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ACCUMULATION OF NONYLPHENOL IN GOLD FISH AND SUCKERMOUTH CATFISH IN THE SEMI STATIC AQUARIUM SYSTEM

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Alkylphenol polyethoxylates (APEs), environmental endocrine (estrogenic) disruptors (Nimrod and Benson, I996 are widely used as non-ionic surfactants and anti-oxidants in detergents, pesticides, herbicides, paints, cosmetics, plastic wares, and in jet-fuel (Bernabei *et al.*, 2000). The production of APEs exceeds 500,000 metric tons in the world, annually. It has been estimated that 60% of the total production ends up in water around the world. In this study, differences in the bioaccumulation rate of nonylphenol (NP), one of the ubiquitous derivatives of APEs, in two different species of freshwater fishes namely Suckermouth catfish (S. catfish) *(Hypo-tomus plecostomus)*, a demersal fish, and in gold fish (G. fish) *(Carassius auratus)*, surface fish, was determined.

Materials and Methods

Shimadzu HPLC pump (model LC-9A), spectrophotometer (model UV-I60A) and integrator (model CR-6A) were employed in the study. HPLC grade methanol was obtained from Merck (Dormstad, Germany); Luna C18 HPLC column (250 x 4.6 mm, 5µl) and C18 BOND ELUT octadecyl silica cartridge (100mg/ml) were obtained from Phenomenex (Aschaffenburg, Germany) and 4-nonylphenol was purchased from Aldrich (Southampton, UK). Gold fish and Suckermouth catfish were employed as models in this research since they were inexpensive and were readily available from any aquarium shop in Turkey.

Fish were maintained in 35 l aquarium filled with charcoal filtered tap water and rested overnight. S. catfish and G. fish, were also rested for four days prior to experiment. Fishes were then exposed to 0 (control), 66 µg NP/l for 1, 2, and 3 weeks. Five fish from each species were sampled at the end of the first, second and third week during experimentation. Fish were anesthetized with MS-222, frozen in liquid nitrogen and stored at -80°C until measurement.

Different concentrations of NP with an aliquots of 25 µl were injected into the HPLC column and the peaks obtained at Shimadzu integrator connected to UV light spectrophotometer at λ = 277 nm with 0.2 ml/min solvent flow rate in the column and retention time was determined. Peak generated in HPLC 8y in response to different concentration of nonylphenol (x's) were used to calculate the regression equations which were Y=-370.63+53238.305x, r^2=0.99 for NP. This equation was used to calculate and quantify the NP accumulation in fish tissues. Alkylphenol compounds were extracted from water samples by using C18 BOND ELUT

cartridge as described by Marcomini *et al.* (I987) nonylphenol was extracted from fish tissues and measured by using techniques as previously described by Zhao *et al.* (1999).

Results and Discussion

Nonylphenol could be found up to 1000 μg/l water (Tyler *et al.*, 1998) and 12,000 μg NP/kg in sediment (Hale *et al.*, 2000) in the aquatic environment. In this study, 66 and 220 μg NP/l concentrations along with control (0 μg NP/l) were chosen as experimental doses as they were found to be sublethal to both the fishes. Significant time and dose dependent bioaccumulation occurred in S. catfish. Both time and dose dependent accumulation was significantly different (Table). Although NP accumulation appeared to be time dependent in both fishes, the concentration dependent NP accumulation seemed to be more important than that of time dependent accumulation in G. fish since significant NP accumulation in the tissues of G. fish can only be attained in higher concentrations of NP (220 μg) upon three weeks of exposure (Table). On the other hand, even lower concentrations of waterborne NP (66 μg NP/l) could lead to significant NP accumulation in the tissues of S. catfish at the same exposure time. This suggests that NP rapidly settle down to the bottom of the tanks and then, S. catfish may be exposed to more NP. In water samples collected from 0,66 and 220 μg NP/1 treatment groups, NP concentrations started to decline in water. This decline of NP in water samples appeared to increase after 10 hr of administration into the tap water. Decreases in NP concentrations in water and increases in NP accumulation in the tissues of fishes were in accordance with the previous findings. For example, Ahel *et al.* (1994 a and b) reported that alkylphenolic compounds do not accumulate in water, instead, they do accumulate in aquatic organisms. Alkylphenolic compounds, including NP, are hydrophobic, they tend to settle down and accumulate in the sediment. Furthermore, these compounds are lipophilic; therefore, they accumulate in the aquatic biota including fish. As mentioned,

Table - Time and dose dependent NP bioaccumulation in S. catfish and G. fish

NP concentrations (μg/l)		N	Exposure time (week) and μg NP bioaccumulation in gr tissue of fish (mean±SE)		
			1st Week	2nd Week	3rd Week
S. catfish	control	5	0	0	0
	66	5	0.81±0.24[a]	1.29±0.44[ab]	2.63±0.81
	220	5	2.79±0.11	4.26±0.23	6.75±0.67[c]
G. fish	control	5	0	0	0
	66	5	0.47±0.08[a]	0.87±0.11[a]	1.37±0.51[a]
	220	5	0.72±0.07[a]	1.24±0.09[a]	3.90±1.13[b]

Different letters indicates significant difference in Turkey's analysis at P<0.05.

S. catfish are demersal fish so that they could be exposed to more of these compounds since they accumulate in the sediment. Therefore, NP accumulates more in S. catfish than that of G. fish.

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