SETAC PRESS
Environmental Toxicology and Chemistry, Vol. 15, No. 10, pp. 1702–1708, 1996

Printed in the USA
0730-7268/96 $6.00 + .00

STRUCTURE–ACTIVITY RELATIONSHIPS FOR CHLORO- AND NITROPHENOL TOXICITY IN THE POLLEN TUBE GROWTH TEST

GERRIT SCHÜÜRMANN,*† RAYASAMUDA K. SOMASHEKAR‡ and UDO KRISTEN§
†UFZ Centre for Environmental Research, Department of Chemical Ecotoxicology, Permoserstrasse 15, D-04318 Leipzig, Germany
‡Department of Botany, Bangalore University, Bangalore 560056, Karnataka, India
§Institut für Allgemeine Botanik, Universität Hamburg, Ohnhorststrasse 18, D-22609 Hamburg, Germany

(*Received* 30 *November* 1995; *Accepted* 8 *April* 1996)

Abstract—Acute toxicity of 10 chlorophenols and 10 nitrophenols with identical substitution patterns is analyzed with the pollen tube growth (PTG) test. Concentration values of 50% growth inhibition (IC50) between 0.1 and 300 mg/L indicate that the absolute sensitivity of this alternative biotest is comparable to conventional aquatic test systems. Analysis of quantitative structure–activity relationships using lipophilicity (log K_{ow}), acidity (pK_a), and quantum chemical parameters to model intrinsic acidity, solvation interactions, and nucleophilicity reveals substantial differences between the intraseries trends of log IC50. With chlorophenols, a narcotic-type relationship is derived, which, however, shows marked differences in slope and intercept when compared to reference regression equations for polar narcosis. Regression analysis of nitrophenol toxicity suggests interpretation in terms of two modes of action: oxidative uncoupling activity is associated with a pK_a window from 3.8 to 8.5, and more acidic congeners with *diortho*- substitution show a transition from uncoupling to a narcotic mode of action with decreasing pK_a and log K_{ow}. Model calculations for phenol nucleophilicity suggest that differences in the phenol readiness for glucuronic acid conjugation as a major phase-II detoxication pathway have no direct influence on acute PTG toxicity of the compounds.

Keywords—Pollen tube growth test Phenols QSAR Narcosis Oxidative uncoupling

INTRODUCTION

Assessment of the environmental hazard potential of xenobiotics requires generation and interpretation of ecotoxicologic data. In addition to detailed experimental studies for individual compounds, investigation of selected biological endpoints for series of compounds is particularly helpful in identifying relationships between structural features and biological activity.

With weak organic acids such as phenols, both bioavailability and toxicity depend on ionization [1–3]. For a certain range of pK_a values, uncoupling of oxidative phosphorylation becomes the dominating mode of toxic action in aquatic organisms [3]. Uncoupling activity results from increased proton transport across energy-transducing membranes, where moderately acidic chemicals may function as particularly efficient carriers [4]. Classic examples of uncoupling agents are pentachlorophenol and 2,4-dinitrophenol. However, uncoupling activity may be masked through sufficiently high lipophilicity and correspondingly high nonspecific toxicity contributions, as was observed for the toxicity pattern of pentachlorophenol (with a log K_{ow} value ≈5) towards nine aquatic organisms [5].

In the present study, acute toxicity of 10 chlorophenols and 10 nitrophenols is examined with the pollen tube growth (PTG) test and analyzed in terms of narcosis and uncoupling activity. The PTG test using in vitro growing pollen tubes of tobacco (*Nicotiana sylvestris*) has been introduced earlier as an alternative test system for assessing cytotoxicity of chemicals [6,7], and it is currently being evaluated in the American CTFA (Cosmetic, Toiletry and Fragance Association) Evaluation of Alternatives program and in the European MEIC (Multicenter Evaluation of In Vitro Cytotoxicity) program for its potential for replacing certain animal tests in the notification phase of cosmetic and pharmaceutical chemicals [8,9]. Due to the lack of chloroplasts and consequently of photosynthetic activity, the PTG test is designed to mimic vertebrate toxicity rather than plant-specific effects of xenobiotics. Furthermore, because of the high mitochondrial respiration rate of pollen tubes, the test system response is extremely sensitive to oxidative uncouplers [10].

Molecular descriptors for the quantitative structure–activity relationship (QSAR) analysis include lipophilicity (log K_{ow}), acidity (pK_a) and quantum chemical parameters for the intrinsic molecular acidity and respective solvation effects. In addition, the potential influence of phase-II *O*-glucuronide formation as a major detoxication pathway for phenols is addressed through computational analysis of a corresponding model reaction, yielding a parameter for the nucleophilicity of the phenolic hydroxyl group. The results reveal substantial differences between PTG toxicity of chlorophenols and nitrophenols, which can be traced back to corresponding differences in the physicochemical compound properties. In particular, QSAR analysis leads to the identification of two different modes of toxic action within the nitrophenol series.

MATERIALS AND METHODS

Acute toxicity of 10 chlorophenols and 10 nitrophenols was determined with the PTG test as described below. The chemicals were used as purchased, with purity above 98% in all cases. Both compound series have the same substitution pattern and are listed in Table 1.

Experimental procedure

Pollen of tobacco plants (*Nicotiana sylvestris* Spegazz and Comes) were collected during early stages of anthesis and sus-

* To whom correspondence may be addressed.

