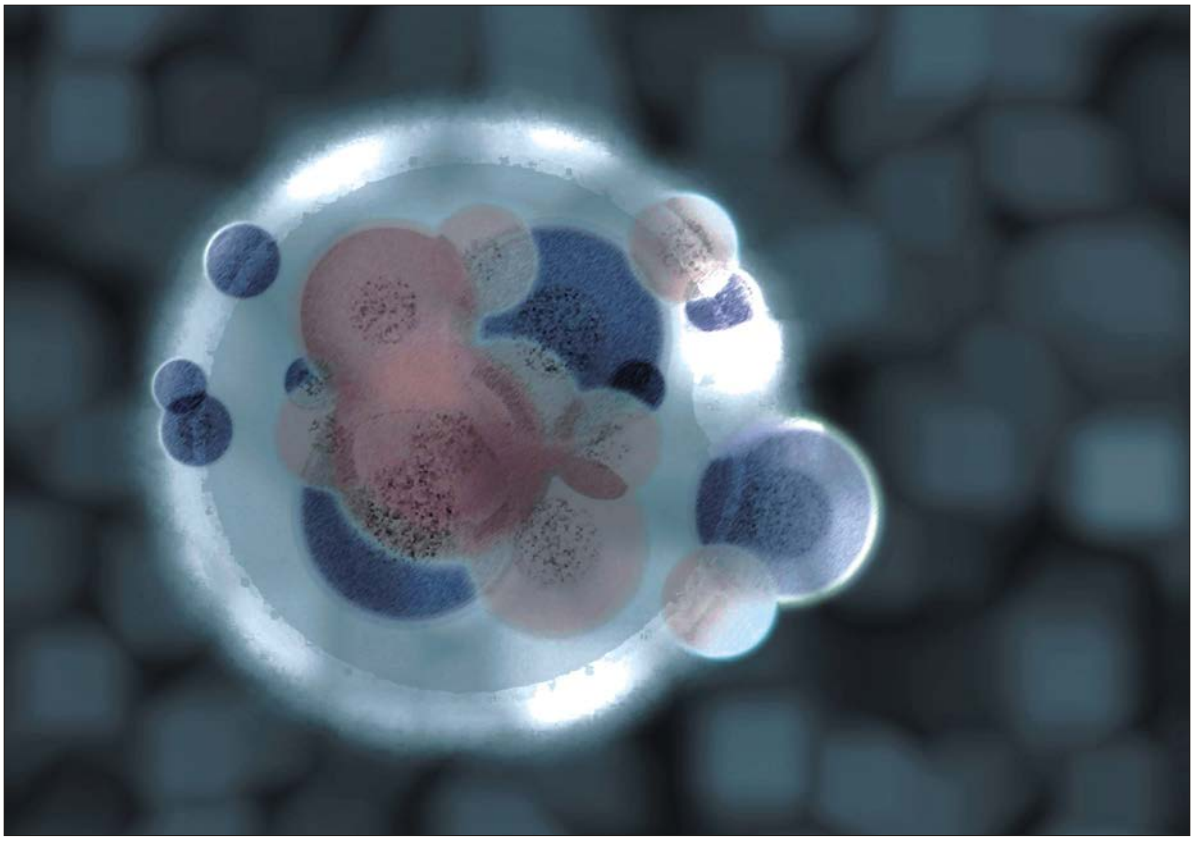

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The Role of Three Dimensional Geometric Descriptors of Selected PAHS on Inducimg Mortality in Juvenile Angel Fish (*Pterophyllum scalare*)

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Abstract: Juvenile angelfish (*Pterophyllum scalare*) was exposed to six different concentrations of Naphthalene, Anthracene, Phenanthrene and Pyrene for a period of 132 h. Mortalities were recorded each 12 h and an average of total mortalities for three replicates of each PAHs was designated as the endpoint of toxicity for angelfishes. This parameter correlated with geometric descriptors (length, width, thickness, length/breadth ratio, molecular volume and molecular surface area), physiochemical descriptors (molar volume, molecular weight, octanol-water partition coefficient and boiling point) and an electronic descriptor (resonance energy). The regression equations showed the significant correlation for three dimensional geometric descriptors, physiochemical descriptors and resonance energy versus mortality. These results indicate that at the range of log $K_{ow} \leq 5.2$, raising three dimensional geometric descriptors values will result in more mortality.

