THE ACUTE TOXICITY OF PULSE-DOSED PARA-SUBSTITUTED PHENOLS TO JUVENILE AMERICAN FLAGFISH (JORDANELLA FLORIDAE): A COMPARISON WITH PREDICTED TOXICITY USING LOG K_{ow} DEPENDENT QSARs AND TOXICITY TO PHOTOLUMINESCENT BACTERIA

D.A. Holdway[1]*, D.G. Dixon[1]** and K.L.E. Kaiser[2]

NWRI Contribution No. 88-29

[1] Department of Biology
University of Waterloo
Waterloo, Ontario N2L 3G1
Canada

[2] Lakes Research Branch
National Water Research Institute
Canada Centre for Inland Waters
Burlington, Ontario L7R 4A6
Canada

* Present address: Office of the Supervising Scientist
Alligator Rivers Region Research Institute
Jabiru East, Northern Territory 5796, Australia
** To whom correspondence may be addressed

The acute toxicity of pulse-dosed para-substituted phenols to juvenile American flagfish (Jordanella floridae): a comparison with predicted toxicity using log K_{ow} dependent QSARs and toxicity to photoluminescent bacteria

D.A. Holdway[1*], D.G. Dixon[1**] and K.L.E. Kaiser[2]

[1]Department of Biology, University of Waterloo,
Waterloo, Ontario N2L 3G1, Canada

[2]Lakes Research Branch, National Water Research Institute,
Burlington, Ontario L7R 4A6, Canada

* Present address: Office of the Supervising Scientist, Alligator Rivers Region Research Institute, Jabiru East, Northern Territory 5796, Australia

** To whom correspondence may be addressed

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Dr. D. George Dixon
Associate Professor
Department of Biology
University of Waterloo
Waterloo, Ontario
N2L 3G1
Canada

Tel. 519-885-1211 (ext. 2531)

Abstract - The acute toxicity of 9 para-substituted phenols was determined using a pulse-exposure testing protocol and 8-d-old American flagfish. Relative tolerance was assessed by determining the 2-h pulse exposure concentration causing 20 and 50% mortality (PE LC20 and PE LC50) over the subsequent 96 h. Four bioassays were run for each phenol and yielded the following mean PE LC20s (mg l^{-1}) in descending order of toxicity: *p*-amino-phenol, 0.06; hydroquinone, 0.13; phenol, 0.70; *p*-nitro-phenol, 0.81; *p*-cyano-phenol, 3.0; *p*-chloro-phenol, 3.3; *p*-hydroxy-acetophenone, 4.2; *p*-hydroxy-benzyl alcohol, 6.4; and *p*-hydroxy-benzoic acid, 170. These toxicities did not correlate significantly with either the log octanol-water partition coefficient or with previously reported toxicity values for the photoluminescent bacteria Photobacterium phosphoreum. For some of the compounds, however, sensitivities were quite close to previously reported rainbow trout chronic no-observed-effect-concentrations based on continuous exposure. Caution is urged with respect to applying either quantitative structure-activity correlations or "low-level" biota techniques when attempting to predict the toxicity of specific chemicals to fish.

Key words - flagfish, phenols, acute toxicity, QSAR, photoluminescent bacteria.

Résumé - La toxicité aiguë de neuf phénols substitués en para a été déterminée à l'aide d'une méthode d'essai par exposition à des doses pulsées et à l'aide d'un fondule J. floridae de huit jours. La tolérance relative a été évaluée en déterminant la concentration de 2 h à des expositions pulsées entraînant un taux de mortalité de 20 et de 50 % (CL_{20} EP et CL_{50} EP) pendant 96 h. Quatre bio-essais ont été effectués pour chaque phénol et ont donné les valeurs moyennes suivantes de CL_{20} EP (mg/L), par ordre décroissant de toxicité; p-aminophénol, 0.06; hydroquinone, 0.13; phénol, 0.70; p-nitrophénol, 0.81; p-cyanophénol, 3.0; p-chlorophénol, 3.3; p-hydroxyacétophénone, 4.2; p-hydroxybenzyl alcool, 6.4; et acide p-hydroxybenzoïque, 170. Ces valeurs ne présentaient pas de corrélation significative avec le coefficient de partition logarithmique octanol-eau ou avec les valeurs de toxicité déjà signalées pour les bactéries photoluminescentes Photobacterium phosphoreum. Toutefois, pour certains de ces composés, les valeurs des sensibilités étaient très voisines des valeurs chroniques déjà signalées pour la truite arc-en-ciel (concentrations sans effet observé), basées sur une exposition continue. On recommande toutefois une certaine prudence pour l'application des corrélations quantitatives structure-activité ou des techniques basées sur les études du biote "aux faibles concentrations" pour la prévision de la toxicité de produits chimiques particuliers pour le poisson.

