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Toxicity assessment of organic pollutants: Reliability of bioluminescence inhibition assay and univariate QSAR models using freshly prepared *Vibrio fischeri*

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ABSTRACT

The toxicity of 14 industrially relevant organic chemicals was determined using freshly grown *Vibrio fischeri* bioluminescence inhibition assay. The results were compared to lyophilized *V. fischeri*, 96 h fish, 48 h *Daphnia magna* and 95 h green algae bioassays. Reliability of octanol–water partition coefficient (K_{ow}), and first order simple and valence molecular connectivity index ($^1\chi$, $^1\chi^v$) based regression models for predicting toxicity to *V. fischeri* was studied. Correlations were obtained between freshly grown *V. fischeri* data (Log(EC_{50})) and Log(K_{ow}), molecular connectivity indices ($^1\chi$, $^1\chi^v$), energy of the highest occupied (E_{HOMO}) and lowest unoccupied (E_{LUMO}) molecular orbitals, and their difference ($E_{LUMO} - E_{HOMO}$). A good match was observed between *V. fischeri* assay conducted with freshly grown and lyophilized culture ($r^2 = 0.90$). Good correlations ($r^2 > 0.95$) were obtained with all the other bioassays after excluding compounds with Log(K_{ow}) less than 2.0. Available regression models based on Log(K_{ow}) and $^1\chi^v$ yielded lower toxicity values. *V. fischeri* bioassay showed fairly good correlation with Log(K_{ow}), $^1\chi$ and $^1\chi^v$ ($r^2 > 0.75$) but poor correlation with E_{HOMO}, E_{LUMO} and ($E_{LUMO} - E_{HOMO}$) in presence of polar compounds. E_{HOMO} and E_{LUMO} values are affected by polarity and can be used along with Log(K_{ow}) and $^1\chi^v$ for generating better predictive models.

1. Introduction

Industries such as organic chemicals, textile-dyes, pulp-paper and petroleum refineries generate large quantity of toxic effluents. A large number of industrial effluents have potent toxic properties (Prakash et al., 1996). Houk (1992) summarized the characteristics of effluents, and genotoxic potencies of effluents from various industrial sources. Organic chemical industry is the second largest producer of toxic chemicals (Wypych, 2002). The textile industry utilizes about 10,000 different dyes and pigments worldwide (Wang et al., 2002). Consequently, textile industry releases a large amount of phenolic and chlorinated organic compounds in the wet processes, i.e., bleaching and washing of equipments. The toxic organic chemicals contained in pulp and paper industry effluents are resins, fatty acids, biocides, surfactants, phenolic compounds and other by-products generated during bleaching, such as, dioxins and furans. Cooling operations and other miscellaneous processes in petroleum refineries contribute to release of toxic organics, such as, benzene, methylethylketone, toluene, xylenes, phenols and propylene in the wastewaters (TRI, 1993).

The analysis of effluents in terms of lumped parameters, such as, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) is inadequate since such analysis cannot indicate the biological effects (Coombe et al., 1999). In contrast, bioassays can directly indicate the toxicity of a wide range of industrial chemicals (Claxton et al., 1998). More than 200 *in vivo* assays utilizing insects, plants, and animals are available for identification of toxic chemicals (Waters et al., 1988). However, *in vivo* tests are more expensive and labor-intensive hence their application for studying industrial wastes have been limited (DeMarini, 1991). There is an urgent need for more reliable, sensitive and quick bioassays for detection of toxic compounds (Schmitt et al., 2005). In recent years, the use of *Vibrio fischeri* based bioluminescence inhibition assay (ISO, 1998) for toxicity measurement of polluted water