Table 1. Compound sets with experimental and calculated data[a]

No.	Compound	CAS RN	IC50 (mg/L)	log IC50 (mol/L)	log K_{OW}	pK_a	ΔH_g^{AM1}	$\Delta\Delta G_s^{SM2}$	ΔH_{nuc}^{AM1}
Chlorophenols									
1	2-Cl	95-57-8	184 ± 10.5	−2.84	2.15	8.40	1,419.3	−1,358.6	−52.1
2	3-Cl	108-43-0	79.1 ± 9.1	−3.21	2.50	9.09	1,422.6	−1,348.9	−78.1
3	4-Cl	106-48-9	41.0 ± 6.0	−3.50	2.39	9.38	1,421.2	−1,347.2	−64.2
4	2,3-diCl	576-24-9	11.9 ± 1.6	−4.14	2.84	7.58	1,396.0	−1,345.5	−59.4
5	2,4-diCl	120-83-2	14.1 ± 2.4	−4.06	3.06	7.87	1,391.2	−1,341.2	−45.9
6	2,5-diCl	583-78-8	23.2 ± 0.8	−3.85	3.06	7.58	1,393.2	−1,343.0	−54.8
7	2,6-diCl	87-65-0	19.4 ± 1.4	−3.92	2.75	6.89	1,397.7	−1,353.6	49.0
8	3,4-diCl	95-77-2	3.24 ± 0.35	−4.70	3.33	8.56	1,397.7	−1,334.9	−71.5
9	2,3,6-triCl	933-75-5	0.12 ± 0.02	−6.22	3.77	6.06	1,376.0	−1,341.7	47.8
10	2,4,6-triCl	88-06-2	1.25 ± 0.26	−5.20	3.69	6.35	1,371.5	−1,337.6	50.8
Nitrophenols									
11	2-NO_2	88-75-5	13.8 ± 1.9	−4.00	1.79	6.80	1,347.0	−1,327.8	−36.8
12	3-NO_2	554-84-7	66.1 ± 5.0	−3.32	2.00	8.27	1,375.1	−1,313.1	−45.2
13	4-NO_2	100-02-7	17.0 ± 0.7	−3.91	1.91	7.15	1,339.9	−1,300.3	−65.1
14	2,3-diNO_2	66-56-8	4.82 ± 0.98	−4.58	1.98[b]	5.15	1,288.6	−1,292.4	−18.4
15	2,4-diNO_2	51-28-5	1.71 ± 0.13	−5.03	1.67	4.03	1,257.5	−1,272.5	−33.2
16	2,5-diNO_2	329-71-5	6.87 ± 1.0	−4.43	1.75	5.15	1,280.7	−1,281.7	−20.6
17	2,6-diNO_2	573-56-8	3.12 ± 0.49	−4.77	1.37	3.68	1,284.2	−1,304.9	−53.0
18	3,4-diNO_2	577-71-9	3.05 ± 0.3	−4.78	1.80[b]	5.50	1,280.9	−1,265.5	−44.9
19	2,3,6-triNO_2	603-10-1	17.2 ± 3.2	−4.12	1.10[b]	2.03	1,228.8	−1,271.8	−60.7
20	2,4,6-triNO_2	88-89-1	302 ± 49.3[c]	−2.88	0.89	0.91	1,205.7	−1,257.6	−60.0

[a] The IC50 is the experimental concentration value of 50% growth inhibition of the PTG test after 18 h of exposure. The purity of the compounds was above 98% in all cases. Gas-phase dissociation enthalpy ΔH_g^{AM1}, difference in solvation free energy $\Delta\Delta G_s^{SM2}$, and nucleophilic reaction enthalpy ΔH_{nuc}^{AM1} (see Theory section and references [17,19]) are given in kJ/mol. Acidity constant pK_a is calculated according to [16], and log K_{OW} is taken from the Starlist of the Daylight databank or calculated with CLOGP-4.34 [15] (see superscript b).

[b] Log K_{OW} of compounds identified through superscript b is calculated with CLOGP-4.34, where with 3,4-dinitrophenol and 2,3,6-trinitrophenol, the calculated values have been corrected by the average error of CLOGP-4.34 for the isomeric nitrophenols of the present compound list.

[c] A second test performed later gave an IC50 of 277 ± 49.2 mg/L, based on five replicates.

pended in aqueous culture medium containing 10% sucrose, 0.01% boric acid, and 3 mmol/L $Ca(NO_3)_2$. Suspensions of pollen, while producing pollen tubes, were incubated with increasing concentrations of the test chemical for 18 h at 27°C in the dark [11]. The Alcian Blue staining method for the photometric quantification of pollen tube mass production was used as reported previously [12]. Alcian Blue binds to water-insoluble polysaccharides of the pollen tube walls when added to the suspension after the 18-h incubation period. After centrifugation of the pollen tube suspension and washing of the pellet, the dye was redissolved by acidification with citric acid and quantified photometrically in the supernatant. The extinction values were shown to linearly correlate with the amounts of pollen tube material produced [12].

Dosage range-finding experiments were performed in the assay and each test was repeated at least three times. Moreover, each dosage group was assayed in triplicate. The 50% growth inhibitory concentrations (IC50s) were extrapolated from concentration–effect curves using linear regression analysis. When the IC50 value was not bracketed in the initial dosage range used for the test chemical, the experiments were repeated and the concentrations were adjusted as necessary. Additional test tubes with nongerminating pollen grains (totally inhibited by 20% ethanol) were processed in parallel as reference blanks. For a more detailed description of the PTG test the reader is referred to reference [13].

Molecular descriptors

Octanol/water partition coefficients in logarithmic form (log K_{ow}), were calculated using the Leo/Hansch scheme [14] in the Daylight implementation [15]. Solution-phase acidity of phenols in terms of pK_a was calculated by the classical Hammett-type relationship [16].

Semiempirical quantum chemical schemes AM1 [17], AM1-COSMO [18], and SM2 [19] as implemented in the packages MOPAC [20,21] and AMSOL [22] have been used to calculate gas-phase dissociation enthalpies, ΔH_g, and corresponding differences in the free energy of aqueous solvation, $\Delta\Delta G_s$, as outlined in the following section. In addition, AM1 reaction enthalpies of the nucleophilic attack of phenolic oxygen at $^+CH_3$ to give R–O(H)–CH_3^+ were calculated to analyze the possible influence of a major phase-II detoxification pathway on the toxicity of the compounds.

All quantum chemical calculations have been performed on IBM RISC 6000 workstations and include geometry optimization under the constraint of transoid configurations of the hydroxyl group without intramolecular hydrogen bonding to *ortho* substituents. This conformation was selected to facilitate analysis of the electrophilic addition of $^+CH_3$ at the phenolic oxygen site. For the quantum chemical calculation of acidity, both cisoid and transoid conformations of phenol derivatives yield very similar results [23]. The starting geometries have been generated and preoptimized with the SYBYL modeling package [24], and AM1-COSMO geometries served as input for the final solution-phase geometry optimization with SM2.

