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Chromatographic retention–activity relationships for prediction of the toxicity pH-dependence of phenols

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Abstract

An investigation of the use of the chromatographic retention ($\log k$) as an in vitro approach for modeling the pH-dependence of the toxicity to Guppy of phenols is developed. A data set of 19 phenols with available experimental toxicity–pH data was used. The importance of the mechanism of toxic action (MOA) of phenols was studied. $\log k$ data at three pH values were used for the phenols classification and two groups or 'MODEs' were identified. For one 'MODE' a quantitative retention–activity relationship (QRAR) model was calculated. Finally, the model was used to assess the toxicity to Guppy of phenols at different pH values. The results of this investigation suggest that chromatographic retention data allows fish toxicity modeling, in the 5.5–8 pH range of interest.

Keywords: Quantitative retention–activity relationships; Biopartitioning micellar chromatography; Ecotoxicity pH-dependence; Phenols

1. Introduction

The relationship between chemical structure and biological activity has drawn the attention of many investigators since the end of the last century. The basis for any (quantitative) structure–activity relationship, (Q)SAR, is that the biological activity of a new or untested chemical can be inferred from the molecular structure, or properties, of similar compounds whose activities have already been assessed. The use of SAR or QSAR models in environmental chemistry and toxicology is not novel, however, it has been growing with special emphasis in the past 10 years as scientifically-credible tools for predicting the toxicity of chemicals (Schultz et al., 2003) and to assist regulatory agencies in toxicological assessment of chemical substances (Cronin et al., 2003).

The application of chromatographic parameters as descriptor and/or predictor variables in (Q)SARs gives rise to a new field, conventionally called (quantitative) retention–activity relationships, (Q)RARs. Although there are relatively few applications of QRAR models in the environmental field respect to the QSAR ones, it has been reported successful QRAR models using retention factors ($\log k$) obtained in RP-HPLC with conventional hydro-organic mobile phases (Hsieh and Dorsey, 1995; Chilmonczyk et al., 1998; Szabó et al., 1999), micellar liquid chromatography, MLC (Breyer et al., 1991) and its particular version using micellar mobile phases of polioxyethylene(23)lauryl ether, Brij35, mimicking physiological conditions, the so-called biopartitioning micellar chromatography, BMC (Bermúdez-Saldaña et al., 2005a).

An important aspect to take into account in the development of toxicity predictive models is the mechanism of toxic action, MOA, of chemicals (Ren, 2003; Schultz and Cronin, 2003). Therefore, the a priori assignation of MOAs to chemicals based on their chemical structures is a common practice in the development of toxicity predictive models. However, correctly determining MOA of a compound is not always straightforward (Schultz et al., 1990;

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Cronin, 2003) and errors in such assignation would be transferred to the QSARs (and QRARs) toxicity estimation.

Toxicity tests are normally conducted at a single pH, while the pH of natural waters in the environment varies approximately from 5 to 8 (Akkanen et al., 2001). Since it is well-known that pH affects the toxicity of ionizable substances, an important aspect to bear in mind in the development of toxicity-QSAR/QRAR models involving these compounds should be the pH at which toxicity tests are conducted. However, little attention has been paid to the influence of the pH in toxicity-QSAR studies (Könemann and Musch, 1981; Saarikoski and Viluksela, 1982; Cronin et al., 2000) and no references exist about the use of QRAR models for this purpose.

Phenols form a large and structurally diverse group of compounds. They are interesting from a toxicological point of view, since phenols are widely used industry and consumer products and they elicit a number of toxicities to different species (Garg et al., 2001). Thus, there has been much interest in QSARs for phenols (Cronin, 2003). The toxicity of phenols involves a number of different MOAs comprising polar narcosis (the most common and less complex MOA), respiratory uncouplers and electrophilicity (Cronin, 2003). Furthermore, some phenols show acidic properties that also influence in the toxicity response (Könemann and Musch, 1981; Saarikoski and Viluksela, 1982; Cronin et al., 2000).

The aim of this study is to derive (Q)RAR models based on the BMC chromatographic retention ($\log k$) to predict the toxicity to Guppy of phenols as function of the aquatic pH for phenols being susceptible of ionization. In addition, the role of the mechanism of action in toxicity of phenols is evaluated.

