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Acute toxicity of benzoic acids to the crustacean *Daphnia magna*

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Abstract

The acute immobilization toxicity of benzoic acids substituted with hydroxyl and/or methoxyl groups on the aromatic ring was determined for the freshwater crustacean *Daphnia magna* under neutralized condition (initial pH: 7.45 ± 0.05). Toxicity, expressed as EC_{50} value, varied depending largely on the number and position of phenolic hydroxyl groups. Especially, benzoic acids with *ortho*-substituted hydroxyl groups were more toxic than benzoic acids with *meta*- and/or *para*-substituted hydroxyl groups. Whereas the limited data indicated that methoxyl substitution had relatively small and variable effects on the toxicity. Of the tested compounds, 2,4,6-trihydroxybenzoic acid showed the highest toxicity with the 48 h EC_{50} of 10 μmol l^{-1}. This was 700 times as toxic as the parent benzoic acid (48 h EC_{50} = 7.0 mmol l^{-1}) and about two orders of magnitude higher than those previously reported for monohalogenated benzoic acid derivatives in *Daphnia*. Within the subgroups based on the number of hydroxyl groups (N_{OH}), the toxicity variations due to the position of hydroxyl groups appeared to be correlated with the logarithms of *n*-octanol/water partition coefficients ($\log P_{ow}$). The toxicity of benzoic acids existing almost entirely as their ionized forms could be expressed as simple structure–toxicity relationships using these two descriptors (N_{OH} and $\log P_{ow}$).

Keywords: *Daphnia magna*; Toxicity; Benzoic acids; Hydroxyl group; $\log P_{ow}$

1. Introduction

Benzoic acid derivatives having a general structure C_6–C_1 are widely used as industrial chemicals, agrochemicals, pharmaceuticals and consumer products. On the other hand, a number of benzoic acid derivatives with variations including hydroxylation and methoxylation are known to be a group of secondary plant metabolites and also aerobic microbial degradation products of lignin, an important plant cell wall polymer (Chen and Chang, 1985). Some of these naturally occurring benzoic acid derivatives are shown to have various biological activities (Tomás-Barberán, 2000).

Furthermore, significant environmental sources of benzoic acids are the microbial metabolism of a variety of natural and anthropogenic aromatic compounds, since benzoic acid derivatives are very common structural units among the identified or estimated degradation products of aromatic compounds by aerobic microorganisms (Smith, 1990; Habe and Omori, 2003). However, toxic effects of stable degradation intermediates from aromatic compounds are not fully elucidated.

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Numerous studies have been made on the antibacterial, antifungal, and antiviral activities of natural and synthetic phenolic compounds including various substituted benzoic acids (Friedman et al., 2003, and references cited therein). However, studies on the effects of benzoic acids, as well as other carboxylic acids, to aquatic organisms have been limited (Fiorentino et al., 2003). Toxicity data for some halogenated benzoic acids in the bacteria, ciliate, daphnids, and fish have been reported (Zhao et al., 1996; Muccini et al., 1999). Whereas the toxicity of substituted phenols to *Daphnia* has been investigated by many researchers (Kopperman et al., 1974; LeBlanc, 1980; Devillers and Chambon, 1986; Devillers, 1988; Kühn et al., 1989; Jin et al., 1998; Abe et al., 2000).

The objective of this study was to establish data for acute toxicity of benzoic acids substituted with hydroxyl and/or methoxyl groups to the crustacean *Daphnia magna*. Furthermore, the data were compared with those of halobenzoic acids in the literature, and used to gain some information regarding the structure–toxicity relationships underlying the toxicity of these compounds. *D. magna*, an important freshwater invertebrate species in aquatic food webs, has been used world-wide for many years as a representative test species for ectoxicological evaluation of industrial chemicals (OECD, 1984).

2. Materials and methods

2.1. Organism and culture conditions

Daphnia magna obtained from the National Institute for Environmental Studies (NIES), Tsukuba, Japan was used throughout this study. Neonatal daphnids were obtained from continuous cultures in 1 liter glass beakers at 21 ± 0.3 °C, in dechlorinated and conditioned tap water (total hardness, ≈100 mg l^{-1} as $CaCO_3$; pH 7.5 ± 0.1), 16 h light: 8 h dark photoperiod and a density of below 20 per beaker. The medium was renewed three times a weak and daphnids were fed daily with the green alga *Selenastrum capricornutum* NIES-35 ($3.0–3.5 \times 10^8$ cells l^{-1}), cultured also in our laboratory.