Management Perspective

Title: The acute toxicity of pulse-dosed para-substituted phenols to juvenile American flagfish (*Jordanella floridae*): a comparison with predicted toxicity using K_{ow} dependent QSAR and toxicity to photoluminescent bacteria

Authors: D.A. Holdway (Univ. Waterloo)
D.G. Dixon (Univ. Waterloo)
K.L.E. Kaiser (LRB, NWRI)

Date: April, 1988

Perspective:

This manuscript reports new research results on the acute toxicity of nine substituted phenols to the flagfish using pulse-dosed exposure. The results show that the effects of the individual chemicals are different from those obtained in more traditional bioassays, such as 48-hr and 96-hr lethal concentrations to rainbow trout and the protozoan *Tetrahymena pyriformis* and the Microtox tests.

As organisms in the environment, particularly in rivers, are often exposed to strongly fluctuating contaminant levels, simulated by this pulse-dosed exposure, this work demonstrates the need for a variety of bioassays to determine the effects of toxic substances in the environment.

Ce manuscrit présente les nouveaux résultats de recherches portant sur la toxicité aiguë de neuf phénols substitués pour le fondule J. floridae, obtenus par exposition à des doses pulsées. Les résultats indiquent que les effets des produits chimiques particuliers sont différents de ceux obtenus à l'aide de bio-essais plus habituels, par exemple les concentrations létales de 48 h et de 96 h pour la truite arc-en-ciel, la protozoaire Tetrahymena pyriformis, ainsi que les essais Microtox.

Etant donné que les organismes dans l'environnement, et plus particulièrement dans les rivières, sont souvent exposés à des concentrations de contaminants présentant d'importantes fluctuations, simulées par l'exposition à des doses pulsées, ce travail démontre la nécessité d'une diversité de bio-essais afin de déterminer les effets des substances toxiques dans l'environnement.

INTRODUCTION

The use of Quantitative Structure-Activity Correlations (QSAR) to predict toxicity of various chemicals has been reasonably successful for fish [1, 2, 3] photoluminescent bacteria [4, 5] and the ciliated protozoan Tetrahymena pyriformis [3, 6]. This success is, however, generally statistically dependent upon large sets of toxicity data and very often requires selective pre-screening of compounds, to include only those that are "non-reactive" and "non-ionic" [6]. The use of non-fish (usually "lower biota") test systems has evolved both to fill the requirement for large data sets and to provide increased accuracy and precision of the bioassays [5]. It should be noted that the real requirement of QSAR and rapid non-fish test systems is to predict the toxicity of individual compounds to "higher" aquatic organisms, most notably fish. Since classical flow-through fish bioassays require comparatively large quantities of compounds which are expensive and often difficult to purify, a rapid and sensitive fish test system is needed. The use of pulse (as opposed to continuous) toxicant exposure with juvenile flagfish represents one such possible system.

This study was undertaken to test the null hypotheses that 1) the acute toxicity of para-substituted phenols to juvenile flagfish (Jordanella floridae) is not affected by the type of substitution; 2) any observed toxicity is not predictable using QSARs based on the log of the octanol-water partition coefficient (K_{ow}); any observed fish toxicity is not predictable from previously reported [4] toxicity to the photoluminescent bacteria (Photobacterium phosphoreum; and 4) the level of sensitivity of pulse exposed fish to para-substituted phenols does not compare with levels reported for continuously exposed fish. Flagfish were chosen for this work since they are continuous spawners which provide a year-round supply of test organisms of known age and since they exhibit thresholds of toxicant response similar to northern species [7].

MATERIALS AND METHODS

The relative tolerance of 8-d-old juvenile American flagfish (Jordanella floridae Goode and Bean) was assessed by determining the 2 h pulse-exposure concentration of each of 9 different phenols causing 20 and 50% mortality (PE LC20 and PE LC50) over the subsequent 96 h. Each phenol bioassay was replicated 4 times. Procedures adhered closely to those previously established for acute pulse-exposure tests with American flagfish [8]. All fish used were obtained from the same batch of eggs. Eggs were collected daily from 4 brood-stock tanks, each containing 2 male and 5 female flagfish. Egg batches were therefore the pooled reproductive product of potentially 8 males and 20 females. The broodstock was 3 generations removed from a wild Florida stock, making all experimental fish 4 generations removed from the wild.