2. Materials and methods

2.1. Instrumental

An Agilent 1100 chromatograph with a quaternary pump and an UV–Visible detector (variable wavelength detector) was employed. It is equipped with a column thermostat with 9 μl extra-column volume for preheating mobile phase prior to the column and an autosampler with a 20 μl loop. All the assays were carried out at 25 °C. Data acquisition and processing were performed by means of an HP Vectra XM computer (Amsterdam, The Netherlands) equipped with an HP-Chemstation software (A.07.01 [682] ©HP 1999).

Two Kromasil C_{18} columns (5 μm, 150 mm × 4.6 mm i.d.; Scharlab SL, Barcelona, Spain) and (5 μm, 50 mm × 4.6 mm i.d.; Scharlab) were used. The mobile phase flow rate was 1.0 or 1.5 ml min^{-1} for the 150 mm and 50 mm column length, respectively. The detection was performed in UV at 254 nm for acetanilide, antipyrine and propiophenone (reference compounds), and 240 nm for phenols.

2.2. Reagents and standards

Micellar mobile phases were prepared by dissolving the adequate amount of polyoxyethylene(23)lauryl ether (Brij35, Fluka, Buchs SG, Switzerland) in aqueous solution of 0.025 M phosphate buffer and 0.025 M citrate buffer to get a final surfactant concentration of 0.04 M. The buffer solutions were prepared with sodium dihydrogen phosphate (analytical reagent, Panreac, Barcelona, Spain) and trisodium citrate (analytical reagent, Guinama, SL, Valencia, Spain). The pH was potentiometrically adjusted by addition of either sodium hydroxide (97%, purissimum, Panreac) or hydrochloric acid (for analysis, Merck, Darmstadt, Germany) aqueous solutions to get the final pH values 5.50, 6.05, 7.00, 7.35 and 7.90. Ionic strength of the mobile phase was adjusted at 0.25 M by addition of the appropriate amount of sodium chloride (analytical reagent, Panreac).

Compounds used in this study were obtained from different sources. Standards of the reference compounds acetanilide and antipyrine were obtained from Fluka and propiophenone from Aldrich (St. Louis, Missouri, USA). The test chemicals (phenols), 2,6-dichlorophenol, 2,5-dinitrophenol and 2,3,6-trimethylphenol were obtained from Aldrich; 4-chlorophenol from Fluka; 3,5-dichlorophenol, 2,3,4,6-tetrachlorophenol, 2,3,5,6-tetrachlorophenol, 2,3,5-trichlorophenol and 2,3,6-trichlorophenol from Riedel-de Haën (Seelze, Germany); 3,4,5-trichlorophenol from Supelco (Bellefonte, Pennsylvania, USA); 2,4,6-trichlorophenol, 4,6-dinitro-2-methylphenol, pentachlorophenol, 2,4-dichlorophenol, phenol, 4-nitrophenol, 2-nitrophenol, 2-chlorophenol, 3-nitrophenol and 2,4,5-trichlorophenol from Acros Organics (Geel, Belgium) and 2,3,4,5-tetrachlorophenol from Dr. Ehrenstorfer (Augsburg, Germany).

Stock standard solution of every compound was prepared by dissolving 10 mg of each compound in 10 ml of acetonitrile or methanol. Working solutions were prepared by dilution of the stock standard solutions using the mobile phase solution. The solutions were stored under refrigeration at 5 °C. As reference solutions, two binary mixtures (acetanilide–propiophenone and antipyrine–acetanilide) were prepared.

Barnstead E-pure, deionized water (Sybron, Boston, MA, USA) was used throughout. The mobile phase and the solutions injected into the chromatograph were vacuum-filtered through 0.45 μm nylon membranes (Micron Separations, Westboro, MA, USA).

2.3. BMC measurements

The retention factor of reference compounds were obtained according to the IUPAC approach (García-Domínguez and Díez-Masa, 2001), based on the extra-column time, t_{ext}, correction:

$$k = \frac{t_R^g - t_M^g}{t_M^g - t_{ext}} \quad (1)$$