2.2. Chemicals and test solutions

Most chemicals (purity, minimum %) were used as purchased from the following companies: benzoic acid (99.5), 4-hydroxybenzoic acid (97), 2,3-dihydroxybenzoic acid (98), 2,5-dihydroxybenzoic acid (98), 4-hydroxy-3-methoxybenzoic acid (95), 4-methoxybenzoic acid (98), 3,4-dimethoxybenzoic acid (97), 3,4,5-trimethoxybenzoic acid (98), methyl 4-hydroxybenzoate (99), and 4-hydroxybenzaldehyde (98), from Wako Pure Chemical Industries, Osaka, Japan; 3-hydroxybenzoic acid (98), 2-hydroxybenzoic acid (99.5), 3,4-dihydroxybenzoic acid (98), and 3,4,5-trihydroxybenzoic acid (98) from Nacalai tesque, Kyoto, Japan; 2,4-dihydroxybenzoic acid (98), 2,6-dihydroxybenzoic acid (98), 3,5-dihydroxybenzoic acid (98), 2,3,4-trihydroxybenzoic acid (95), 2,4,6-trihydroxybenzoic acid (98), and 3-hydroxy-4-methoxybenzoic acid (98) from Tokyo Chemical Industries, Tokyo, Japan; and 4-hydroxy-3,5-dimethoxybenzoic acid (98) from Sigma, St Louis, MO, USA. 4-Hydroxybenzyl alcohol was prepared by the reduction of 4-hydroxybenzaldehyde with $NaBH_4$ in methanol at 0 °C in almost quantitative yield. Other reagents used for *Daphnia* medium were of the highest purity available and purchased from Wako.

Measured values of the logarithm of the *n*-octanol/water partition coefficient ($\log P_{ow}$) were obtained from on-line interactive demo version of SRC physical properties database (Syracuse Research Corporation Web site, http://esc.syrres.com). If a measured value was unavailable, the $\log P_{ow}$ was estimated using the online version of the CLOGP program (Daylight Chemical Information Systems Web site, http://www.daylight.com). The experimentally determined negative logarithms of the first dissociation constants (pK_a) were also obtained from the SRC database. If a measured value was unavailable, the pK_a value was estimated using the SPARC on-line calculator (http://ibmlc2.chem.uga.edu/sparc). Table 1 shows some physico-chemical properties of benzoic acids and related compounds tested in the present study.

To prepare the highest test solution, a test compound was dissolved in the aerated test medium and the pH was, where necessary, adjusted carefully with 1 mol l^{-1} NaOH solution to 7.45 ± 0.05, and then diluted to prepare a series of test solutions. Test medium used was "moderately hard water" prepared from deionized and distilled water (total hardness, ≈100 mg $CaCO_3$ l^{-1}; USEPA, 1993), and after aeration the pH was adjusted to 7.45 ± 0.05 with 1 mol l^{-1} HCl.

The solution pH decreased below 6 at concentrations over 1 mmol l^{-1} of benzoic acids when dissolved in the test medium employed here, and daphnids exposed to the solutions showed 100% immobility. Consequently, the toxic effects of benzoic acids were not related to the exposed concentrations alone, under the non-neutralized condition. It was further noted that the pH adjustment of some test solutions containing catechol and hydroquinone type structures such as 2,3-, 2,5-, and 3,4-dihydroxybenzoic acids, and 2,3,4-, and 3,4,5-trihydroxybenzoic acids resulted in slightly colored preparations.

