-5.17	-3.47
11	1,3-Dinitrobenzene	99-65-0	1.63	-3.76	-3.59	-4.64
12	2-Chloroaniline	95-51-2	1.72	-2.75	-5.19	-4.31
13	3-Chloroaniline	108-42-9	1.72	-3.01	-6.11	-3.98
14	4-Chloroaniline	106-47-8	1.72	-4.35	-6.41	-3.67
15	Nitrobenzene	98-95-3	1.81	-3.14	-3.48	-2.97
16	2-Methylphenol	95-48-7	2.06	-2.71	-4.05	-3.77
17	3-Methylphenol	108-39-4	2.06	-2.97	-3.04	-3.48
18	4-Methylphenol	106-44-5	2.06	-2.79	-3.68	-3.74
19	2-Ethylaniline	578-54-1	2.11	-2.65	-4.18	-3.21
20	4-Ethylaniline	589-16-2	2.11	-3.05	-6.13	-3.52
21	2-Chloro-4-nitroaniline	121-87-9	2.12	-3.75	-4.49	-3.93
22	Quinoline	91-22-5	2.14	-3.09	-3.53	-3.63
23	4-Chlorophenol	106-48-9	2.16	-3.54	-4.42	-4.18
24	2,4-Dinitrotoluene	121-14-2	2.18	-3.64	-3.72	-4.16
25	1-Chloro-2,4-dinitrobenzene	97-00-7	2.27	-4.98	-5.4	-6.19
26	2-Nitrotoluene	88-72-2	2.36	-3.05	-4.14	-3.59
27	3-Nitrotoluene	99-08-1	2.36	-3.05	-4.04	-3.65
28	4-Nitrotoluene	99-99-0	2.36	-3.17	-4.01	-3.67
29	2,5-Dichloroaniline	95-82-9	2.37	-3.58	-4.74	-4.99
30	3,5-Dichloroaniline	626-43-7	2.37	-3.71	-5.16	-4.62
31	2,4-Dichloroaniline	554-00-7	2.37	-3.56	-5.43	-4.41
32	3,4-Dichloroaniline	95-76-1	2.37	-4.37	-5.95	-4.39
33	Tetrachloromethane	56-23-5	2.44	-2.71	-3.64	-3.36
34	2-Chloronitrobenzene	88-73-3	2.46	-3.39	-3.64	-3.72
35	3-Chloronitrobenzene	121-73-3	2.46	-3.63	-3.84	-4.01
36	4-Chloronitrobenzene	100-00-5	2.46	-3.21	-4.31	-4.42
37	Toluene	108-88-3	2.54	-2.5	-2.8	-3.13
38	2,4-Dimethylphenol	105-67-9	2.61	-2.96	-4.43	-3.86
39	Chlorobenzene	108-90-7	2.64	-2.87	-3.77	-3.77

Table 1. Continued.

40	4-Chloro-3-methylphenol	59-50-7	2.70	-3.8	-4.85	-4.33
41	2,3-Dimethylnitrobenzene	83-41-0	2.91	-3.56	-4.56	-4.39
42	3,4-Dimethylnitrobenzene	99-51-4	2.91	-3.59	-3.98	-4.21
43	2-Chloro-6-nitrotoluene	83-42-1	3.00	-3.68	-4.61	-4.52
44	4-Chloro-2-nitrotoluene	89-59-8	3.00	-3.82	-4.27	-4.44
45	2,3,4-Trichloroaniline	634-67-3	3.01	-4.35	-5.43	-5.15
46	2,4,5-Trichloroaniline	636-30-6	3.01	-4.3	-4.76	-4.92
47	4-Xylene	106-42-3	3.09	-3.12	-3.52	-3.48
48	3,5-Dichloronitrobenzene	618-62-2	3.10	-4.13	-4.46	-4.63
49	2,3-Dichloronitrobenzene	3209-22-1	3.10	-4.07	-4.62	-4.66
50	2,4-Dichloronitrobenzene	611-06-3	3.10	-3.99	-4.66	-4.46
51	2,5-Dichloronitrobenzene	89-61-2	3.10	-4.13	-4.26	-4.59
52	3,5-Dichloronitrobenzene	618-62-2	3.10	-4.13	-4.46	-4.58
53	1,2-Dichlorobenzene	95-50-1	3.28	-3.53	-4.81	-4.4
54	2-Phenylphenol	90-43-7	3.28	-4.09	-5.38	-4.76
55	1,2,4-Trichlorobenzene	120-82-1	3.93	-4.08	-4.16	-4.83