Key words: PAHs, QSAR, *Pterophyllum scalare*, geometric, mortality

INTRODUCTION

The increasing concentration of Persistent Toxic Substances (PTSs) has been one of the most important environmental problems since the beginning of the industrial age. PAHs are among chemicals which are well known because of their carcinogenic and toxic potential and being comprehensive contaminants of water, sediment, soil and air (Masih and Taneja, 2006; Pathiratne *et al*., 2007; Taioli *et al*., 2007). These are among the organic pollutants which have two or more fused benzene rings produced during the incomplete burning of coal, oil, garbage and other organic substances. Nowadays the increasing interests are concentrated on Quantitative Structure-Activity Relationship (QSAR) studies which have been frequently used in medicinal chemistry, environmental science, material science etc. Some research groups have studied the relationship between PAHs molecular descriptors and their toxicity effects (Djomo *et al*., 2004; Lee *et al*., 2004; Schirmer *et al*., 1998; Sverdrup *et al*., 2002). The role of physiochemical descriptors on their fate in the environment is well established (Ferreira, 2001) but among these literatures the role of geometric descriptors (length, width, thickness, molecular volume, molecular surface area and length/breadth ratio) has been highly neglected. Therefore the main objective of this research had been on the investigation of effect of these molecular properties on mortality.

Wilcock *et al*. (1996) suggested that the order of persistence of PAHs in surficial sediment of an intertidal sandflat may be predicted on the basis of molecular size parameters, such as molecular weight, molecular volume and area. Gute *et al*. (1999) investigated geometric descriptors and molecular weight as the effective parameters in predicting dermal penetration of PAHs. Sovadinova *et al*. (2006) explained the importance of molecular size of N-heterocyclic derivatives in activating the aryl hydrocarbon receptor (AhR). Eghtesadi *et al*. (2002) showed the correlation of membrane permeability with L/B ratio of PAHs.

The importance of K_{ow} in ecotoxicology was first introduced by Veith *et al*. (1979) and then the increasing toxicity with increasing value of log K_{ow} was showed by Sverdrup *et al*. (2002) to the point of log K_{ow} = 5.2. In present research, four PAHs in this range (Naphthalene,

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Anthracene, Phenanthrene and Pyrene) which are commonly found in the environment (Eghtesadi *et al*., 2002) have been chosen. In the case of direct cytotoxicity, as reported before (Schirmer *et al*., 1998) the membrane impairment is shown up due to different mechanisms, likely the physical disruption of membrane integrity and fluidity by the presence of PAHs molecules. An important mechanism in the uptake of lipophilic compounds is the partitioning of these molecules into the lipid bilayer of the cytoplasmic membrane and accumulation of cyclic hydrocarbons causes swelling of membrane bilayer and increase in membrane fluidity (Sikkema *et al*., 1994). The toxicity of petroleum hydrocarbons for aquatic organisms has been widely investigated, however, the effects on freshwater environments have been largely ignored (Pollino and Holdway, 2002). Many reports on fish kills in freshwater environments have been attributed to oil, but few have been investigated in the scientific literature (Shales, 1989). Therefore in this study the angelfish *Pterophyllum scalare* (Lichtenstein) was selected as the testing organism due to its wide availability, reproductive capacity and adaptability to captivity (Blom *et al*., 2000; Chapman *et al*., 1997; Degani, 1993; Mackay *et al*., 2004) for investigation on effects of structural parameters of four selected PAHs on inducing mortality in it.