The concentrations of the resultant solutions were checked before and after (48 h) exposure experiments by the UV spectra (400–200 nm) using a UV–visible spectrophotometer, UV mini 1240 (Shimadzu, Kyoto, Japan). No significant spectral changes in most test solutions were observed, indicating the stability of these

Table 1
Compounds tested in this study

Compound (common name)	Abbreviation	CAS number[a]	MW[b]	log P_{ow}	pK_a	λ_{max} (nm)[c]
Benzoic acid	BA	65-85-0	122.12	1.87	4.17	224
4-Methoxybenzoic (*p*-anisic) acid	4-M	100-09-4	152.15	1.96	4.29	246.5
3,4-Dimethoxybenzoic (veratric) acid	3,4-DM	93-07-2	182.17	1.61	4.44	250.5, 284.5
3,4,5-Trimethoxybenzoic acid	3,4,5-TM	118-41-2	212.2	1.26[d]	4.44[d]	252.5, 277sh[e]
2-Hydroxybenzoic (salicylic) acid	2-H	69-72-7	138.12	2.26	2.99	230, 295
3-Hydroxybenzoic acid	3-H	99-06-9	138.12	1.50	4.07	223sh,[e] 286.5
4-Hydroxybenzoic acid	4-H	99-96-7	138.12	1.58	4.54	245.5
Methyl 4-hydroxybenzoate [methyl paraben]		99-76-3	152.15	1.96	(8.00)[d]	255.5, 294.5
4-Hydroxy-3-methoxybenzoic (vanillic) acid	VA	121-34-6	168.15	1.43	4.52	251, 285
3-Hydroxy-4-methoxybenzoic (*iso*-vanillic) acid	*i*-VA	645-08-9	168.15	1.36[d]	4.50[d]	250, 286.5
4-Hydroxy-3,5-dimethoxybenzoic (syringic) acid	SA	530-57-4	198.17	1.04	4.21	261
4-Hydroxybenzyl alcohol		623-05-2	124.14	0.25	(9.82)	273
4-Hydroxybenzaldehyde		123-08-0	122.12	1.35	(7.67)	221.5, 286.5, 330.5
2,3-Dihydroxybenzoic acid	2,3-DH	303-38-8	154.12	1.20	2.91	238, 306
2,4-Dihydroxybenzoic (β-resorcylic) acid	2,4-DH	89-86-1	154.12	1.63	3.11	247.5, 290.5
2,5-Dihydroxybenzoic (gentisic) acid	2,5-DH	490-79-9	154.12	1.74	2.95	224sh,[e] 318.5
2,6-Dihydroxybenzoic (γ-resorcylic) acid	2,6-DH	303-07-1	154.12	2.20	1.05	245, 305.5
3,4-Dihydroxybenzoic (protocatechuic) acid	3,4-DH	99-50-3	154.12	1.15	4.26	250, 287.5
3,5-Dihydroxybenzoic (α-resorcylic) acid	3,5-DH	99-10-5	154.12	0.86	4.04	241, 295
2,3,4-Trihydroxybenzoic acid	2,3,4-TH	610-02-6	170.12	1.05	3.13[d]	255.5, 292
2,4,6-Trihydroxybenzoic acid	2,4,6-TH	83-30-7	170.12	1.62[d]	2.48[d]	255, 289
3,4,5-Trihydroxybenzoic (gallic) acid	3,4,5-TH	149-91-7	170.12	0.70	3.13	258.5

[a] Chemical Abstract Service registry number.
[b] Molecular weight.
[c] UV absorbance in test solution.
[d] Calculated value.
[e] Shoulder peak.

compounds under the test conditions. However, 2,3,4- and 3,4,5-trihydroxybenzoic acids showed an increase of the absorbance and the peak broadening in UV region, with the darkening of their solution colors. At pH > 7, 3,4,5-trihydroxybenzoic acid and its analogues are readily oxidized by atmospheric oxygen, as shown in a spectrometric study by Friedman and Jürgens (2000). In the present study no further investigation was, however, made on the structural and toxicological evaluation of products causing such observed changes.

2.3. Acute immobilization toxicity test

Neonates (<24 h old) from 2–3-week-old mothers were placed in a 50 ml glass beaker containing 40 ml of a test solution. All experiments for exposure and controls without chemicals were made in four replicates and performed at 21 ± 0.3 °C under 16 h light: 8 h dark photoperiod. Immobility was used as the endpoint for determining acute toxicity; the daphnids showing no movement within 15 s after gentle stirring were defined to be immobile. After 24 and 48 h, the number of immobile daphnids was recorded to determine the concentration able to achieve 50% immobilization and it was indicated as EC_{50}. The EC_{50} values were calculated by Probit analyses (USEPA, 1993), based on nominal concentrations.