Log K_{ow} = decadic logarithm of the octanol/water partition coefficient calculated with EPISuite [21], LC50 [mol/L] / IG_{50} [mol/L] = effective concentration yielding 50% inhibition. [a,c]96-h guppy (*P. reticulata*) mortality (LC_{50}) and the 40-h ciliate (*T. pyriformis*) population growth impairment (IGC_{50}) data was collated in an Appendix by Seward (2002). [b]Medium Lethal Concentrations (LC_{50} Immobilization) for essays of 48-h, were collated in an Appendix by Von der Ohe (2005).

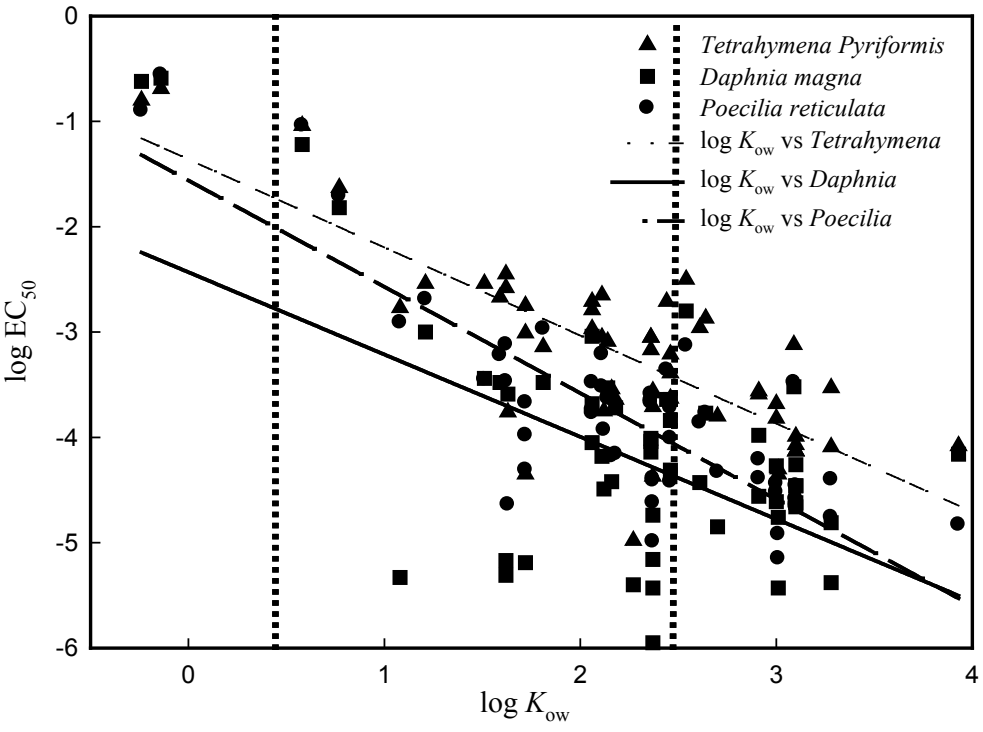

Fig. 1. Acute toxicity of 55 organic chemicals toward protozoan *Tetrahymena pyriformis*, water flea *Daphnia magna,* and fish *Poecilia reticulata* vs. their hydrophobicity values (log K_{ow}); the two vertical lines represent the area with distinguish toxic response among all three bio-tests.