THEORY

Dissociation of phenols in aqueous solution is governed by their intrinsic molecular acidity and solvation interactions of the acid, its conjugate base, and the proton. The former can be quantified through the dissociation in the gas phase, which may be written as

$$R\text{–}OH \rightarrow R\text{–}O^- + H^+ \quad (1)$$

Equation 1 corresponds to a free energy change, ΔG_g, which can be decomposed into the heats of formation, H_f, of the relevant molecular species, and the entropic contribution, $\Delta S_g T$, at temperature T:

$$\Delta G_g = H_f(RO^-) + H_f(H^+) - H_f(ROH) + \Delta S_g T \quad (2)$$

Neglecting the entropy term and introducing $H_f(H^+) = 1{,}530$ kJ/mol, according to experimental values for the heat of formation of atomic hydrogen in the gas phase and its ionization potential [25], leads to the gas-phase dissociation enthalpy in kJ/mol:

$$\Delta H_g = H_f(RO^-) - H_f(ROH) + 1{,}530 \quad (3)$$

The relevant heats of formation can be calculated with quantum chemical schemes. With the assumption of a sufficient accuracy of the semiempirical AM1 scheme it follows that

$$\Delta G_g \approx \Delta H_g^{AM1} \quad (4)$$

Proton transfer in aqueous solution involves water as the reaction partner,

$$R\text{–}OH_{aq} + H_2O_{aq} \rightarrow R\text{–}O^-_{aq} + H_3O^+_{aq} \quad (5)$$

and the respective dissociation free energy can be written as

$$\Delta G_{aq} = H_f(RO^-_{aq}) - H_f(ROH_{aq}) + H_f(H_3O^+_{aq}) - H_f(H_2O_{aq}) + \Delta S_{aq} T \quad (6)$$

Decomposing the solution-phase heat of formation into its gas-phase counterpart and the respective solvation energy

$$H_f(RO^-_{aq}) - H_f(ROH_{aq}) = H_f(RO^-) - H_f(ROH) + \Delta G_s(RO^-) - \Delta G_s(ROH) \quad (7)$$

introducing $H_f(H_3O^+_{aq}) - H_f(H_2O_{aq}) = 415$ kJ/mol [23], and applying Equation 3 as well as neglecting the entropy term $\Delta S_{aq} T$ leads to the following expression of the solution-phase dissociation enthalpy in kJ/mol:

$$\Delta H_{aq} = \Delta H_g + \Delta G_s(RO^-) - \Delta G_s(ROH) - 1{,}115 = \Delta H_g + \Delta\Delta G_s \quad (8)$$

Quantum chemical continuum-solvation models enable approximate calculation of $\Delta\Delta G_s$. The semiempirical SM2 scheme yields an acceptable level of accuracy if the results are scaled properly through linear regression [23]:

$$\Delta G_{aq} \approx \Delta H_{aq} = a \times \Delta H_g^{AM1} + b \times \Delta\Delta G_s^{SM2} + c \quad (9)$$

Acidity constant pK_a is related to ΔG_{aq} according to

$$pK_a = 0.175\ \Delta G_{aq} - 1.74 \quad (10)$$

(see textbooks and reference [23]). It follows that Equation 9 offers a way to analyze pK_a in terms of the intrinsic molecular acidity and solvation interactions. Furthermore, the influence of solution-phase acidity on biological effects of the compound can be studied from a mechanistic basis, which will be helpful in further elucidating the role of pK_a as molecular descriptor in structure–activity relationships. With the level of computational accuracy currently available, it will be preferable to derive the relevant regression parameters of Equation 9 separately for individual compound classes [23].

After uptake of phenols into organisms, a major detoxication pathway is given by *O*-glucuronide formation as part of phase-II metabolism [26]. The critical step in this conjugation reaction is the attack of the phenolic hydroxyl group at the relevant ring carbon of uridine diphosphate glucuronic acid (UDPGA), leading to a nucleophilic displacement (S_N2 reaction) of the uridine diphosphate (UDP) moiety by the substrate:

$$R\text{–}OH + UDP\text{–}GA \rightarrow R\text{–}O\text{–}GA + UDP \quad (11)$$

It follows that differences in nucleophilic activity of phenols may contribute to corresponding differences in toxicity. For a computational analysis of the corresponding S_N2 transition state formation as the critical step, one option is to construct a simple model reaction that may still reflect differences in nucleophilic power of the substituted phenols. For convenience, nucleophilic addition of phenols at a carbonium ion was selected:

$$R\text{–}OH + {}^+CH_3 \rightarrow R\text{-}O(H)CH_3^+ \quad (12)$$

The corresponding reaction enthalpy, ΔH_{nuc}, can be calculated on the AM1 level, referring to optimized geometries of the nonbonded structure (left) and the adduct (right):

$$\Delta H_{nuc}^{AM1} = H_f^{AM1}(RO(H)CH_3^+) - H_f^{AM1}(ROH + {}^+CH_3) \quad (13)$$

The potential toxicologic relevance of differences in nucleophilic power according to Equation 13 will be evaluated through multilinear regression of the toxicity data on ΔH_{nuc}^{AM1}, in addition to the other descriptors mentioned above.

RESULTS AND DISCUSSION

The two compound sets are listed in Table 1 together with experimental toxicity data and calculated molecular parameters as described above. Chlorophenols are more lipophilic than nitrophenols by ≈1.3 log K_{ow} units, and within each series variation in log K_{ow} is relatively small with only 1.6 and 1.1 units for chloro- and nitrophenols, respectively.

The PTG toxicity spans 3.4 (chlorophenols) and 2.2 (nitrophenols) orders of magnitude, which is almost twice the range of log K_{ow}. Absolute IC50 values are between 0.1 and 300 mg/L, thus indicating a sensitivity range comparable to aquatic test systems with this type of chemical.

A pronounced difference between the two compound series is observed for pK_a, which covers 3.3 units for chlorophenols and 7.4 units for nitrophenols, respectively. Similarly, the range of calculated gas-phase acidity, ΔH_g^{AM1}, is much larger for nitrophenols (169 kJ/mol) than for chlorophenols (51 kJ/mol).