2.4. Analysis of structure–toxicity relationships

The relationship between the toxicity and the physicochemical and/or structural descriptors was analyzed by least-square regression using JMP 5.0 J software (SAS Institute Inc., USA). Log (EC_{50}, in mol l^{-1}) was taken as the dependent variable, while the structural and/or physicochemical descriptors were used as the independent variables. The following descriptive information was provided: n (number of observations used in analysis), r^2 (square of the correlation coefficient adjusted for degrees of freedom), s (the root of the mean square for error), F (the Fisher statistic), p (the significance level).

3. Results and discussion

Acute immobilization toxicity of benzoic acid derivatives and some related compounds for *D. magna* are summarized in Table 2. In the present study, toxicity of benzoic acid derivatives was determined at (initial)

Table 2
Acute toxicity data for tested compounds

Compound	Tested concentration levels (mmol l^{-1})	48 h EC_{50} (95% C.I.)[a]		24 h EC_{50} (95% C.I.)[a]	
		(mmol l^{-1})	(mg l^{-1})	(mmol l^{-1})	(mg l^{-1})
Benzoic acid	1.25–20	7.04 (6.25–7.85)	860	n.o.[b]	
4-Methoxybenzoic acid	2–12	6.20 (5.60–6.70)	943	n.o.[b]	
3,4-Dimethoxybenzoic acid	3.75–15	10.0 (9.03–11.0)	1820	12.7 (11.6–14.0)	2310
3,4,5-Trimethoxybenzoic acid	5–40	24.5 (22.1–27.2)	5200	n.o.[b]	
2-Hydroxybenzoic acid	2–16	6.30 (5.60–6.90)	870	7.70 (6.50–8.80)	1060
3-Hydroxybenzoic acid	5–40	11.2 (8.90–13.6)	1550	19.9 (16.8–22.9)	2750
4-Hydroxybenzoic acid	3.125–25	12.2 (10.3–14.1)	1690	19.7 (17.8–21.9)	2720
Methyl 4-hydroxybenzoate	0.05–0.5	0.27 (0.23–0.31)	41.1	0.46 (0.41–0.59)	70.0
4-Hydroxy-3-methoxybenzoic acid	1–10	5.40 (4.8–6.0)	908	7.0 (5.5–8.8)	1180
3-Hydroxy-4-methoxybenzoic acid	1.5–15	6.70 (6.10–7.40)	1130	9.2 (8.0–10.8)	1550
4-Hydroxy-3,5-dimethoxybenzoic acid	1.25–20	6.70 (6.20–7.20)	1330	n.o.[b]	
4-Hydroxybenzyl alcohol	0.05–0.6	0.24 (0.22–0.27)	29.8	0.60 (0.54–0.67)	74.5
4-Hydroxybenzaldehyde	0.0625–1.0	0.404 (0.337–0.478)	49.3	0.908 (0.707–1.59)	111
2,3-Dihydroxybenzoic acid	0.25–8	2.9 (2.6–3.2)	447	5.40 (4.90–6.20)	832
2,4-Dihydroxybenzoic acid	0.25–8	0.785 (0.60–1.01)	120	1.22 (0.97–1.55)	188
2,5-Dihydroxybenzoic acid	0.125–2	0.386 (0.304–0.487)	59.5	n.o.[b]	
2,6-Dihydroxybenzoic acid	0.0125–0.1	0.0685 (0.0531–0.090)	10.6	n.o.[b]	
3,4-Dihydroxybenzoic acid	0.25–4	2.4 (2.08–2.69)	370	n.o.[b]	
3,5-Dihydroxybenzoic acid	0.625–10	4.0 (3.57–4.45)	616	5.86 (5.29–6.45)	903
2,3,4-Trihydroxybenzoic acid	0.014–0.339	0.0686 (0.0548-0.0826)	11.7	0.123 (0.099–0.145)	20.9
2,4,6-Trihydroxybenzoic acid	0.003–0.3	0.0100 (0.0082–0.0122)	1.70	0.0142 (0.0110–0.0179)	2.4
3,4,5-Trihydroxybenzoic acid	0.0625–1.0	0.112 (0.094–0.133)	19.1	0.165 (0.150–0.181)	28.1

[a] 95% Confidence interval.
[b] Not obtained.

pH of 7.45 ± 0.05 greater than the pK_a of acids (Table 1). Hence, the acids were almost entirely as their ionized forms under the test conditions. The 48 h EC_{50} values span over three orders of magnitude, ranging from 0.01 mmol l^{-1} for 2,4,6-trihydroxybenzoic acid to 24.5 mmol l^{-1} for 3,4,5-trimethoxybenzoic acid.