MATERIALS AND METHODS

Acclimatization procedure: Juvenile angelfish were collected in August and September 2006 from Kolbeh Abzian breeding center and transported to the laboratory. Mean body weight (±SE) and standard length (±SE) of fishes was, respectively 1.32 g (±0.073) and 2.91 cm (±0.22). The fishes were kept in two 150 L glass aquarium (an average of 0.9 fish L^{-1}) and were maintained for two weeks for acclimatization and investigating any mortality. Two water falls style bio-filter with refining power of 300 L h^{-1} was used for cycling and filtering of water. The temperature of water was maintained at 28±1°C (Pérez-Cruz *et al*., 1998). Juvenile angelfish were fed up twice a day with Tetra Prima pellets (Gómez-Laplaza and Morgan, 2003) and *Artemia* sp. nauplious (Pérez *et al*., 2003) were given at 5% body weight. An increasing number of ornamental fish farmers has used *Artemia* sp. for breeding their fish (Lim *et al*., 2001) due to its ability to increase growth of juvenile angelfish (Degani, 1993). Water was replaced partially twice a week. The characteristics of water were: 6.83-6.95 pH, 8.56-8.58 mg L^{-1} O_2, 2.95-2.96 μS cm^{-1} conductivity. The lightness of laboratory was provided by the indirect sunlight through two 1.1 m^2 windows beside each other and a 50 w lamp. The photoperiod was 14-1:10+1 L: D with gradual dawn and sunset to dusk transition of 30 min. During acclimatization period (two weeks) no mortality was observed among 270 juvenile angelfish.

Solution preparation: PAHs purchased in crystallized form as Naphthalene (purity min 99%), Anthracene (purity>96%), Phenanthrene (purity>96%) and Pyrene (purity>96%) from Merck. Test substances were dissolved in Ethanol 96% (which was used as a carrier of PAHs) from Merck to yield stock solution of 1.28-2.02 g L^{-1}. Ethanol is applied as a co-solvent in many drugs and its hyperosmolar conditions will not hurt the cells (Chen and Lostritto, 1996). These working stocks were stored in 500 mL screw cap vials (closed firmly after each usage to avoid volatilization of toxicants) at room temperature protected from sunlight.

Some molecular descriptors of tested PAHs are shown in Table 1. Values of molecular weight are taken from reference Mackay *et al*. (2004). The length, width, thickness and length to breadth ratio (L/B) are taken from Sanders and Wise database (http address). The resonance energy, boiling point, molecular surface area, molar volume and molecular volume are taken from Jinno Laboratory database (http address) and the log K_{ow} are taken from Güsten and Sabljic (1995).

Test condition and procedures: Totally 78 experiments each of ten juveniles consisting from 26 groups for each

Table 1: Molecular descriptors of the QSAR model for toxicity of Naphthalene, Anthracene, Phenanthrene, Pyrene

CAS name	Naphthalene	Anthracene	Phenanthrene	Pyrene
MW (g mol^{-1})	128.180	178.240	178.240	202.260
bp (°C)	218.000	340.000	338.000	393.000
Log S	1.500	-1.130	0.060	-0.870
Log K_{ow}	3.330	4.540	4.550	4.880
Width (Å)	7.428	7.439	8.031	9.279
Length (Å)	9.915	11.651	11.752	11.662
Thickness (Å)	3.884	3.882	3.888	3.888
L/B	1.238	1.566	1.463	1.257
Molar volume (cm^3 mol^{-1})	148.000	197.000	199.000	214.000
Molecular volume ($Å^3$)	126.900	170.300	169.500	186.000
S Area (Å)	155.800	202.200	198.000	213.500
RE (ev)	1.328	1.606	1.924	2.099

three replacements were used to determine the effects of Naphthalene, Anthracene, Phenanthrene and Pyrene on survival of juvenile angelfish.

Two 150 L of aquaria were filled with dechlorinated tap water and adjusted for two weeks before the experiments (in addition to acclimation period described above). Water was transferred to the twenty six glass aquariums (Santos *et al*., 2006) with the size of 25×25×25 cm until the water reached to a height of 16 cm for each aquarium (10 L). For preventing oxygen deficiency during the test, each aquarium was aerated at the rate of 100 bubbles per minute (US EPA., 1994). Randomly ten fish were transferred to each 10 L aquarium and exposed to six different concentrations of Naphthalene, Anthracene, Phenanthrene and Pyrene namely 0.5, 1, 2, 4, 8 and 16 ppm. Two groups were considered as control groups, respectively: clean water (0.0 ppm PAHs) and water with maximum of ethanol (12.5 ml L^{-1} for presenting concentration of 16 ppm for Naphthalene).