From the viewpoint of molecular structure, PTG toxicity of chlorophenols is expected to be associated with polar narcosis [27], implying a significant correlation with log K_{ow}. With the nitrophenol series, additional influence of ionization in terms of the compound pK_a is expected, because the PTG test system shows sufficient specific sensitivity towards uncouplers of oxidative phosphorylation, as observed after treatment of pollen tubes with pentachlorophenol [28]. Furthermore, factorization of pK_a into ΔH_g^{AM1} and $\Delta\Delta G_s^{SM2}$ may enable differentiation between intrinsic acidity referring strictly to the pure molecular structure, and solvation interactions of the compounds and their deprotonated anions with the aqueous phase.

Before proceeding with an analysis of correlations between PTG toxicity and molecular descriptors for lipophilicity and acidity, a corresponding mechanistic analysis of pK_a will be performed for both compound series. From a practical point of view, such an analysis will be particularly useful for future studies of compounds where substituent constants are not available or Hammett-type relationships are simply not applicable [29].

Table 2. Linear regression relationships for pK_a[a]

	ΔH_g^{AM1}	$\Delta\Delta G_s^{SM2}$	Constant	r^2_{adj}	SD	$F_{1,8}$ or $F_{2,7}$	n
Chlorophenols	0.0558	—	−70.2	0.77	0.53	31.1	10
	0.0780	0.0858	14.1	0.97	0.20	141.7	10
Nitrophenols	0.0417	—	−48.9	0.92	0.64	106.9	10
	0.0569	0.0413	−15.3	0.97	0.41	137.1	10

[a] Coefficients, constant, and statistical information refer to linear one- and two-parameter regressions of pK_a on ΔH_g^{AM1} and $\Delta\Delta G_s^{SM2}$ in kJ/mol. Squared correlations r^2 between ΔH_g^{AM1} and $\Delta\Delta G_s^{SM2}$ are (−)0.41 for chlorophenols and (−)0.73 for nitrophenols, and between the two compound series 0.97 for ΔH_g^{AM1}, 0.90 for $\Delta\Delta G_s^{SM2}$, and 0.86 for pK_a, respectively.

Linear regression of pKa

Regression analysis of pK_a on ΔH_g^{AM1} and $\Delta\Delta G_s^{SM2}$ is summarized in Table 2. With both compound series, two-parameter relationships according to a combination of Equations 9 and 10 yield highly significant statistics with r^2_{adj} of 0.97. However, regression coefficients of ΔH_g^{AM1} and $\Delta\Delta G_s^{SM2}$ differ both within and between the two series, which confirms that calculation accuracy of current semiempirical methods requires scaling of dissociation energies through compound class-specific regression, as suggested earlier [23].

An interesting difference between chloro- and nitrophenols is observed for the correlation between solution-phase and gas-phase acidity. With the more hydrophobic chlorophenols, solvation interactions as quantified by $\Delta\Delta G_s^{SM2}$ yield a significantly larger contribution to pK_a than with nitrophenols. It follows that the suitability of gas-phase calculations for mimicking solution-phase acidity differs for different classes of compounds. Furthermore, these results of Table 2 seem to indicate that with increasing hydrophobicity of a compound, differences in aqueous solvation between the acidic and anionic form are increasingly important for solution-phase acidity. Note in this context, that semiempirically calculated solvation energies can also be used to model log K_{ow} if combined with a term representing solute–solvent dispersion interactions [30].

From the identical substitution pattern of both compound series, similar trends for physicochemical properties such as log K_{ow} and pK_a would be expected. However, correlation analysis reveals interseries r^2 of only (−)0.59 for log K_{ow}, 0.86 for pK_a, 0.90 for $\Delta\Delta G_s^{SM2}$, and 0.97 for ΔH_g^{AM1} (Table 2). These statistics suggest again that the above-mentioned differences in solvation interaction form a major cause for deviations between intraseries trends of log K_{ow} and pK_a. The results further demonstrate that quantum chemical calculations have a particular merit in elucidating mechanistic causes of compound properties associated with certain structural features that go beyond the phenomenological level of Hammett-type relationships [29].

Linear regression of PTG toxicity

The PTG toxicity shows similar levels for the two series of chloro- and nitrophenols (Table 1), but the relative trends differ markedly with almost no interseries correlation (r^2 = (−)0.001). Similarly, there is no meaningful linear or multilinear correlation between log IC50 and any selection of the molecular descriptors listed in Table 1. Consequently, the following analysis will be performed separately for the two compound series.

With log IC50 of the 10 chlorophenols, multilinear regression analysis including all molecular descriptors from Table 1 reveals log K_{ow} as the only significant parameter:

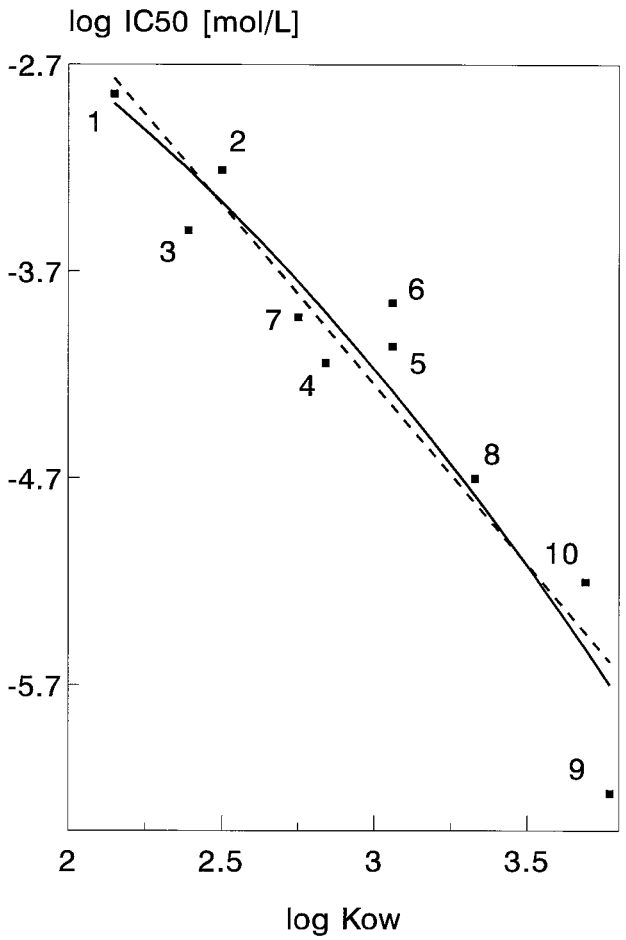

Fig. 1. Linear and parabolic relationship (broken and solid line, respectively) between PTG toxicity in terms of log IC50 (mol/L) and log K_{ow} of 10 chlorophenols (Eqns. 14 and 15). Compound numbering of the experimental data distribution is according to the list in Table 1.