All test chambers used in the bioassays were 100 ml linear polyethylene beakers modified by replacing their bottoms with nylon screen and adding an external styrofoam floatation ring. Ten fish were randomly distributed to each chamber. A group of five chambers constituted one bioassay set. The relative toxicities of the phenols to the bacteria Photobacterium phosphoreum [4] were used to divide the compounds into either "high" or "low" toxicity groups. Bioassays with "high" toxicity phenols (phenol, hydroquinone, *p*-nitro-phenol, *p*-hydroxy-acetophenone and *p*-cyano-phenol) exposed fish to nominal toxicant concentrations of 0, 0.05, 0.20, 1.0 and 10 mg l^{-1}. Nominal exposure concentrations of 0, 0.1, 1.0, 10 and 100 mg l^{-1} were used for the "low" toxicity phenols (*p*-chloro-phenol, *p*-hydroxy-benzoic acid, *p*-hydroxy-benzyl alcohol and *p*-amino phenol).

Before and after toxicant exposure the bioassay chambers were held in 23 l glass aquaria, each of which received a continuous flow of toxicant-free water (120 l d^{-1}). At the start of each bioassay the test chambers were transferred to 23 l toxicant exposure tanks containing one of the 9 phenols at either the "high" or "low" exposure

scheme depending on estimated toxicity. The phenols (recrystallized from best available grade) were dissolved in the exposure tanks 15 min prior to the start of exposure. Exposure tanks were vigorously mixed to ensure even toxicant distribution. All tests employed 2 h exposures, with the entire volume of each chamber manually exchanged by raising and lowering the chambers every 15 min. After 2 h, exposed fish were transferred back to their respective clean-water holding tanks in ascending order of exposure after a quick dip in a clean water tank to remove any residue. Control fish were rinsed and returned to their holding tanks first.

The mean (s.e.) characteristics of the test water during all experiments were: temperature, 25.1°C (0.02); pH, 7.97 (0.03); dissolved oxygen, 8.1 mg l^{-1} (0.01); total hardness, 333 mg l^{-1} as $CaCO_3$ (4.0); and alkalinity, 265 mg l^{-1} as $CaCO_3$ (0.2). These characteristics did not vary significantly either from tank to tank within experiments or between experiments. Food consisted of 2 feedings per day of brine shrimp nauplii. Experimental photoperiod was 16 h light : 8 h dark with 0.5 h of artificial dawn and dusk included in the daylight portion.

All data were analyzed with standard SAS programs from the library of the Department of Computing Services, University of Waterloo. The PE LC20 or PE LC50 for each replicate was determined by probit analysis [9] and a mean PE LC20 (or PE LC50) was calculated for each phenol. The PE LC20 and PE LC50 values were compared by standard error of the difference [10]. Correlations between measurements of toxicity and chemical characteristics of the phenols were assessed by linear regression analysis [11]. The statistical significance of the results was assessed at the 0.05 probability level.

RESULTS

The short term exposure of juvenile flagfish to eight of the nine phenols caused significant acute toxicity which was greatly modified by the type of substituent group (R) in the position para to the phenolic hydroxy group ($HO\text{-}C_6H_4\text{-}R$). The 96 h PE LC20s (a more accurate estimate of toxicity than PE LC50s, since all but one of the mean PE LC20s were bracketed by the actual exposure concentrations) ranged from significantly different values of 0.06 and 0.13 mg l^{-1} for *ρ*-amino-phenol and hydroquinone respectively, to a value of 170 mg l^{-1} for *ρ*-hydroxy-benzoic acid (Table 1). Phenol and *ρ*-nitro-phenol showed similar levels of toxicity with respective PE LC20 values of 0.70 and 0.81 mg l^{-1}. The remaining four substituted phenols (*ρ*-cyano-phenol, *ρ*-chloro-phenol, *ρ*-hydroxy-acetophenone and *ρ*-hydroxy-benzyl alcohol) gave PE LC20s which ranged from 3.0 to 6.4 mg l^{-1} and which were not significantly different from each other.

