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Synergistic Effect of Two- and Four-Component Combinations of the Polycyclic Aromatic Hydrocarbons: Phenanthrene, Anthracene, Naphthalene and Acenaphthene on *Daphnia magna*

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An important phenomenon of the environmental fate of polyaromatic hydrocarbons (PAHs) is the extent to which they are accumulated in aquatic organisms, the hydrophobic character being important to the uptake processes for PAHs. Interest in detecting PAHs in trace quantities in water, sediments, and biota has considerably increased in recent years owing to the fact that freshwater and marine organisms are frequently exposed to PAHs from point sources, such as petroleum drilling activities, oil spills, or chronic leakage, and also because the bioconcentration factor for PAHs, is about 1000 times its concentration in water (Onuska 1989). Numerous studies have been carried out to determinate, their behaviour in the environment (Martel et al. 1986), the human risk due to their accumulation pattern in some steps of the chainfood (Uthe 1986), as well as the relative ranking for the compounds potentially hazardous to some environments (Smith et al. 1988).

The greatest concern about PAHs is their carcinogenic potential. There seems to be a relationship between structure and carcinogenic activity (Onuska 1989).

Regarding acute toxicity, it appears that the structure also plays an important role. So toxicity increases with increasing molecular size until the 4- and 5-ring molecules are reached. Aqueous solubility, low water solubility, and more importantly, octanol/water partition coefficients (Kow) are well correlated with partitioning and bioaccumulation processes (Chin et al. 1986; Onuska 1989).

In saltwater species, acute toxicity occurs at concentrations as low as 300 ug/L, and for freshwater system it can be expected in a wide range in specific

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PAHs, so acute toxicity produced by acenaphthene would occur at concentrations as low as 1,700 ug/L or lower and at 2,300 ug/L by naphthalene (USEPA 1986).

The purpose of this study was to determine the acute toxicity of four PAH compounds (anthracene, phenanthrene, acenaphthene and naphthalene), frequently detected in freshwater systems due to oil contamination, as well as their synergistic effects, on Daphnia magna, an invertebrate commonly used as test organism (Devillers and Chambon 1986).

MATERIAL AND METHODS

Daphnia magna were obtained from continuous cultures maintained in our laboratory. Both stock and experimental animals were kept in spring water, aerated, in a chamber at 20 ± 1ºC on a 14L:10D photoperiod with approximately 1000 lux. All stock cultures were fed with a diet of 0.3 mg/L Chlorella vulgaris per individual at a concentration of 300-600 mg carbon/L.

Acute toxicity tests were performed with PAHs purchased from Chem Service Inc, USA, with a minimum purity of 97%, in 120 mL glass beakers containing 100 mL of the solution in reconstituted water (EEC 1984). Even though beakers were closed with teflon screw caps, the oxygen concentration was not less than 2 mg/L at the end of the test. Daphnids younger than 24 hr were selected for tests, 20 animals, divided into two groups of ten animals, were used in chemical concentration and control. Chemicals were dissolved in methanol, and the solvent was also added in a maximum of 2% to control flasks, concentration used in the highest concentration of the test substances. Solutions were added before the introduction of the animals. The nominal concentrations selected to perform the acute toxicity were: 500, 1000, 1500, 2000, 2500 and 3000 ug/L for acenaphthene; 31.25, 62.5, 125, 250, 500, 1000 ug/L for phenanthrene; 1000, 1500, 2000, 2500 ug/L for naphthalene; 31.25, 62.5, 125, 250 ug/L for anthracene. The median effective concentration after 48 hr (48-hr EC50) for immobilization was calculated by computerized log-probit analysis. Tests were performed according to EEC Directive (EEC 1984).

Afterwards synergistic effects were measured by combining different rates of each chemical using the toxic unit (TU) concept of Sprague and Ramsay (1965), the following combinations were tested:

Combinations of two PAHs:
(0.25 TU: 0.75 TU)
(0.5 TU: 0.5 TU)
(0.75 TU: 0.25 TU)

Combinations of four PAHs:
(0.5 TU: 0.5 TU: 0.5 TU: 0.5 TU).
(0.25 TU: 0.25 TU: 0.25 TU: 0.25 TU).
(0.125 TU: 0.125 TU: 0.125 TU: 0.125 TU).