$$\log \text{IC50} = -1.74\ (\pm 0.22) \log K_{ow} + 0.98\ (\pm 0.65)$$
$$r^2_{adj} = 0.87, \quad r^2 = 0.89,$$
$$\text{SD} = 0.35, \quad F_{1,8} = 63.9, \quad n = 10 \qquad (14)$$

The corresponding data distribution is shown in Figure 1. The two largest deviations are observed for 2,3,6-trichlorophenol (No. 9) and 2,5-dichlorophenol (No. 6) with regression errors of 0.63 and −0.50 log units, respectively. When introducing log K_{ow} in quadratic form, the linear term becomes insignificant, resulting in

$$\log \text{IC50} = -0.29\ (\pm 0.03)\ (\log K_{ow})^2 - 1.53\ (\pm 0.31)$$
$$r^2_{adj} = 0.90, \quad r^2 = 0.91,$$
$$\text{SD} = 0.32, \quad F_{1,8} = 79.1, \quad n = 10 \qquad (15)$$

Equation 15 is statistically superior to Equation 14, but the data set is probably too small to allow a definite decision between the linear and parabolic relationship. However, other aquatic test systems often show a clearly linear dependence, with polar narcotics in the log K_{ow} range of 2 to 3.5.

Further inspection of Equation 14 reveals quite unusual values for slope and intercept as compared to typical regression relationships between toxicity and lipophilicity for narcotic chemicals, which (to the authors' knowledge) have not been observed

Table 3. Linear regression of logarithmic acute toxicity on log K_{OW} for narcotic modes of toxic action[a]

Mode of action	Organism	Endpoint log (mol/L)	log K_{OW} coefficient	Constant	CBR (mmol/kg)	log K_{OW} range	n	r^2	SD	Literature reference of regression equation
Nonpolar narcotic	Guppy	14-d LC50	−0.87	−1.13	3.7	−1.3–5.7	50	0.98	0.24	Könemann and Musch [1]
	Daphnia magna	48-h IC50	−0.91	−1.28	2.6	−1.3–5.7	19	0.98	0.24	Hermens et al. [34]
	Tetrahymena pyriformis	48-h IC50	−0.80	−0.83	7.4	−0.8–5.7	20	0.99	0.14	Schultz et al. [35]
	Vibrio fisheri	5-min EC50	−1.17	−0.52	15	−0.8–4.2	12	0.97	0.38	Schultz et al. [35]
Ethoxylate narcotic	Crustacean	48-h LC50	−0.74	−2.01	0.48	0.8–5.1	8	0.98	0.18	Schüürmann [36]
Polar narcotic	Fathead minnow	96-h LC50	−0.65	−2.29	0.26	1.3–6.4	39	0.90	n.d.	Veith and Broderius [27]
	Tetrahymena pyriformis	48-h IC50	−0.63	−1.95	0.56	0.5–5.3	36	0.91	0.24	Schultz et al. [37]
Polar narcotic-type	Pollen tube	18-h IC50	−1.74	0.98	480	2.2–3.8	10	0.89	0.35	Present work

[a] The general form of the regression equation is given in Equation 16, and the critical body residue referring to wet weight (CBR) is calculated from the respective QSARs according to Equation 18, as described in the text.

before with any aquatic test system. However, this finding may be considered somewhat preliminary due to the rather narrow log K_{ow} range. In Table 3, reference QSARs for nonpolar narcosis, ethoxylate narcosis, and polar narcosis according to the general regression equation

$$\log \text{Tox} = a \times \log K_{ow} + c \tag{16}$$

where Tox is the toxic exposure concentration, are compared with the present result of Equation 14. The slope of −1.74 is steeper than typical polar narcosis slopes by a factor of three, indicating a correspondingly enhanced sensitivity of the PTG test to log K_{ow} differences in the (small) range analyzed here. On the other hand, the intercept of 0.98 (referring to concentration values in mol/L) is three orders of magnitude greater than with other polar narcosis QSARs. This would imply a correspondingly decreased sensitivity for hydrophilic compounds.

The latter is seen more clearly when converting the exposure-related toxicity concentrations to dose-related concentration values in terms of critical body residues (CBRs). As shown by McCarty [31], toxicant concentrations in aquatic organisms can be estimated from corresponding exposure concentrations in the water through multiplication by the bioconcentration factor (BCF), assuming steady-state conditions. In the (familiar) exposure scale, toxicant potency may vary by up to five or more orders of magnitude, whereas corresponding lethal body burdens show much less variation and can be regarded approximately constant for unspecific modes of toxic action such as nonpolar and polar narcosis [31–33].

In case of lacking BCF data, an alternative way of estimating CBRs from narcosis-type QSARs (Eqn. 16) can be derived as follows. If $K_{ow} = 1$ and thus log $K_{ow} = 0$ in Equation 16, the compound concentration in water (c_w) equals the compound concentration in octanol (c_o) and, following narcosis theory, also the compound concentration in the lipid fraction of the organism (c_L), which is related to the total internal concentration based on wet weight (CBR) through the lipid fraction (f_L) of the organism:

$$c_w = c_o = c_L = \frac{1}{f_L} \times \text{CBR} \tag{17}$$

As c_w is known as the toxic exposure concentration (Tox in Eqn. 16), it follows that the critical body residue (referring to wet weight) can be estimated from narcosis-type QSARs according to

$$\text{CBR} = f_L \times 10^c \tag{18}$$

with c representing the regression constant of Equation 16 (or any corresponding regression equation with a power function of log K_{ow}). Assuming $f_L = 5\%$ and a density of the organism of 1, application of Equation 18 leads to the CBR values in mmol/kg wet weight as listed in Table 3. With nonpolar and polar narcotics, these calculated CBRs agree quite well with the experimentally determined ranges of 2 to 8 mmol/kg [32] and 0.4 to 1.0 mmol/kg [33], respectively. In contrast, the CBR of 480 mmol/kg for the pollen tube estimated from the linear QSAR seems unrealistically high, corresponding to an extraordinary insensitivity of the PTG test for hydrophilic compounds.