For simple monohydroxylated benzoic acid derivatives, 2-hydroxybenzoic (salicylic) acid showed higher toxicity (48 h EC_{50} = 6.3 mmol l^{-1}) than the other isomers, 3- and 4-hydroxybenzoic acids (48 h EC_{50} = 11.2 and 12.2 mmol l^{-1}, respectively). But salicylic acid had a similar toxicity to that of the parent benzoic acid (48 h EC_{50} = 7.0 mmol l^{-1}). Compounds with additional methoxyl groups, vanillic, *iso*-vanillic and syringic acids, also had similar toxic levels to that of benzoic acid, but the toxicites were higher than those of the corresponding monohydroxylated benzoic acids.

The toxic effects of dihydroxybenzoic acids in descending order were 2,6- > 2,5- > 2,4- > 2,3- > 3,4- > 3,5-dihydroxybenzoic acids, suggesting the toxicity enhancement by the *ortho*-hydroxyl substitution. All dihydroxy derivatives showed higher toxicity than non-hydroxylated and monohydroxylated benzoic acids.

As for trihydroxy derivatives, 2,4,6-trihydroxybenzoic acid was the most toxic isomer, followed by 2,3,4- and 3,4,5-trihydroxybenzoic acids. Again, the number of *ortho*-hydroxyl groups appeared to enhance the toxicity of substituted benzoic acids. The toxicity (48 h EC_{50} = 10 μmol l^{-1}) of 2,4,6-trihydroxybenzoic acid with two *ortho*-hydroxyl groups was unexpectedly higher than those of 2,3,4- and 3,4,5-trihydroxybenzoic acids containing a more reactive and biologically active catechol structure (Schweigert et al., 2001). This was 700 times as toxic as the parent benzoic acid. It should be noted that 2,3,4-and 3,4,5-trihydroxybenzoic acids were suggested to be air-oxidized under the test conditions as described in materials and methods section. Therefore, the observed toxicity may have been partly affected by some oxidation products from the parent acid.

Very recently, Friedman et al. (2003) reported the results of their extensive studies on the bactericidal activities of benzaldehyde and benzoic acid derivatives substituted with hydroxyl and/or methoxyl groups. They found that the carboxyl group was less active than the aldehyde group and the compounds had the increased activity with the increasing number of hydroxyl groups in the molecule. Interestingly, 2,4,6-trihydroxybenzoic acid was inactive, while 3,4,5-trihydroxybenzoic acid showed relatively higher activity among hydroxybenzoic acids tested. They showed that 2-OH and

4-OH groups enhance the antimicrobial activities of benzaldehyde but not those of benzoic acid. Furthermore, they found that compounds with mixed hydroxyl and methoxyl groups exhibited variable results, i.e., in some cases methoxyl groups enhanced activity and in other cases they did not.

The esterification of 4-hydroxybenzoic acid to its methyl ester (so-called methyl paraben) resulted in a significant 45-fold increase in toxicity, giving the 48 h EC_{50} of 0.27 mmol l^{-1} for methyl paraben. Methyl paraben is used as an antimicrobial preservative in food, drug, and cosmetics for over 50 years, and in animals it is known to be practically non-toxic by both oral and pareteral routs (Soni et al., 2002).

On the other hand, methylation of the phenolic hydroxyl group of 4-hydroxybenzoic acid to 4-methoxybenzoic acid resulted in 2-fold increase in toxicity. These increase may be related to the increased hydrophobicity ($\log P_{ow}$) of these derivatives.

However, the results with methoxybenzoic acids indicated that the toxicity of benzoic acid decreased with increasing methoxyl groups.