The PE LC20 and PE LC50 values for the nine phenolics were converted to log (c^{-1}), where c is the concentration in mmol l^{-1}, to facilitate correlation with log K_{ow} and previously reported [4] 30 min EC50 values for P. phosphoreum (Table 2). The relative toxicities of the nine compounds were then ranked from 1 to 9 with the lowest number representing the highest measured toxicity in the case of the fish and bacteria, or predicted toxicity in the case of log K_{ow} data (Table 3). Thus, *ρ*-amino-phenol is the most toxic compound to pulse-exposed juvenile flagfish as indicated by the PE LC20, while *ρ*-hydroxy-benzoic acid is the least toxic.

The PE LC50 results indicate essentially the same patterns of toxicity relative to the PE LC20 results with a few minor differences. Hydroquinone was marginally more toxic than *ρ*-amino phenol (PE LC50 of 0.24 compared to 0.34 mg l^{-1}), phenol was more toxic than *ρ*-nitro-phenol (PE LC50 of 5.1 compared to 16 mg l^{-1}) which in turn was more toxic than either *ρ*-hydroxy-acetophenone (PE LC50 of 190 mg l^{-1}) or *ρ*-cyano-

phenol (PE LC50 of 380 mg 1^{-1}). The latter two phenol derivatives were much more toxic than either *p*-chloro-phenol (PE LC50 of 10,500 $mg.1^{-1}$) or *p*-hydroxy-benzoic acid (PE LC50 exceeded saturation levels) (Tables 1, 2, 3).

Simple linear regressions between log K_{ow}, P. phosphoreum EC50s and flagfish PE LC20s and LC50s yielded only one significant regression (Table 4). There was a highly significant positive correlation ($r=0.981$, $p<0;0001$) between PE LC20 and PE LC50 values as would be expected. There was no significant correlation between log K_{ow} values and PE LC20 ($r=-0.416$, $p=0.27$), PE LC50 ($r=0.337$, $p=0.38$), or EC50 values ($r=-0.505$, $p=0.17$), nor between EC50 values and PE LC50 ($r=0.407$, $p=0.28$) or PE LC20 values ($r=0.460$, $p=0.21$).

DISCUSSION

This work demonstrates that short-term pulse-exposure of juvenile flagfish to para-substituted phenols causes significant acute toxicity, toxicity which is modified by the type of substituent group in the position para to the phenolic hydroxy group. The concentrations which resulted in mortality during the 4-d period following a 2-h exposure were, for the most part, as low or lower than concentrations previously reported toxic under continuous exposure regimes [1, 2, 6, 12]. For example, phenol has a 48-h LC50 to rainbow trout (Salmo gairdneri) of 9.3 mg l^{-1} [13], 1.8 and 13.3 times higher than the respective PE LC50 and PE LC20 values reported here. The mean no effect concentration (NOEC) of phenol and *p*-nitrophenol reported for chronic exposure of rainbow trout fry [2] are, respectively, 2.7 and 3.6 times lower than the PE LC20 values reported in this study for juvenile flagfish. In the same study, the NOEC for *p*-chlorophenol was found to be some 30 times lower than the PE LC20 reported here. The apparent low toxicity of *p*-chlorophenol in this study is possibly due to the short exposure time utilized in pulse-exposure. With a log K_{ow} of 2.42, *p*-chlorophenol is the most hydrophobic of the nine phenols tested in this study. It has been argued [2] that toxicity tests of relatively short, fixed duration underestimate the toxicity of hydrophobic organic compounds due to their decreased rates of uptake.

Our finding that *p*-amino-phenol and hydroxyquinone were by far the most toxic of the 9 phenols is interesting, since both compounds are very strong electron-releasing derivatives and for this reason have been excluded from other studies comparing the relative toxicity of para-substituted phenols with log K_{ow} [6]. In a comparison of QSAR predictions with fish toxicity screening data for some 110 phenols, *p*-amino-phenol exhibited lethal toxicity at a concentration 35 times lower than the QSAR predicted 96 h LC50 value [1] supporting our finding that *p*-amino-phenol is extremely toxic to fish.