Recall that the underlying assumption with this estimated CBR for the PTG test is linear extrapolation outside the log K_{ow} range analyzed here, which would (of course) not be justified statistically and need not be the case. For example, a sigmoid dependence of lipophilicity-driven PTG toxicity for relatively unreactive compounds would bring the absolute sensitivity of this test system more in line with conventional aquatic test systems at the high and low end of log K_{ow}. This is illustrated by calculating CBR from parabolic Equation 15 in the corresponding manner, yielding a value of 1.5 mmol/kg, which is much closer to expectation, and between the above-mentioned experimental ranges for nonpolar and polar narcosis. At present, additional experimental work is needed to assess the response characteristic of the PTG test in a more general way. For this purpose, selection of nonpolar or polar narcotics in the low and high log K_{ow} range can be recommended from the present findings.

With the 10 nitrophenols, regression of log IC50 on log K_{ow} gives no meaningful correlation ($r^2 = 0.08$, $r^2_{adj} = 0$). Inspection of the data distribution (Table 1) reveals the following pattern: *diortho*-substituted nitrophenols (having log K_{ow} below 1.55) show a sharp increase in toxicity with an only small increase in log K_{ow}, and an opposite trend is observed for the other mono- and dinitrophenols (having log K_{ow} above 1.55).

As can be seen from Table 1, the three *diortho* congeners are the most acidic compounds of the present test set, with pK_a values below 3.8. The plot of log IC50 against pK_a in Figure 2

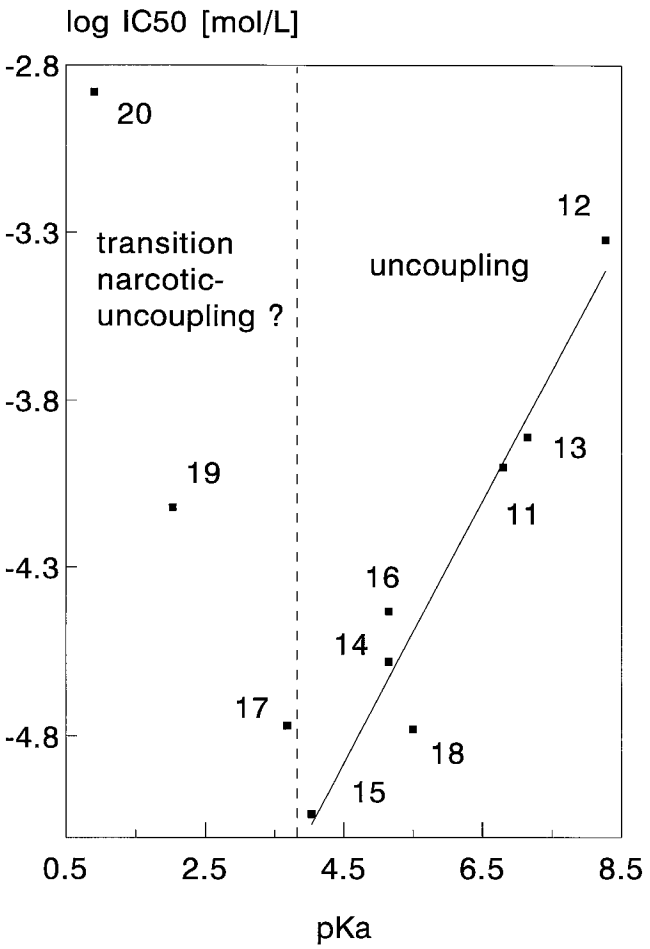

Fig. 2. Relationship between PTG toxicity in terms of log IC50 (mol/L) of 10 nitrophenols and pK_a (compound numbering according to Table 1). The solid line represents the linear regression relationship for the subset of seven nitrophenols without *diortho* substitution (Eqn. 19).

reveals two distinct regions. Moderate acidity with pK_a above 3.8 apparently leads to oxidative uncoupling activity, which increases with decreasing acidity (i.e., increasing pK_a). For stronger acidic nitrophenols with pK_a below 3.8, the data distribution suggests a transition between polar narcosis and uncoupling of oxidative phosphorylation as dominant modes of toxic action. In this high-acidity range, increase in acidity makes the compounds less effective as uncouplers due to the instability of the protonated species in solution. In other words, the narcosis contribution to overall toxicity probably increases with decreasing pK_a for sufficiently acidic compounds.

Linear regression of PTG toxicity for the subset of seven nitrophenols without *diortho* substitution (i.e., without compound Nos. 17, 19, and 20) gives the following result:

$$\log \text{IC50} = 0.39\ (\pm 0.05)\ \text{p}K_a - 6.63\ (\pm 0.29)$$
$$r^2_{adj} = 0.92, \qquad r^2 = 0.93,$$
$$\text{SD} = 0.17, \qquad F_{1,5} = 69.5, \qquad n = 7 \qquad (19)$$

Equation 19 strongly supports the hypothesis that with this subset of nitrophenols, uncoupling of oxidative phosphorylation is the dominating mode of toxic action. Equation 19 also implies that the PTG test is indeed well suited to detecting uncoupling activity of chemicals, due to its sufficient specific sensitivity [5,10].

