Acute toxicity of four alkylphenols (3-tert-butyl-, 2-isopropyl-, 3-propropyl-, and 4-isopropyl-phenol) and their binary mixtures to Microtox, with comparisons to *Ceriodaphnia dubia* and *Pimephales promelas*

Kyungho Choi[1*] · Leonard I. Sweet[2] · Brian E. Olseski[2] · Peter G. Meier[2]

[1]School of Public Health, Seoul National University, Seoul, 110-799 Korea
[2]School of Public Health, University of Michigan, Ann Arbor, MI 48109 USA

Abstract

Toxicity evaluations of 3-tert-butyl-, 2-isopropyl-, 3-isopropyl- and 4-propyl-phenol and their binary mixtures were performed with the Microtox® assay and compared to invertebrates and fish. The single chemical, 4-isopropylphenol, exhibited the greatest relative toxicity to the Microtox organism (*Vibrio fischeri*).

The relative electrophilicity (LUMO) of the phenols, in contrast to the lipophilicity (Log P), was strongly correlated with toxicity to *V. fischeri* (r^2=0.96, $p<0.01$). In contrast, relative electrophilicity alone could not explain variances in toxicity of the phenols to *Ceriodaphnia dubia*. Results suggest that electrophilicity in conjunction with lipophilicity provide better correlation with toxicity to *C. dubia* and *Pimephales promelas*.

Microtox results from the binary mixture toxicity tests of selected phenolics indicate a mechanism of interaction governed by suppression/antagonism.

Introduction

It is estimated that about 60% of the industrial chemicals entering the aquatic environment exert toxicity by means of narcosis. For narcosis, the lethal concentrations of a compound are often close for similar species (e.g. for the guppy and the fathead minnow), which is not surprising considering the nonspecific nature of narcotic toxins. Phenols produce toxicity syndromes similar to those from inert narcotic pollutants but have greater toxicities. They act by a so-called "polar narcosis" mechanisms, which is associated with the presence of a strong hydrogen bond doner (polar) group in the molecule. (Class 2 chemicals)

It is reported that hydrophobicity (Log P) and electrophilicity (Energy of the Lowest Unoccupied Molecular Orbitals, LUMO) could explain the toxicity of Class 2 chemicals to ciliate *T. pyriformis*. However, with Microtox organinsm, *V. fischeri*, this was not evident. In

a study, Log P showed highly collinear relationship with EC50s for 267 compounds for fathead minnows, *Daphnia magna*, and *V. fischeri*. (Kaiser and Esterby, 1991) The difference in QSAR models for different organisms suggests that different mode of action (MOA) might be involved in toxicity of Class 2 chemicals to these organisms. Therefore, there is a need to evaluate the QSAR model for different test species.

In the present report, we evaluated the toxicity of four phenolic compounds to aquatic organisms of different trophic levels, including microbes, macroinvertebrates, and fish. In addition, toxicity of binary mixtures of the four alkylphenols was investigated.

Materials and Method

The following test organisms were employed for toxicity testing of four alkylphenols. For Microtox test with *V. fischeri*, Microtox protocol was followed. For daphnid and fish tests, US EPA's guidance was followed.

- *V. fischeri*. A marine bacterium. 5, 15 min exposure
- *C. dubia*. A macroinvertebrate. 48 hr exposure
- *P. promelas*. A fish. 96 hr exposure

Binary mixture test was conducted with *V. fischeri*. Detailed of the test is as follows.

- Two concentrations of each compound (~3 and 10x Microtox EC50)
- Binary mixtures were prepared in every possible combination

Results and Discussion

Acute toxicities of four alkylphenols were determined and are summarized in Table 1. QSAR parameters used for modeling include LUMO (Energy of the lowest unoccupied molecular orbitals) and Log P (log of octanol water partition coefficient). These parameters are also summarized in Table 1.

Table 1. Acute Toxicity of Alkylphenols to Test Organisms

	Mtox_5min	Mtox_15min	Cerio_48hr	Fish_96hr	LUMO	logP
Phenol*	2.76E-01	3.61E-01	4.57E-02	3.22E-01	0.396	1.475
3-tertiary-butyl phenol	7.06E-04	6.74E-04	8.11E-02	4.79E-02	0.433	3.301
2-methylethyl phenol	2.00E-02	2.09E-02	7.72E-02	6.15E-02	0.407	2.902
3-isopropyl phenol	1.94E-03	2.22E-03	5.28E-02	9.64E-02	0.418	2.902
4-isopropyl phenol	9.99E-05	1.01E-04	7.42E-02	4.04E-02	0.439	2.902

Units in mM, *Data obtained from other literatures, Cerio_48hr = *Ceriodaphnia dubia* 48 hr EC50, Fish_96hr = Fathead minnows 96hr LC50, LUMO = Energy of Lowest Unoccupied Molecular Orbitals, LogP = Logarithm of octanol-water partition coefficient

Relative electrophilicity (LUMO) of the alkylphenols was strongly correlated with toxicity measured at 5 and 15 min of exposures. Figure 1 shows correlation between LUMO and

toxicity results at 5 min exposure. The correlation with data at 15 min exposure were similar.