Mortalities were recorded each 12 h during a period of 132 h. The static condition was considered; therefore no water was replaced during test period. The reasons for selecting the static condition were at first the variation the level of naphthalene was not significant in relation to the lapse of time and its concentration was reduced only 22% over the first 12 h (Santos *et al*., 2006). Among the selected PAHs, naphthalene has the least boiling point; therefore for the other mentioned PAHs reduction of their concentration will not be significance. Another reason for this decision was to simulate the actual presentation of PAHs in the natural environment (not renewal condition) same as the shipwrecks or discharge of oil products into the water bodies.

Data analysis: Data analysis were done by using Microsoft excel 2003 version 11.0 software and SPSS version 13.0.

RESULTS AND DISCUSSION

No mortality was observed in 125 mL ethanol control groups and clean water groups (0.0 ppm PAHs) during 132 h of three replicates.

The average of total mortalities (during 132 h) for three replicates of each PAHs was designated as the endpoint of toxicity for juvenile angelfishes.

The average of total mortalities for each PAHs during three replicates are shown in Table 2.

Based on analysis of variance (one way ANOVA with three replications) the difference of mortalities for Pyrene, Phenanthrene, Naphthalene and control groups was significant (respectively: $p<0.01$, $F = 4.969$; $p<0.01$, $F = 2.900$; $p<0.01$, $F = 449.755$). Among these results, Pyrene had the highest total average mortality value and the least belongs to Anthracene. Present results on the priority of inducing toxicity (Pyrene> Phenanthrene> Naphthalene) agrees with the order reported by Lee *et al*. (2004) who investigated the toxicity of six PAHs in fish and amphipods and reported the toxicity of the tested substances as: Pyrene> Fluoranthene > Phenanthrene > Fluorene > Acenaphthene > Naphthalene.

Table 2: Average mortality of Naphthalene, Anthracene, Phenanthrene and Pyrene for three replicates

CAS name	Average mortality pieces±SE
Naphthalene	10.66±0.25
Anthracene	2.66±0.65
Phenanthrene	21.00±0.69
Pyrene	24.00±0.43

Present results showed that the Effects of Pyrene, Phenanthrene and Naphthalene on mortality started at the concentration of 8 ppm at the time of 12 h ($p<0.01$). The average mortality of Naphthalene at the first 12 h was highest among the other PAHs. Therefore the most direct and rapid mode of toxicity belongs to smallest PAH, Naphthalene. Bateman *et al*. (1986), who studied the uptake of naphthalene by a *Pseudomonas* species, showed that neither ATP nor an electrical potential was required for the uptake of this apolar compound and Naphthalene can easily penetrate the lipid bilayer.

Based on analysis of variance (one way ANOVA with three replications), the difference of mortalities for Anthracene and control groups was not significant ($p>0.05$, $F = 0.959$), therefore Anthracene does not exhibit toxic effects on juvenile *P. scalare* while removing this data point yielded some significant regressions among death average versus some molecular properties. Perhaps this observation was because of lower solubility of Anthracene (in water and ethanol) (Harvey, 1991).

Octanol-water partition coefficient (K_{ow}) vs. Mortality (M): The most widely used molecular structure descriptor in QSAR and QSPR (Quantitative Structure-Properties Relationship) studies is K_{ow}. Compounds for which $K_{ow}<1$ are hydrophil or lipophobe and $K_{ow}>1$ are considered as hydrophobe or lipophil. Lipophilicity can be expressed as the K_{ow} or as the membrane aqueous partition coefficient (Sikkema *et al*., 1994).

Hutchinson *et al*. (1979) and Lacaze *et al*. (1987) had showed that K_{ow} values of PAHs are proportional to their toxic effects on photosynthetic activity. Sverdrup *et al*. (2002) tested some PAHs with log K_{ow} = 5.2 and showed that toxicity significantly goes up together with increasing lipophilicity (increasing log K_{ow}) of the substances. Djomo *et al*. (2004) showed a direct relation between

PAHs toxicity and their K_{ow}. Present results were in agreement with the mentioned researchers based on the following regression equation:

$$M = 8.5436 \log K_{ow}\text{-}17.444;\ R^2 = 0.998;\ p = 0.02$$

Resonance Energy (RE) vs. Mortality (M): Resonance energy is the differences in potential energy between the actual molecular entity and the contributing structure of the lowest potential energy. The regression of resonance energy vs. mortality yielded the following significant regression:

$$M = 17.242\ RE\text{-}12.184;\ R^2 = 1;\ p = 0.005$$

As a result of adding a benzene fused ring to PAHs, resonance energy is increased in normal condition, consequently, for Naphthalene, Phenanthrene and Pyrene, increasing Resonance energy means increasing molecular sizes.

