Preliminary Risk Assessment of Several Major Pharmaceutical Products In Aquatic Ecosystem

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Abstract

Acute toxicities of five pharmaceutical products were evaluated with aquatic microbes, invertebrates, and fish. The test pharmaceuticals, i.e., cimetidine, carbamazepine, diltiazem, acetaminophene, and metformin have been often detected in aquatic environment, but their ecological hazard on receptors of various trophic levels has seldom been evaluated. In the present study, we conducted acute toxicity assays with a marine bacterium, *Vibrio fischeri*, an invertebrate, *Daphnia magna*, and a fish, Japanese medake (*Oryzias latipes*).

In general, *D. magna*, showed the most sensitive response to the test chemicals. Diltiazem exhibited the lowest EC50 value after 96 hr of exposure at 7.6 mg/L, followed by cimetidine > acetaminophen > metformin = carbamazepine in an order of decreasing susceptibility.

With the fish, diltiazem and carbamazepine showed the 96 hr EC50 values at 14.1~35.4 mg/L while acetaminophen, cimetidine, and metformin did not cause 50% mortality at 100 mg/L. Similar pattern was noted with the Microtox Assay, with which the median effective concentrations for acetaminophen, cimetidine, and metformin were found at the range between 301.8 and 755.4 mg/L. Carbamazepine and diltiazem exposure to the microbes resulted in EC50 values around 50 mg/L.

Predicted no effect concentrations (PECs) of these pharmaceuticals derived from the EC50 values obtained from this study, and predicted environmental concentrations (PECs) obtained from available literatures were utilized to estimate ecological risks of the test compounds. No test pharmaceuticals resulted in risk quotients (PEC/PNEC) greater than 1, which suggests no serious potential ecological concerns. It should be noted however that further studies including the refinement of PEC derivation, identification and toxicity assessment of the metabolites and/or their interactions with other stressors may be warranted to better understand the environmental consequences of the residual pharmaceutical discharge to the waterway.

Introduction

Pharmaceuticals and Personal Care Products (PPCPs) are developed and manufactured for specific biological effects, and administered for human and animal health care, and livestock

farming[1]. Recently numerous reports have been published indicating the occurrence of many pharmaceuticals in various environmental media, however, ecological consequences of the discharge of these pharmaceutical residues have seldom been thoroughly investigated. The PPCPs are designed to cause physiological effects, therefore an impact on aquatic and terrestrial organisms would not be surprising. Because of their potential effects on the ecosystems, there are growing concerns about the ecological risks of the pharmaceutical residues.

In the present study, we chose five human pharmaceutical products, i.e., cimetidine, carbamazepine, diltiazem, acetaminophene, and metformin based on the reports of their frequent occurrences in the environment, and evaluated their acute aquatic toxicities using a marine bacterium, *Vibrio fischeri*, an invertebrate, *Daphnia magna*, and a fish, Japanese medake (*Oryzias latipes*). We aimed at estimation of their potential ecological risks through derivation of their predicted no effect concentrations (PNECs) and potential environmental concentrations (PECs) in water[2]. The information gleaned from this study will be useful for formulation of appropriate risk management decisions for protection of aquatic ecosystem from these pharmaceutical residues.

Materials and Method

Test Compounds and Organisms

Five test pharmaceuticals, i.e., acetaminophene, metformin, diltiazem, cimetidine, and carbamazepine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions were prepared in 99% dimethylsulfoxide (DMSO), and used for toxicity evaluation after dilution with appropriate medium. Lyophilized *V. fischeri* was obtained from the commercial vendor (SDI, Newark, DE, USA), therefore no culturing was required. *D. magna* and Japanese medaka were cultured in house, and were subject to routine quality control and quality assurance. Either U.S. Environmental Protection Agency guideline[3] or OECD method[4] were followed for the culture and maintenance of the test species.

Bioassays

Microtox Assay

Microtox Model 500 analyzer was used for the bacterial toxicity assay. The "81.9% Basic test" procedure was utilized after a minor modification. The bacterial luminescence, the endpoint of this assay, was measured for each pharmaceutical product after five and fifteen minutes of exposure at 15℃. Water quality parameters such as pH and dissolved oxygen of the test solutions were measured before the exposure[5),8)].