Comparison between the physicochemical profiles of chlorophenols and nitrophenols (Table 1) leads to a further observation: in the present set of compounds, oxidative uncoupling activity of nitrophenols is associated with log K_{ow} below 2 and pK_a between 3.8 and 8.5. Eight of the 10 chlorophenols also have pK_a values within that range, but have log K_{ow} values above 2, resulting in a narcotic-type mode of action. It follows that oxidative uncoupling occurs only for proper combinations of lipophilicity and acidity, which themselves vary with the individual property values. With increasing lipophilicity, increasing acidity is required to exert uncoupling activity, with an upper pK_a limit due to the increasing degree of ionization. In other words, from the chemical viewpoint, moderate acidity (in this case with pK_a between 3.8 and 8.5) is a necessary but not sufficient condition for uncoupling oxidative phosphorylation. Within a given acidity window, only selected ranges of lipophilicity will lead to the activation of this mode of toxic action.

As outlined above, solution-phase acidity can be factorized into intrinsic (gas-phase) acidity and solvation interactions of the relevant chemical species with the aqueous phase. Multilinear regression analysis of log IC50 on ΔH_g^{AM1} and $\Delta\Delta G_s^{SM2}$ reveals that only ΔH_g^{AM1} gives a significant relationship with PTG toxicity:

$$\log \text{IC50} = 0.0130\ (\pm 0.0015)\ \Delta H_g^{AM1} - 21.3\ (\pm 1.9)$$
$$r^2_{adj} = 0.93, \qquad r^2 = 0.94,$$
$$\text{SD} = 0.16, \qquad F_{1,5} = 76.9, \qquad n = 7 \qquad (20)$$

In connection with the pK_a modeling results of Table 2, it follows that with this series of nitrophenols, the component of macroscopic pK_a relevant for uncoupling activity is the molecular intrinsic acidity as quantified through ΔH_g^{AM1}. Further investigations of the two-dimensional uncoupling window of pK_a and log K_{ow} may reveal whether the dependence of uncoupling activity on solution-phase acidity can be traced back to suitable proportions of ΔH_g^{AM1} and $\Delta\Delta G_s^{SM2}$.

Finally, the potential role of molecular nucleophilicity on PTG toxicity will be discussed. As noted above, *O*-glucuronide formation represents a major phase-II detoxication pathway of phenolic xenobiotics, implying that increasing S_N2 nucleophilicity as modeled through ΔH_{nuc}^{AM1} may contribute to decreasing acute toxicity of the compounds. Comparison between ΔH_{nuc}^{AM1} and pK_a in Table 1 shows that the information encoded in ΔH_{nuc}^{AM1} (representing the ability of phenolic OH to add at electrophilic carbon sites) is indeed significantly different from solution-phase acidity (representing the ability of phenolically bound hydrogen to leave as proton). A major difference between the two compound sets is observed for the *diortho* congeners, where strong inhibition of S_N2 reactivity through chlorine substituents (compound Nos. 7, 9, and 10) contrasts with strong enhancement through nitro substituents (compound Nos. 17, 19, and 20).

Multilinear regression of log IC50 including all descriptors listed in Table 1 reveals that ΔH_{nuc}^{AM1} shows no significant relationship with PTG toxicity for both chloro- and nitrophenols. In particular, the only moderate narcotic-type relationship with chlorophenols cannot be improved through inclusion of molecular nucleophilicity. It follows that despite the well-known involvement of UDP conjugation in the phase-II detoxication of phenols, a direct impact on acute toxicity cannot be seen with the present test design and the model parameter for nucleophilicity.

CONCLUSIONS

The PTG toxicity of chlorophenols shows a narcotic-type relationship with log K_{ow}. However, slope and intercept indicate marked differences to corresponding QSARs with conventional aquatic test systems: the steeper slope reveals a substantially greater relative sensitivity in the (small) log K_{ow} tested, and the greater intercept suggests a significantly reduced sensitivity for hydrophilic compounds as well as a correspondingly greater CBR. Further testing of selected narcotic-type chemicals at the low and high log K_{ow} end will be required to further characterize the response characteristic of the alternative PTG test.

Moderately acidic nitrophenols with pK_a between 3.8 and 8.5 exert uncoupling of oxidative phosphorylation, as indicated by a significant relationship of PTG toxicity with molecular acidity. This relationship further implies that the PTG system is particularly suited for detecting this mode of toxic action. For higher acidic congeners, data analysis suggests a shift from uncoupling to a narcotic mode of action. Comparison with the property profile of chlorophenols reveals that a distinct two-dimensional property window of pK_a and log K_{ow} is required for substantial oxidative uncoupling activity.