$Log(1/EC50_{Mtox}) = -28.2 + 73.3\ LUMO\ (r^2=0.96.\ p<0.01)$

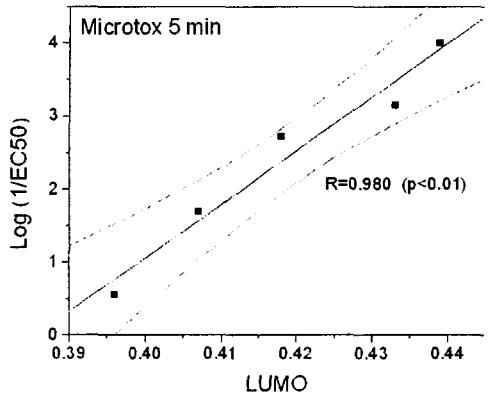


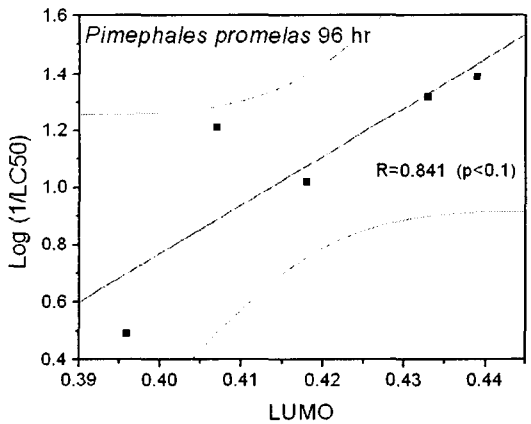
Figure 1. Electrophilicity (LUMO) of Alkylphenols and Their Toxicity to Microtox

Fish toxicity results did show LUMO related QSAR, but correlation was not as strong as that of Microtox. (Figure 2) When Log P parameter was included, correlation was improved. For *Ceriodaphnia*, LUMO alone could not explain the observed toxicity well. Addition of Log P parameter improved explanation of the toxicity, but statistical significance was not noted. (Figure 2)

$Log(1/LC50_{FISH}) = -6.0 + 17.0\ LUMO\ (r^2=0.71.\ p<0.10)$

$Log(1/LC50_{FISH}) = -2.6 + 0.4\ Log\ P + 6.6\ LUMO\ (r^2=0.90.\ p<0.10)$

$Log(1/EC50_{DAPHNID}) = 1.75 - 0.12\ Log\ P + 0.59\ LUMO\ (r^2=0.65.\ p>0.10)$



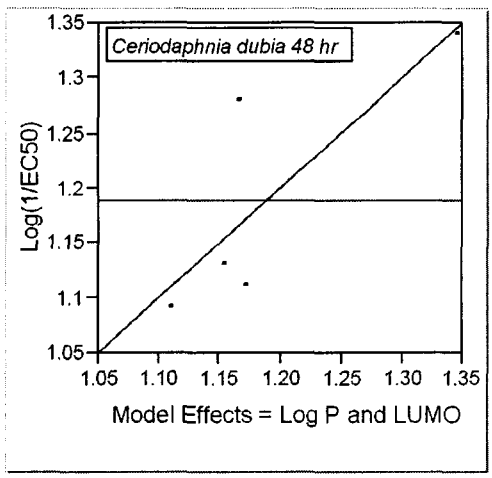


Figure 2. Electrophilicity (LUMO) of alkylphenols and their toxicity to P. promelas and C. dubia.

Addition of lipophilicity parameter (Log P) did not improve correlation to a significant extent.

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Acute toxicity of alkylphenol binary mixtures

Interaction within binary mixtures appears to mitigate the combined toxicity of the alkylphenols to *V. fischeri.* (Figure 3)

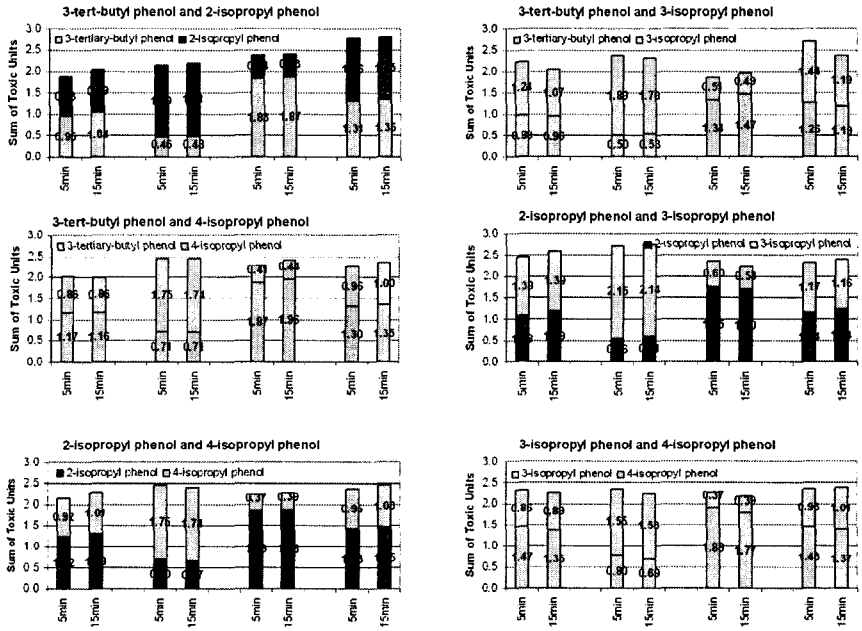

Figure 3. Acute toxicity of alkylphenol binary mixtures.

Conclusion

- Electrophilicity (LUMO) alone explains a majority of alkylphenol toxicity.
- Microtox organism showed LUMO related QSAR with alkylphenols.

When present in binary mixtures, these alkylphenols show antagonistic or less-than-additive toxicity interactions to *V. fischeri.*