and functioning by partitioning into the membrane [10, 33]. Certain compounds on availability of specific targets may additionally exhibit more specific and selective mechanisms. The presence of weak organic acids may destroy the electrochemical proton gradient, which is an intermediate in energy transduction, and on the other hand compounds with specific functional groups and reactive moieties may bind to membrane-bound enzymes and receptors [34]. Essentially, the adverse effects of pollutants in aquatic ecosystems depend on the total concentration of a chemical in aqueous environment, the bio-accessible fraction, and the final concentration reaching the target site(s). Furthermore, the fate of the environmental pollutants not only affected by their physico-chemical properties but also the characteristics of the environment and biological processes also play a critical role in the uptake of these chemicals [35-38]. Hydrophobic ionizable organic compounds (HIOC) such as weak organic acids (e.g., phenolic pesticides) can in addition destroy the electrochemical proton gradient through short-circuiting the energy cycle that in response may influence their bio-avalaibility and bioaccumulation, as well as sorption to organic matter and particles [12]. Therefore, the hydrophobic compound can disturb membrane energization by non-specific membrane perturbation, exerting a narcotic-level effect or baseline toxicity. The lipophilic compounds most likely penetrate and are captured in hydrophobic membranes, in which cytochromes P450 are ingrained, thereby gaining access to these enzymes and pointing to a specific mode of action. The partitioning into the lipid phases is not very dominant for overall partitioning of the low hydrophobic molecules having log K_{ow} values less than 2, therefore the contribution of the pollutant concentration in the aqueous compartments of the organism has to be considered in calculating the overall internal effective concentration [39]. Mostly, pesticides are moderate to weak acids in nature, and strong acid pollutants are fully ionized at ambient pH, i.e., trifluoroacetic and chloroacetic acids used as herbicides have been banned, but these still occur as solvent degradation products [40-42]. Polar compounds exhibit particularly lower IEC values than non-polar molecules in different organisms because the polar compounds may undergo strong H-donor/H-acceptor interactions [12]. Although the 50% internal lethal concentration (ILC50) found for polar narcotics range from 0.6 to 2 mmol/kg body weight [10], in an in-vitro test system the effective membrane concentrations of chemicals were indistinguishable between the non-polar and polar compounds, where the test system contains only energy-transducing membranes (target lipid membrane with intercalated proteins) [10]. However, the most important factor related to whole body concentration in both polar and non-polar compounds is the distribution of the environmental pollutants between target and non-target compartments [12]. As baseline toxicity is a reversible mechanism, where response is directly related to concentration of the pollutants in the membrane. The time of exposure has significant influence in response to hydrophobic pollutants, which is directly related to the concentration in the membrane [9]. The concentration is determined by the time it takes to reach equilibrium with the surrounding aqueous phase, i.e., by the bioaccumulation kinetics. The relatively short exposure time in most acute tests may not be sufficient to reach a time-independent EC_{50} value in the hydrophobic chemicals, where time to reach equilibrium increases with hydrophobicity, and longer test durations have been recommended [9-10]. Furthermore, it has been observed from LC_{50}-values for more hydrophobic electrophiles (the positively charged or neutral species having vacant orbitals that are attracted to an electron rich centre like nucleophile) that they tend to deviate less from baseline models than the hydrophilic electrophiles do. Although many factors influence the bio-accessibility of the environmental pollutants, hydrophobicity has a substantial effect among physico-chemical properties. The potential chemicals could only meet the classification of toxic compounds while reaching the target site (by crossing all physico-chemical and biological barriers) in such quantity that they can pose an adverse effect in aquatic species [10]. The quantity of potential chemicals translated into response may vary from species to species and from chemical to chemical, as can be seen from Fig. 1. At a certain scale of hydrophobicity (log K_{ow} values 0.5 to 2.5), all environmental pollutants have the utmost ability to reach biological compartments such as cytosole and target sites in membranes, to interfere with normal cell functioning by effecting normal enzymatic activity, and directly to biological macromolecules.

Conclusions

The significant relationship was found among physico-chemical (hydrophobicity) parameters and toxicity. It was demonstrated that the toxic response of all three bio-tests was distinguished at a certain level of hydrophobicity (log K_{ow} values 0.5 to 2.5). There seems to be no obvious reason but possibly, at this level of hydrophobicity of all environmental pollutants, it has the utmost ability to reach biological compartments to react with target sites. Secondly, the presence of proper aqueous concentrations of chemicals ensures the continuous availability of chemicals to target sites, which leads to wider biological action spectra and is translated in distinguished toxic response. Furthermore, due to proper dose administration, all chemicals get appropriate time to reach equilibrium between the inner and outer environments in order to follow the chemo-availability concept. For future study, along hydrophobicity, additional factors also are considered, such as steric hindrance, rate of hydrolysis, charge, mono- versus polyfunctionality, and molecular size.

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Conflict of Interest

The authors declare no conflict of interest.

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