Molecular weight vs. Mortality (M): Molecular weight is the sum of atomic weights which forms a molecule. Toxicity will be increased as a result of increasing molecular weight of PAHs (ATSDR, 1994; Eisler, 1987; Moring, 1996).

The regression equations for molecular weight vs. mortality were:

$$M = 0.1841\ MW\text{-}12.661;\ R^2 = 0.9885;\ p = 0.068$$

This regression was significant at the level of 0.06 ($p = 0.06$). Therefore our results were in agreement with the mentioned researcher.

Boiling point (bp) vs. Mortality (M): Boiling point is the temperature at which the thermal energy of the particle is sufficient to break the cohesion forces and allow an estimative of the atmospheric dispersion of chemicals. PAHs with higher rings have larger surface area so leads to a greater number of intramolecular contacts, increasing the boiling point. The intramolecular wan der Waals interactions are influenced by the contact area available for these interactions, expressed by the molecular weight, molecular volume and molecular surface area (Ribeiro and Ferreira, 2003). The values of regression equations were:

$$M = 0.077\ bp\text{-}6.0706;\ R^2 = 0.9907;\ p = 0.061$$

The significance of this regression was at the level of 0.06 ($p = 0.06$). Therefore with increasing boiling point, mortality increased.

Length vs. Mortality (M): This regression correlation yield not a significant regression ($M = 6.5317\ Length\text{-}54.012$; $R^2 = 0.934$; $p = 0.157$). Therefore according to White *et al.* (1981) who reported the effect of length of linear hydrocarbons on membrane permeability and recently, Eghtesadi *et al.* (2002) reported the effect of length to breadth on changes in membrane permeability, we suggest that molecular length exerts its effect in association with other molecular factor(s) for inducing mortality.

Width vs. Mortality (M): The correlation of width vs. mortality was not significant ($M = 6.4839\ Width\text{-}34.913$; $R^2 = 0.765$; $p = 0.322$).

Thickness vs. Mortality (M): Thickness vs. mortality did not exhibit a significant regression equation: $M = 0.0003\ Thickness + 3.8807$; $R^2 = 0.954$; $p = 0.137$. This seems to be natural as the values of thickness for Naphthalene; Phenanthrene and Pyrene are almost the same (respectively 3.884, 3.888, 3.888).

Length to breadth (L/B) vs. Mortality (M): The L/B value is defined as the ratio of the longer to the shorter side of the rectangle, which has a minimum area among all the rectangles drown to enclose the van der Waals radii of the atoms in the molecule (Ferreira, 2001). The regression equation for L/B vs. mortality was not significant ($M = 20.688\ L/B - 8.616$; $R^2 = 0.141$; $p = 0.752$).

Molecular volume vs. Mortality (M): This correlation yielded a significant equation ($M = 0.229\ Molecular\ volume\text{-}18.287$; $R^2 = 0.996$; $p = 0.036$). These results explain a direct correlation between molecular volume and mortality.

Molar volume vs. Mortality (M): Molar volume is the volume in which one mole of the substance occupies in the form of solid, liquid or gas. The correlation between molar volume and mortality yielded the significant equation ($M = 0.2023\ Molar\ volume\text{-}19.273$; $R^2 = 1$; $p = 0.001$).

Molecular surface area vs. Mortality (M): Molecular surface area is the sum of atomic surfaces. Correlation between molecular surface area and mortality gave rise to a significant equation ($M = 0.2341\ Molecular\ Surface\ Area\text{-}25.716$; $R^2 = 0.99$; $p = 0.03$) which suggests a positive correlation of molecular surface area vs. mortality. These results suggest the powerful impact of molecular surface area on mortality.