Abbreviations: $^1\chi$, First order simple molecular connectivity index; $^1\chi^v$, First order valence molecular connectivity index; A, Aniline; Ac, Acetaldehyde; B, *n*-Butanol; BOD, Biochemical oxygen demand; Ca, Catechol; COD, Chemical oxygen demand; Cr, *p*-Cresol; DMN, 1,3-Dimethylnaphthalene; Eb, Ethylbenzene; EC_{50}, Effective concentration causing 50% bioluminescence inhibition; EC_{50_Exp}, EC_{50} values determined experimentally using fresh cultures; EC_{50_Lit}, Literature reported EC_{50} values based on lyophilized cultures; E_{HOMO}, Energy of the highest occupied molecular orbital; E_{LUMO}, Energy of the lowest unoccupied molecular orbital; HOCs, Hydrophobic organic compounds; K_{ow}, Octanol–water partition coefficient; LC_{50}, Lethal concentration causing death in 50% of the population; 1MN, 1-Methylnaphthalene; 2MN, 2-Methylnaphthalene; N, Naphthalene; P, Phenol; QSAR, Quantitative structure activity relationship; Tmb, 1,2,4-Trimethylbenzene; T, Toluene; X, *o*-Xylene.

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and soil samples has increased tremendously due to its good reproducibility and sensitivity (Devare and Bahadir, 1994; Boluda et al, 2002; Soldan, 2003). This assay uses a marine Gram negative bacterium, *V. fischeri* (NRRL B-11177) (Parvez et al., 2006; Lappalainen et al., 2001). This strain has been widely used in several commercial test kits i.e., Microtox, Aboatox, LUMIStox and ToxAlert (Backhaus et al., 1997). Wang et al. (2002) and Rigol et al. (2004) evaluated the toxicity of textile mill, and pulp and paper mill effluents, respectively, using LUMIStox 300, Microtox and ToxAlert kits. They demonstrated that toxicity assessment with luminescent bacteria is effective and of practical use for determining toxicity of industrial effluents. Toxicity studies performed using commercial toxicity kits (Microtox, LUMIStox, ToxAlert) based on lyophilized *V. fischeri* (Farre et al., 2001; Jennings et al., 2001; Hernando et al., 2006) are very expensive and have a limited shelf life. However, very limited toxicity studies have been performed using freshly prepared *V. fischeri* (NRRL B-11177). No significant literature is available on comparison between the *V. fischeri* toxicity assay and other standard toxicity assays.

A variety of molecular descriptors have been used in predictive quantitative structure activity relationship (QSAR) based toxicity models, (Perkins et al., 2003). The molecular descriptors used include octanol–water partition coefficient (Log(K_{ow})), first order valence molecular connectivity ($^1\chi^v$) index, the energy of the lowest unoccupied molecular orbital (E_{LUMO}), and the energy of the highest occupied molecular orbital (E_{HOMO}). The octanol-water partition coefficient which is invariably related to the water solubility of compounds is one of the most important molecular descriptors in predictive toxicity. The first order valence molecular connectivity ($^1\chi^v$) index is a numerical descriptor of molecular shape, size, symmetry and heterogeneity of substituents in a molecule (Agarwal and Khadikar, 2002). Compounds with high Log(K_{ow}) accumulates in the cell membrane due to preferred interaction with the phospholipid membrane. Larger molecular size increases the toxicity while a greater degree of hydrogen bonding in a molecule reduces toxicity by increasing the polarity of the molecule. The energy of the lowest unoccupied molecular orbital (E_{LUMO}), represents the electrophilicity of compounds and measures the ability of molecules to act as electron acceptors. The lower the E_{LUMO}, stronger will be the electrophilicity and hence, higher will be the toxicity. The highest occupied molecular orbital (E_{HOMO}) measures the ability of a molecule to donate an electron pair. A large difference in E_{HOMO} and E_{LUMO} represents stability of a molecule and lower reactivity of the molecule (Zhang et al., 2007). QSAR models based on freshly prepared *V. fischeri* assay are unavailable and it is not known how well the available models based on the lyophilized culture assay are applicable.