RESULTS AND DISCUSSION

When we studied acute toxicity on Daphnia magna, the reduction of its normal mobility was very common. However immobility was assessed in all tests as previously recommended (EEC 1984) when daphnids were unable to swim 15 sec after stimulation by gentle agitation of water, even if they could only move their antennae.

With regard to acute toxicity, our data was similar to data previously reported (USEPA 1986; Slooff et al. 1989; Edsall 1991). Three ring PAHs, phenanthrene and anthracene, were more toxic than two ring PAHs, acenaphthene and naphthalene, with close differences in EC_{50} values of an order of magnitude; anthracene being the most toxic and naphthalene the least toxic (Table 1).

Table 1. Acute toxicities of nominal concentrations of acenaphthene, phenanthrene, naphthalene and anthracene to the Daphnia magna and aqueous solubilities (Sw).

Chemical	EC50 (95% confidence intervals) µg/L 24 hr	EC50 (95% confidence intervals) µg/L 48 hr	log Sw* mol/L
Acenaphthene	---	1275 (1102-1475)	-4.59
Phenanthrene	861 (632-1173)	383 (253-317)	-5.15
Naphthalene	2305 (2128-2497)	2194 (1958-2459)	-3.61
Anthracene	211 (189-236)	95 (81-112)	-6.38

* log Sw values from Chin et al. (1986)

The joint action for two-components mixtures was studied using different combinations, being the sum of TU equal to unity, where TU was defined as the 48-hr EC50 for each compound. In all the combinations immobilization percentages were lower than 50, percentage expected in additive effects, showing less than additive join action for these combinations. In some cases, i.e., between naphthalene: anthracene and phenanthrene: anthracene, immobilitation percentage near zero indicated that antagonism occurs. Only the combination of acenaphthene: naphthalene and acenaphthene: anthracene in which acenaphthene was the main toxic contribution immobilitation percentage near 50, shown lower but similar to those expected if toxic effect were additive.

The joint action for four-component mixtures, showed similar results to those expected from the results of the two-component studies. Slightly less than additive joint action was found in equitoxic concentrations of the four PAHs under the toxic unit concept.

Table 2. Immobilization percentages of <u>Daphnia magna</u> after acute exposure to various PAHs two- and four-combinations.

Chemical	Toxic Units (TU)	Immobilization Percentage
Naphthalene:Acenaphthene	0.25: 0.75	40
	0.5: 0.5	25
	0.75: 0.25	5
Naphthalene:Anthracene	0.25:0.75	5
	0.5: 0.5	0
	0.75: 0.25	5
Naphthalene:Phenanthrene	0.25: 0.75	0
	0.5: 0.5	0
	0.75: 0.25	25
Acenaphthene:Phenanthrene	0.25: 0.75	0
	0.5 : 0.5	0
	0.75: 0.25	15
Acenaphthene:Anthracene	0.25: 0.75	0
	0.5: 0.5	0
	0.75: 0.25	45
Phenanthrene:Anthracene	0.25: 0.75	0
	0.5: 0.5	5
	0.75: 0.25	0
Anthr:Acen:Phen:Naph	0.5:0.5:0.5:0.5	100
Anthr:Acen: Phen: Naph	0.25:0.25:0.25:0.25	5
Anthr:Acen:Phen:Naph	0.125:0.125:0.125:0.125	0

Different PAHs are usually found in polluted waters and sediments (Cartoni et al. 1986; Onoska 1989; Gomez-Belinchon 1991) and therefore synergistic effects between different PAHs must be expected. This fact has also been considered in some water quality criteria such as those of WHO and EEC for drinking water and of USEPA for natural water (WHO 1977; EEC 1980; USEPA 1986), which use a single criteria for total PAH concentration, represented by the sum of different individual compounds. Some authors have found at the field level, correlations between total PAHs concentration and some sublethal effect indicators such as the level of total cytochrome P-450 and EROD activities (van Veld et al. 1990).

Our results show that the toxic action expected for the four PAHs studied would result in a effect slightly less than the additive effect and therefore a criteria based on additive effect will be sufficiently protective. But we must take into account, that when the sum of individual compounds have to be used, the differences in acute toxicities between individual chemicals could be higher than an order of magnitude.

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