The effect of carboxyl group (COOH) was examined by the comparative study with other *p*-hydroxylated C_6–C_1 compounds such as 4-hydroxybenzaldehyde (C_1 = formyl, CHO), 4-hydroxybenzyl alcohol (C_1 = hydroxymethyl, CH_2OH), and 4-methylphenol (C_1 = methyl, CH_3; *p*-cresol). The 48 h EC_{50} values of formyl and hydroxymethyl substituted derivatives were experimentally determined and a 48 h EC_{50} value of 0.071 mmol l^{-1} for *p*-cresol was obtained from Kühn et al. (1989). These data indicated that carboxyl group is considerably less toxic than other C_1 groups. The toxic effects of C_1 substituents in descending order were $CH_3 > CH_2OH > CHO > COOH$. This also indicates that oxidative transformation of aromatic methyl group leading to carboxyl group, a common pathway in aerobic microbial degradation of aromatic methyl group (Smith, 1990), is the detoxification process for daphnids. Similar toxic trend for such compounds has also been reported for the bacteria, fish, and ciliate (Ribo and Kaiser, 1983; Schultz et al., 1996), although respective sensitivities to individual compounds are different.

The pH of test medium is an important factor especially to assess the ionizable compounds, because the unionized form of a weak acid such as benzoic acid is generally thought to be more toxic than the ionized form. Wang and Lay (1989) reported that sodium 2-hydroxybenzoate (24 h_{10} = 304 mg l^{-1}) was significantly less toxic than the corresponding acid with the 24 h EC_{50} value of 230 mg l^{-1} in *Daphnia*. The higher toxicity values for some hydroxybenzoic acids in the literature (Kühn et al., 1989; Fiorentino et al., 2003) than our present data may be ascribed to the differences in their test conditions, especially the actual pH of exposure solutions.

Muccini et al. (1999) studied the toxicities of benzoic acid and its 14 halogenated derivatives for the ciliate *Tetrahymena pyriformis* under neutralized and non-neutralized conditions, and found that the ionized and unionized forms of halobenzoic acids had different contributions to toxicity. Zhao et al. (1996, 1998), studying the acute toxicities of benzoic acid, 6 monohalogenated benzoic acids, and three aminobenzoic acids to *D. magna* at three different pH values (6.0, 7.8, and 9.0), found that the toxicities decreased as the pH increased. The 24 h EC_{50} values for monohalogenated benzoic acids in *Daphnia* ranged from 0.78 mmol l^{-1} for 3-bromobenzoic acid to 7.08 mmol l^{-1} for 4-fluorobenzoic acid. The toxicity (48 h EC_{50}) of 2,4,6-trihydroxybenzoic acid was about two orders of magnitude higher than those of monohalogenated benzoic acids. As for halobenzoic acids, compounds substituted at *meta*- and/or *para*-positions are more toxic than those substituted at *ortho*-positions. They explained the difference by the general concept that the *ortho*-halogenated benzoic acids having lower pK_a values are more ionized, hence less toxic than *meta*- and/or *para*-substituted ones. As for the hydroxylated benzoic acids considered here, *ortho*-hydroxylated benzoic acids also have lower pK_a values, but they showed higher toxicities than the *meta*- and/or *para*- hydroxylated ones, indicating that the hydroxybenzoate derivatives behaved differently from the halobenzoates. Especially, polyhydroxybenzoates may have higher affinity to daphnids than expected from their low $\log P_{ow}$ and pK_a values.

As shown in Fig. 1A, there are not so significant differences in relative magnitudes of toxicities among benzoic, methoxybenzoic, monohydroxybenzoic, and methoxylated monohydroxybenzoic acids. On the contrary, di- and tri-hydroxybenzoic acids appeared to have higher toxicity than these compounds. These results suggested the importance of the number of hydroxyl groups in the toxicity. However, there were substantial variations depending on the position of hydroxyl groups. Within the subgroups based on the number of hydroxyl groups, the toxicity variations appeared to be well correlated with the $\log P_{ow}$s, as shown in Fig. 1B. $\log P_{ow}$ is a hydrophobicity parameter determined for the unionized form of a chemical. Probably, $\log P_{ow}$ functions here as a relative indicator of partitioning of benzoate molecules. Moreover, limited data in Fig. 1B showed that benzoates substituted with both hydroxyl and methoxyl groups (VA, *i*-VA, and SA) seemed to form a disparate group from the other non-phenolic and mono-phenolic benzoic acids.