REFERENCES

1. **Könemann, H.** and **A. Musch.** 1981. Quantitative structure–activity relationships in fish toxicity studies. Part 2: The influence of pH on the QSAR of chlorophenols. *Toxicology* **19**:223–228.
2. **Saarikoski, J.** and **M. Viluksela.** 1982. Relation between physicochemical properties of phenols and their toxicity and accumulation in fish. *Ecotoxicol. Environ. Saf.* **6**:501–512.
3. **Schultz, T.W.** 1987. The use of the ionization constant (pK_a) in selecting models of toxicity in phenols. *Ecotoxicol. Environ. Saf.* **14**:178–183.
4. **Terada, H.** 1990. Uncouplers of oxidative phosphorylation. *Environ. Health Perspect.* **87**:213–218.
5. **Schüürmann, G.** and **H. Segner.** 1994. Wirkungsforschung in der Chemischen Ökotoxikologie. *UWSF—Z. Umweltchem. Ökotox.* **6**: 351–358.
6. **Kristen, U., U. Joos, J.P. van Aken** and **R. Kappler.** 1991. Bestimmung der Zytotoxizität von Detergentien, Pestiziden und anderen Chemikalien mit dem Pollen-Test (pollen tube growth test). *VDI Berichte* **901**:1191–1210.
7. **Kristen, U., U. Hoppe** and **W. Pape.** 1992. The pollen tube growth test: A new alternative to the Draize eye irritation assay. *J. Soc. Cosmet. Chem.* **44**:153–162.
8. **Gettings, S.D., et al.** 1994. The CTFA evaluation of alternatives program: An evaluation of in vitro alternatives to the Draize primary eye irritation test. (Phase II) Oil/water emulsions. *Food Chem. Toxicol.* **32**:943–976.
9. **Clemedson, C., et al.** 1996. MEIC evaluation of acute systemic toxicity for the first 30 reference chemicals: Part II. In vitro results from 68 toxicity assays used to test the first 30 reference chemicals and a comparative cytotoxicity analysis. *ATLA* **24(Suppl.1)**:273–311.
10. **Strube, K., D. Janke, R. Kappler** and **U. Kristen.** 1991. Toxicity of some herbicides to in vitro growing tobacco pollen tubes (the pollen test). *Environ. Exp. Bot.* **31**:217–222.
11. **Kappler, R.** and **U. Kristen.** 1987. Photometric quantification of in vitro pollen tube growth: A new method suited to determine the cytotoxicity of various environmental substances. *Environ. Exp. Bot.* **27**:305–309.
12. **Kappler, R.** and **U. Kristen.** 1988. Photometric quantification of water-insoluble polysaccharides produced by in vitro grown pollen tubes. *Environ. Exp. Bot.* **28**:33–36.
13. **Kristen, U.** and **R. Kappler.** 1995. The pollen tube growth test. In S. O'Hare and C. K. Atterwill, eds., *In Vitro Toxicity Testing Protocols*, Vol. 43—Methods in Molecular Biology. Humana, Totowa, NJ, USA, pp. 189–198.
14. **Leo, A.J.** 1993. Calculating log P_{oct} from structures. *Chem. Rev.* **93**:1281–1306.
15. **Daylight Chemical Information Systems.** 1994. *Daylight Software 4.34.* Irvine, CA, USA.
16. **Perrin, D.D., B. Dempsey** and **E.P. Serjeant.** 1981. *pK_a Prediction for Organic Acids and Bases.* Chapman and Hall, London, UK.
17. **Dewar, M.J.S., E.G. Zoebisch, E.F. Healy** and **J.J.P. Stewart.** 1985. AM1: A new general purpose quantum mechanical molecular model. *J. Am. Chem. Soc.* **107**:3902–3909.
18. **Klamt, A.** and **G. Schüürmann.** 1993. COSMO: A new approach to dielectric screening in solvents with explicit expressions for the screening energy and its gradient. *J. Chem. Soc. Perkin Trans. 2*, pp. 799–805.
19. **Cramer, C.J.** and **D.G. Truhlar.** 1992. An SCF solvation model for the hydrophobic effect and absolute free energies of solvation. *Science* **256**:213–217.
20. **Stewart, J.J.P.** 1990. MOPAC: A semiempirical molecular orbital program. *J. Comput.-Aided Mol. Des.* **4**:1–105.
21. **Fujitsu Limited.** 1993. *MOPAC 93.* Stewart Computational Chemistry, Colorado Springs, CO, USA.
22. **Cramer, C.J., G.C. Lynch, G.D. Hawkins, D.G. Truhlar** and **D.A. Liotard.** 1993. AMSOL 4.0: An SCF program for free energies of solvation (QCPE 606). *Quantum Chem. Prog. Exchange Bull.* **13**:78.
23. **Schüürmann, G.** 1996. Modelling pKa of carboxylic acids and chlorinated phenols. *Quant. Struct.–Act. Relat.* **15**:121–132.
24. **Tripos Associates** 1992. *SYBYL Molecular Modelling Software 6.0.* St. Louis, MO, USA.
25. **Lide, D.R.,** ed. 1992–1993. *CRC Handbook of Chemistry and Physics*, 73rd ed. CRC, Boca Raton, FL, USA.
26. **Hodgson, E.** and **P.E. Levi.** 1987. *Modern Toxicology*. Elsevier, New York, NY, USA.
27. **Veith, G.** and **S. Broderius.** 1990. Rules for distinguishing toxicants that cause Type I and Type II narcosis syndromes. *Environ. Health Perspect.* **87**:207–211.
28. **Kandasamy, M.K.** and **U. Kristen.** 1987. Pentachlorophenol affects mitochondria and induces formation of Golgi apparatus–endoplasmatic reticulum hybrids in tobacco pollen tubes. *Protoplasma* **141**:112–120.
29. **Schüürmann, G.** 1991. Do Hammett constants model electronic properties in QSARs? *Sci. Total Environ.* **109/110**:221–235.
30. **Schüürmann, G.** 1995. Quantum chemical approach to estimate physicochemical compound properties: Application to substituted benzenes. *Environ. Toxicol. Chem.* **14**:2067–2076.
31. **McCarty, L.S.** 1986. The relationship between aquatic toxicity QSARs and bioconcentration for some organic chemicals. *Environ. Toxicol. Chem.* **5**:1071–1080.
32. **Sijm, D.T.H.M., M. Schipper** and **A. Opperhuizen.** 1993. Toxicokinetics of halogenated benzenes in fish: Lethal body burden as a toxicological end point. *Environ. Toxicol. Chem.* **12**:1117–1127.
33. **McKim, J.M.** and **P.K. Schmieder.** 1991. Bioaccumulation: Does it reflect toxicity? In R. Nagel and R. Loskill, eds., *Bioaccumulation in Aquatic Systems, Contributions to the Assessment.* VCH, Weinheim, Germany, pp. 161–188.
34. **Hermens, J., H. Canton, P. Janssen** and **R. de Jong.** 1984. Quantitative structure–activity relationships and toxicity studies of mixtures of chemicals with anaesthetic potency: Acute lethal and sublethal toxicity to *Daphnia magna. Aquat. Toxicol.* **5**:143–154.
35. **Schultz, T.W., N.L. Wyatt** and **D.T. Lin.** 1990. Structure–toxicity relationships for nonpolar narcotics: A comparison of data from the *Tetrahymena*, *Photobacterium* and *Pimephales* systems. *Bull. Environ. Contam. Toxicol.* **44**:67–72.
36. **Schüürmann, G.** 1991. Acute aquatic toxicity of alkyl phenol ethoxylates. *Ecotoxicol. Environ. Saf.* **21**:227–233.
37. **Schultz, T.W., M. Cajina-Quezada** and **S.K. Wesley.** 1989. Structure–toxicity relationships for mono alkyl- or halogen-substituted anilines. *Bull. Environ. Contam. Toxicol.* **43**:564–569.