Data on the toxicity of five of the phenols used in this work (phenol, ρ-nitro, ρ-cyano-, ρ-chloro-, and ρ-hydroxy-acetophenone) to Tetrahymena pyriformis have been reported [3]. The T. pyriformis toxicity measurement (60 h 50% inhibitory growth concentration; IGC50) does not correlate significantly with either flagfish PE LC20 ($r=0.278$, $n=5$) or P. phosphoreum EC50 ($r=0.178$, $n=5$). Admittedly the number of phenols compared is small, but along with the results reported here, this raises a strong warning over the use of "lower biota" test systems or chemical descriptors like K_{ow} to predict the toxic impact of any particular phenol on "higher biota" such as fish. The danger of using regressions involving large numbers of related compounds to make such predictions lies with the fact that the very large aberrations from these regressions which can exist on an individual chemical basis can be ignored or overlooked. Although some value can be derived by classifying the general toxicity of categories of compounds, there exists a real danger that this value will be greatly overshadowed by the large differences in toxicities found between compounds, even within narrowly defined groups. Reasons for such aberrations and a basis for their prediction has recently been proposed [14].

In conclusion, the pulse-exposure of juvenile flagfish to various para-substituted phenols resulted in significant substituent-dependent toxicity. Neither the ranking of toxicity nor the acute toxicity to flagfish could be successfully predicted using K_{ow} based QSAR or P. phosphoreum EC50. Caution is urged in the use of QSAR and "lower biota" test systems in predicting the toxicities of individual compounds to "higher biota". In the case of the 9 para-substituted phenols tested in this study, any prediction of fish toxicity based on the above mentioned systems would be incorrect and misleading. The pulse-exposure of juvenile flagfish yielded results much closer to continuously-exposed trout chronic results than to the "lower biota" screening tests discussed.

REFERENCES

1. Lipnick, R.L., C.K. Bickings, D.E. Johnson and D.A. Eastmond. 1985. Comparison of QSAR predictions with fish toxicity screening data for 110 phenols. In R.C. Bahner and D.J. Hansen, eds. Aquatic Toxicology and Hazard Assessment : Eighth Symposium. STP 891. American Society for Testing and Materials, Philadelphia, PA, pp. 153-176.
2. McCarty, L.S., P.V. Hodson, G.R. Craig and K.L.E. Kaiser. 1985. The use of quantitative structure-activity relationships to predict the acute and chronic toxicities of organic chemicals to fish. Environ. Toxicol. Chem. 4:595-606.
3. Schultz, T.W., G.W. Holcombe and G.L. Phipps. 1986. Relationships of quantitative structure-activity to comparative toxicity of selected phenols in the Pimephales promelas and Tetrahymena pyriformis test systems. Ecotoxicol. Environ. Safe. 12:146-153.
4. Ribo, J.M. and K.L.E. Kaiser. 1983. Effects of selected chemicals to photoluminescent bacteria and their correlations with acute and sublethal effects on other organisms. Chemosphere 12:1421-1442.
5. Kaiser, K.L.E. and J.M. Ribo. 1985. QSAR of toxicity of chlorinated aromatic compounds. In M. Tichy, ed. QSAR in Toxicology and Xenobiochemistry, Elsevier, Amsterdam, pp. 27-38.
6. Schultz, T.W. 1987. Relative toxicity of para-substituted phenols: Log K_{ow} and pKa-dependent structure-activity relationships. Bull. Environ. Contam. Toxic. 38:994-999.
7. McKim, J.M. 1985. Early life stage toxicity tests. In G.M. Rand and S.R. Petrocelli, eds. Fundamentals of Aquatic Toxicology : Methods and Applications. Hemisphere Pub. Corp., New York, NY, pp 58-95.

8. Holdway, D.A. and D.G. Dixon. 1985. Acute toxicity of pulse-dosed methoxychlor to juvenile American flagfish (Jordanella floridae Food and Bean) as modified by age and food availability. Aquat. Toxic. 6:243-250.

9. Finney, D.J. 1971. Probit Analysis, 3rd ed. University Press, Cambridge.

10. Sprague, J.B. and A. Fogels. 1977. Watch the Y in bioassay. In Proceedings, Third Aquatic Toxicity Workshop. EPS-5-AR-77-1. Environmental Protection Service, Environment Canada, Ottawa, Ontario, pp. 107-118.

11. Zar, J.H. 1984. Biostatistical Analysis, 2nd ed. Prentice-Hall, Toronto.

12. Holcombe, G.W., G.L. Phipps, M.L. Knuth and T. Felhaber. 1984. The acute toxicity of selected substituted phenols, benzenes and benzoic acid esters to fathead minnows Pimephales promelas. Envir. Poll. (A) 35:367-381.

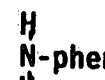
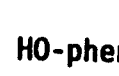
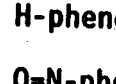
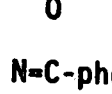
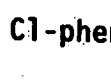
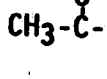
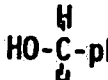
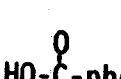
13. Brown, V.M., D.G. Shurben and J.K. Fawell. 1967. The acute toxicity of phenol to rainbow trout in saline waters. Water Res. 1:683-685.