This study is focused on industrially relevant toxic hydrophobic organic compounds (HOCs) released from polluting industries, specifically, organic chemical, textile-dye, pulp-paper and petroleum refinery. The chemicals were selected based on the mass discharged and inherent acute toxicity as discussed by Parvez (2008). In addition, a set of substituted naphthalenes were also included in the study. The 14-chemicals included in this study were: *o*-xylene (X), ethylbenzene (Eb), acetaldehyde (Ac), 1,2,4-trimethylbenzene (Tmb), toluene (T), phenol (P), aniline (A), naphthalene (N), *n*-butanol (B), catechol (Ca), *p*-cresol (Cr), 1-methylnaphthalene (1MN), 2-methylnaphthalene (2MN) and 1,3-dimethylnaphthalene (DMN). This study focuses on acute effects. However, the chemicals selected are also known to manifest chronic health effects, such as, cancer, birth defects, reproductive anomalies and neurological disorders (James, 1985; Claxton et al., 1998; Wypych, 2002). The specific objectives of this study were: to determine the effectiveness of freshly grown *V. fischeri* assay for acute toxicity measurement by comparing with other toxicity bioassays; to study the reliability of existing univariate regression models; and to characterize the effective physicochemical parameters for obtaining better predictive models.

2. Materials and methods

2.1. Toxicity assessment with freshly grown V. fischeri (NRRL B-11177)

The International Standards Organization (ISO) method (ISO, 1998) was used for toxicity measurement. A lyophilized culture obtained from Aboatox, Finland was grown in the ISO recommended medium. This freshly grown culture was used to prepare the bacterial reagent for the toxicity experiments. The culture was stabilized using an ISO recommended protective medium and a reagent dilution medium. The protective medium was prepared fresh and added to the bacterial suspension for preventing damage to cells during preservation at −20 °C. The reagent dilution medium required for reconstitution of reagent was composed of $C_6H_{12}O_6 \cdot H_2O$ (8.0 mg/l), NaCl (20.0 mg/l) and $MgCl_2 \cdot 6H_2O$ (2.035 mg/l). This reconstituted reagent was stabilized at 15 °C for 1 h before it was used in the toxicity experiments. Stock and working solutions, containing a toxicant were prepared in 2% NaCl and 4.5% methanol. All the samples and bacterial reagent were stabilized at 15 °C for at least 45 min. After stabilization, equal volumes of sample and bacterial reagent (200 µl each) were combined. The control used in this assay was 2% NaCl with no toxicant added. Measurement was performed after 30 min exposure and the Sirius luminometer (Berthold Detection Systems, Germany) was set at gate time (t_g) and measurement time (t_m) of 0 and 2 s, respectively. A minimum of three replicates were used to ensure repeatability in the results. Percent inhibition effect (%INH) was calculated as per the ISO method (ISO, 1998).

2.2. Calculation of toxicity in other standard bioassays using EcoSAR

For all the 14 compounds used in this study, the toxicity data based on lyophilized *V. fischeri* assay was collected from various sources (Deneer et al., 1989; Zhao et al., 1993; Cronin and Schultz, 1997; Ren and Frymier, 2002; Liu and Yu, 2005). Toxicity of chemicals based on 96 h fish, 48 h *Daphnia magna* and 95 h green algae were obtained from ECOWIN v 0.99 (EcoSAR program, USEPA). This program is designed for expert users and can predict aquatic toxicity of chemicals based on Log(K_{ow}). It relies on group specific regression models based on measured aquatic toxicity data obtained using various types of bioassays.

2.3. Univariate regression models and estimation of molecular descriptors

V. fischeri based regression models were collated from studies reported by various researchers. These models were based on Log (K_{ow}) and $^1\chi^v$. All the available models are listed in Table 1. The Log(K_{ow}) for chemicals were calculated using KOWWIN v 1.66 (EcoSAR program, USEPA). Molecular connectivity indices were calculated by Kier and Hall method. These indices were developed to explain the topological features of a molecule, such as, the total number of atoms in a molecule and the amount of branching in a molecule (Baum, 1997). The energy of the highest occupied molecular orbital (E_{HOMO}) and energy of the lowest unoccupied molecular orbital (E_{LUMO}) were calculated using a windows based molecular modeling package CAChe 7.5.0.85 (Fujitsu Ltd., Japan). The E_{HOMO} and E_{LUMO} values were determined using the molecular orbital package (MOPAC) wave function generated by performing optimized geometry calculations using PM3 parameters. The unit of energy was in electron volts (eV). E_{HOMO} and E_{LUMO} describe