Based on these observations, the relationship was examined between the toxicity ($\log EC_{50}$), and structural and physicochemical descriptors. Regression analysis using both the number of hydroxyl groups (N_{OH}) and the hydrophobicity parameter ($\log P_{ow}$) revealed a good relationship as follows:

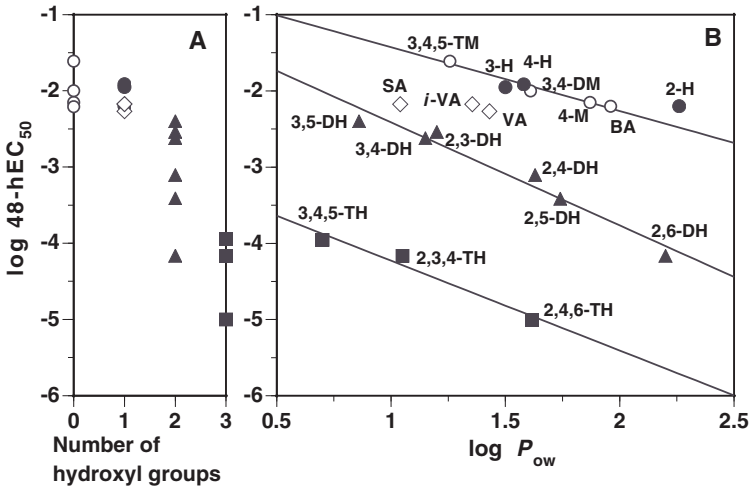

Fig. 1. Plots of the 48 h log EC_{50} (in mol l^{-1}) versus the number of hydroxyl groups (A) and log P_{ow} (B) for benzoic acids substituted with hydroxyl and/or methoxyl groups. For abbreviations of benzoic acids, see Table 1.

$$\log EC_{50} = -0.321(N_{OH})^2 - 0.869(\log P_{ow}) - 0.494,$$
$$n = 19, \quad r^2 = 0.919, \quad s = 0.270, \quad F = 104,$$
$$p < 0.0001.$$

For only hydroxybenzoic acids the following relationship was obtained:

$$\log EC_{50} = -1.55(N_{OH}) - 1.13(\log P_{ow}) + 1.63,$$
$$n = 12, \quad r^2 = 0.949, \quad s = 0.229, \quad F = 103,$$
$$p < 0.0001.$$

Apparently, toxicity increases (with decreasing log EC_{50}) with increasing N_{OH} and log P_{ow}.

Inclusion of an additional structural descriptor, the number of methoxyl groups (N_{OMe}), did not significantly improve the correlation ($r^2 = 0.926$). Methoxyl substitution seemed to be related to the whole toxicity through modification of the hydrophobicity.

4. Conclusions

Study was made on the acute immobilization toxicity to *D. magna* of benzoic acids substituted with hydroxyl (OH) and/or methoxyl (OMe) groups on the benzene ring under neutralized condition. While monohydroxybenzoic, methoxybenzoic and methoxylated monohydroxybenzoic acids were only weakly toxic, polyhydroxybenzoic acids were found to be moderately toxic even in the ionized forms. The toxicity of benzoate derivatives was strongly influenced by the number of OH groups (N_{OH}), and the differences in the toxicities due to the position of OH groups were well related with the differences in their hydrophobicity (log P_{ow}). Thus the toxic potency of all benzoate derivatives could be described by the use of these two descriptors, N_{OH} and log P_{ow} In contrast to the effects of OH groups, OMe groups had relatively smaller effects on the whole toxicity.

References

Abe, T., Saito, H., Niikura, Y., Shigeoka, T., Nakano, Y., 2000. Embryonic development assay with *Daphnia magna* application to toxicity of chlorophenol. Water Sci. Technol. 42, 297–304.

Chen, C.-L., Chang, H.-M., 1985. Chemistry of lignin biodegradation. In: Higuchi, T. (Ed.), Biosynthesis and Biodegradation of Wood Components. Academic Press, Florida, pp. 535–556.

Devillers, J., 1988. Acute toxicity of cresols, xylenols, and trimethylphenols to *Daphnia magna* Sraus 1820. Sci. Total Environ. 76, 79–83.

Devillers, J., Chambon, P., 1986. Acute toxicity and QSAR of chlorophenols on *Daphnia magna*. Bull. Environ. Contam. Toxicol. 37, 599–605.