Because there is not enough literatures about PAHs molecular effects on inducing toxicity in aquatic organisms and because of the presence of phospholipids molecules in all cell membrane structures, in this research the data about inducing toxicity through cell membranes from aquatic and non-aquatic organism was used. As reported before, in order to induce toxicity log K_{ow} should be between 2 to 5 (Incardona *et al*., 2004) or below 5.2 (Sverdrup *et al*., 2002), because the PAHs with higher values of K_{ow} failed to be directly cytotoxic due to their lower water solubility (Lee *et al*., 2004; Sverdrup *et al*., 2002) which prevents them from being presented to the cell at concentrations that would allow them to accumulate in membrane sufficiently to generally disrupt it's function (Schirmer *et al*., 1998). Some literatures have explained the increasing toxicity and persistence of PAHs with increasing the number of benzene fused ring (Bleeker *et al*., 1996; Cerniglia, 1992; Heitkamp and Cerniglia, 1987) but did not consider the role of geometric descriptors in toxicity of PAHs and the probable mechanism(s) of their impact on cell membranes.

In this research finding no significant regression for one dimensional geometric descriptors (Length, width and thickness) vs. mortality and for two dimensional geometric descriptors (L/B) suggested that one and two dimensional geometric descriptors can not play an effective role on mortality (which probably occurs through physical disruption of membrane integrity according to previous reports). It seems that presence of the relationship between three dimensional geometric descriptors (molecular volume and molecular surface area) and physiochemical descriptors (boiling point, molar volume and molecular weight) versus mortality and resonance energy versus mortality have explained the effect of three dimensions of the PAHs molecules (with together) on mortality. Boiling point and resonance energy can be two descriptors for predicting mortality. As a result of increasing benzene fused ring (from Naphthalene to Phenanthrene and to Pyrene) (Fig. 1) boiling point and resonance energy is increased.

It means that bigger PAHs among these molecules have higher boiling point and higher resonance energy.

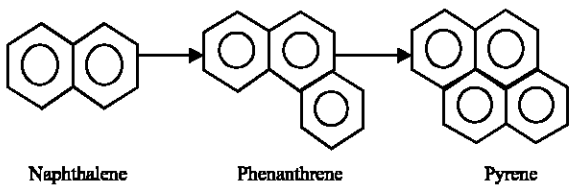

Fig. 1: Increasing the number of benzene fused ring from naphthalene to pyrene

Therefore based on this research, boiling point and resonance energy are physicochemical and electronic, respectively descriptors that can predict mortality because they are exerting their effects through three dimensional descriptors. Phospholipid bilayer of fish cell membrane play an important role in absorption of the PAHs molecules because the lipophilic property of these components (Fismes *et al*., 2002; Kipopoulou *et al*., 1999). The accumulation of lipophilic compounds into lipid bilayers may enhance their availability to the cell but may also cause toxicity problems (Sikkema *et al*., 1992). Since biological membranes are the first contact border for PAHs in the fish cells (and based on mentioned previous reports) this research showed that fish mortality of these molecules correlate with log K_{ow} and three dimensional geometric descriptor values for PAHs. Linear hydrocarbons attach to the acyl chain of phospholipids and change the area which is occupied by each phospholipids molecule (Brenner, 1984; Cornell and Separovic, 1983; van der Meer, 1984). It seems that among polycyclic aromatic hydrocarbons bigger one can occupied more volume (maybe through attaching to the acyl chains) and disrupt membrane integrity more than smaller ones; consequently, higher mortality rate is expected for larger PAHs at mentioned scope (log $K_{ow} \leq 5.2$).

CONCLUSION

QSAR studies are important tools for predicting the fate of PAHs in the environment. Because the experiments of PAHs for assignment of their fate are time consuming, costly and risky, therefore QSAR studies help us to estimate which substances will be active or toxic through calculations and structural analysis. Thus in the framework of our study we suggest that the aquatic toxicity of PAHs is controlled by two factors: first one is log K_{ow} which had been suggested by previous researchers. In this favorable range (log $K_{ow} \leq 5.2$), mortality is controlled by three dimensional geometric descriptors (molecular volume, molecular surface area) or any other molecular properties which can indirectly exert their effects through three dimensional geometric descriptors (molar volume, boiling point, resonance energy) consequently, larger values of three dimensional geometric descriptors means more inducing mortality probably due to more membrane physically disruption.

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