14. Kaiser, K.L.E. and K.M. Gough. 1988. Predictability of unusually high acute toxicity to Photobacterium phosphoreum of 1,4-di-substituted benzene derivatives. In G.W. Suter II, ed. Proceedings 11th Symposium on Aquatic Toxicology and Hazard Assessment. STP 000. American Society for Testing and Materials, Philadelphia, PA, in press.

Table 1. PE LC20s and LC50s for flagfish exposed to one of nine para-substituted phenols for 2 h at 8 d of age and observed over the subsequent 94 h in toxicant-free water. Values are given as means (n=4) followed by the standard error in parentheses. Values within columns without an alphabetical superscript in common are significantly different ($p \leq 0.05$).

Phenol	PE LC20 (mg l^{-1})	PE LC50 (mg l^{-1})
p-amino-phenol	0.06 (0.01)[a]	0.34 (0.03)[b]
hydroquinone	0.13 (0.02)[b]	0.24 (0.01)[a]
phenol	0.70 (0.14)[c]	5.1 (1.48)[c]
p-nitro-phenol	0.81 (0.24)[c]	16 (3.65)[d]
p-cyano-phenol	3.0 (1.34)[d]	380 (136)[e]
p-chloro-phenol	3.3 (0.81)[d]	2400(1070)[f]
p-hydroxy-acetophenone	4.2 (1.15)[d]	189 (58.5)[e]
p-hydroxy-benzyl alcohol	6.4 (2.01)[d]	10500 (5690)[g]
p-hydroxy-benzoic acid	170 (127)[e]	> saturation

Table 2. Chemical and toxicological characteristics of 9 para-substituted phenols. The flagfish PE LC20 and PE LC50 values, as well as the EC50 values for P. phosphoreum, are given as log (c^{-1}) where c=concentration in mmol l^{-1}.

Structure	Molecular Weight	Phenol	CAS[1] No.	PE LC20	PE LC50	EC50[2]	log K_{ow}
	109.14	ρ-amino-phenol	123-30-8	3.24	2.51	2.15	0.04
	110.11	hydroquinone	123-31-9	2.92	2.67	3.46	0.59
	94.11	phenol	108-95-2	2.13	1.27	0.42	1.49
	139.11	ρ-nitro-phenol	100-02-07	2.24	0.94	0.92	1.91
	119.12	ρ-cyano-phenol	767-00-0	1.61	-0.50	2.18	1.60
	128.56	ρ-chloro-phenol	106-48-9	1.59	-1.28	1.19	2.42
	136.15	ρ-hydroxy-acetophenone	99-93-4	1.52	-1.52	1.49	1.35
	124.14	ρ-hydroxy-benzyl alcohol	623-05-2	1.29	-1.93	1.39	0.25
	138.12	ρ-hydroxy-benzoic acid	99-96-7	-0.09	-6.01	1.12[3].	1.58

1. Chemical Abstracts Service Registry Number
2. Data from [4] except for ρ-amino-phenol, which is based on more recent results (Kaiser, unpublished data).
   At low pH.

Table 3. Ranking of observed toxicities of 9 different para-substituted phenols to pulse-exposed flagfish (PE LC20, PE LC50) and P. phosphoreum (EC50) compared to theoretical ranking based on log K_{ow} where 1 represents the most toxic and 9 the least.

Phenol	Rank			
	PE LC20	PE LC50	EC50	log K_{ow}
ρ-amino-phenol	1	2	3	9
hydroquinone	2	1	1	7
phenol	3	3	9	5
ρ-nitro-phenol	4	4	8	2
ρ-cyano-phenol	5	6	2	3
ρ-chloro-phenol	6	7	6	1
ρ-hydroxy-acetophenone	7	5	4	6
ρ-hydroxy-benzyl alcohol	8	8	5	8
ρ-hydroxy-benzoic acid	9	9	7	4

Table 4. Correlation coefficients of linear regressions between flagfish PE LC20s, PE LC50s, P. phosphoreum EC50s and log K_{ow}.

	log K_{ow}	EC50	PE LC50
log K_{ow}	-		
EC50	-0.505	-	
PE LC50	-0.337	0.407	-
PE LC20	-0.416	0.460	0.981*

* Significant $p \leq 0.05$