Fiorentino, A., Gentili, A., Ishidori, M., Monaco, P., Nardelli, A., Parrella, A., Temussi, F., 2003. Environmental effects caused by olive mill wastewaters: toxicity comparison of low-molecular-weight phenol components. J. Agric. Food Chem. 51, 1005–1009.

Friedman, M., Henika, P.R., Mandrell, R.E., 2003. Antibacterial activities of phenolic benzaldehydes and benzoic acids against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. J. Food Protect. 66, 1811–1821.

Friedman, M., Jürgens, H.S., 2000. Effect of pH on the stability of plant phenolic compounds. J. Agric. Food Chem. 48, 2101–2110.

Habe, H., Omori, T., 2003. Genetics of polycyclic aromatic hydrocarbon metabolism in diverse aerobic bacteria. Biosci. Biotechnol. Biochem. 67, 225–243.

Jin, L., Dai, J., Guo, P., Wang, L., Wei, Z., Zhang, Z., 1998. Quantitative structure–toxicity relationships for benzaldehydes to *Daphnia magna*. Chemosphere 37, 79–85.

Kopperman, H.L., Carlson, R.M., Caple, R., 1974. Aqueous chlorination and ozonation studies I. Structure–toxicity correlations of phenolic compounds to *Daphnia magna*. Chem.-Biol. Interact. 9, 245–251.

Kühn, R., Pattard, M., Pernak, K.-D., Winter, A., 1989. Results of the harmful effects of selected water pollutants (anilines, phenols, aliphatic compounds) to *Daphnia magna*. Water Res. 23, 495–499.

LeBlanc, G.A., 1980. Acute toxicity of priority pollutants to water flea (*Daphnia magna*). Bull. Environ. Contam. Toxicol. 24, 684–691.

Muccini, M., Layton, A.C., Sayler, G.S., Schultz, T.W., 1999. Aquatic toxicities of halogenated benzoic acids to *Tetrahymena pyriformis*. Bull. Environ. Contam. Toxicol. 62, 616–622.

OECD, 1984. Guidelines for testing chemicals: *Daphnia* sp. acute immobilisation test and reproduction test. OECD, Paris, France, No. 202, pp.1–16.

Ribo, J.M., Kaiser, K.L.E., 1983. Effects of selected chemicals to photoluminescent bacteria and their correlations with acute and sublethal effects on other organisms. Chemosphere 12, 1421–1442.

Schultz, T.W., Bryant, S.E., Kissel, T.S., 1996. Toxicological assessment in *Tetrahymena* of intermediates in aerobic microbial transformation of toluene and *p*-xylene. Bull. Environ. Contam. Toxicol. 56, 129–134.

Schweigert, N., Zehnder, A.J.B., Eggen, R.I.L., 2001. Chemical properties of catechols and their molecular modes of toxic action in cells, from microorganisms to mammals. Environ. Microbiol. 3, 81–91.

Smith, M.R., 1990. Biodegradation of aromatic hydrocarbon by bacteria. Biodegradation 1, 191–206.

Soni, M.G., Taylor, S.L., Greenberg, N.A., Burdock, G.A., 2002. Evaluation of the health aspects of methyl paraben: a review of the published literature. Food Chem. Toxicol. 40, 1335–1373.

Tomás-Barberán, F.A., Clifford, M.N., 2000. Dietary hydroxybenzoic acid derivatives—nature, occurrence and dietary burden. J. Sci. Food Agric. 80, 1024–1032.

USEPA, 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms (4th ed.). US Environmental Protection Agency, EPA/600/4-90/027F, Office of Research and Development, Washington DC.

Wang, W.H., Lay, J.P., 1989. Fate and effects of salicylic acid compounds in freshwater systems. Ecotoxicol. Environ. Safety 17, 308–316.

Zhao, Y.H., Ji, G.D., Cronin, M.T.D., Dearden, J.C., 1998. QSAR study of the toxicity of benzoic acids to Vibrio fischeri, *Daphnia magna* and carp. Sci. Total Environ. 216, 205–215.

Zhao, Y.H., Yuan, X., Yang, L.H., Wang, L.S., 1996. Quantitative structure–activity relationships of organic acids and bases. Bull. Environ. Contam. Toxicol. 57, 242–249.

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