Daphnid toxicity test

The 96 hr static-renewal tests were conducted with *D. magna*, following U.S. EPA guideline[3]. Immobility of daphnids was the measurement endpoint. Temperature was maintained at 21 ±1℃. *Chlorella* and YCT (alfalfa:yeast:Tetramin® = 1:1:1) were fed shortly before the change of exposure media after the first 48 hr of exposure. Water quality parameters such as pH, temperature, dissolved oxygen, and specific conductivity of the test solutions and control were

measured, and recorded over the exposure period[5].

Medaka toxicity test

The 96 hr static-renewal tests were conducted with 1-3 mo old Japanese medaka (*Oryzias laptipes*) following OECD guideline[4]. Mortality, the endpoint of this test, was recorded daily during the exposure. The test temperature was maintained at 25±1℃and the solutions were renewed at 48 hr without feeding[5]. Water quality parameter including, pH and dissolved oxygen of the test solutions were measured daily during the exposure.

Derivation of risk quotients

Risk quotients of each pharmaceutical products were calculated by dividing PEC with PNEC. PEC can be obtained from the equation (1)[6],[7].

$$PEC_w = \frac{A \times (100 - R)}{365 \times P \times V \times D \times 100} \quad (1)$$

where A is the amount used per year (kg/yr), R the removal in percent (set to zero), P the number of inhabitants, the volume of waste water per day per capita (0.2 m^3) and D is the dilution factor in the environment (a default factor of 10 is used). PNECs of each pharmaceuticals were estimated by dividing the lowest EC50 values obtained from the test organisms, with an assessment factor of 1000.

Results and Discussion

Acute toxicities of individual pharmaceuticals

The results of acute toxicity assays with five pharmaceuticals are summarized in Table 1. Daphnids were, in general, the most sensitive among the test organisms toward the pharmaceutical compounds under investigation: All test pharmaceuticals showed their 96 hr EC50s at <100 mg/L, among which diltiazem exhibited the lowest EC50 value of 7.6 mg/L, followed by cimetidine > acetaminophen > metformin = carbamazepine in an order of decreasing susceptibility.

With the fish, diltiazem and carbamazepine were most toxic, of which 96 hr EC50 values were found at 14.1 and 35.4 mg/L, respectively. However, acetaminophen, cimetidine, and metformin did not cause 50% fish mortality even at the highest concentration of 100 mg/L.

Similar pattern was noted with the Microtox Assay, with which the median effective concentrations for acetaminophen, cimetidine, and metformin were found at the range between 301.8 and 755.4 mg/L. Carbamazepine and diltiazem exposure to the microbes resulted in EC50 values around 50 mg/L.

Table 1. Summary of the median effective concentrations (EC50s) of five pharmaceuticals obtained from V. fischeri, D. magna, and Japanese medaka

		Acetaminophene	Carbamazepine	Cimetidine	Diltiazem	Metformin
V. fischeri						
5 min	EC50	301.8 (171.3~531.6)	52.52 (49 19~56 06)	340.0 (163.1~710 9)	58.57 (30.43~77 58)	497.2 (223 1~1108)
15 min	EC50	390.2 (241 5~630 7)	52.22 (45 84~59.47)	372.9 (254.2~546.9)	47.63 (38 88~58 33)	755.4 (129.7~4400)
D. magna						
48hr	EC50	54.40 (45 30~63 50)	>100	35.36 (30.27~41.29)	24.82 (19.55~31 53)	70.70 (67 10~74 60)
96hr	EC50	47.40 (37.80~57 00)	69.40 (56 93~84 60)	13.93 (11.83~16 40)	7.637 (5 310~10.99)	63.73 (56 94~71 33)
O. latipes						
48hr	EC50	>100	35.35	>100	21.01 (16 23~27 09)	>100
96hr	EC50	>100	35.35	>100	14.14	>100

Units in mg/L Values in parentheses are 95% confidence intervals.

Estimation of risk quotient of each pharmaceuticals

Risk quotients of each pharmaceuticals were estimated from their respective PEC and PNEC values (Fig. 1). Since information required for PEC derivation lacks in Korean environment, we used estimates from literatures, which were for United Kingdom (U.K.)[9]. PECs may be only valid for U.K., however, these values are likely to be applicable to other countries with similarly developed healthcare provisions and wastewater treatment infrastructure.

For PNEC calculation, we used the 96 hr exposure data from the most sensitive species, to which an assessment factor of 1000 was applied. Table 2 shows the estimates of risk quotients of each test pharmaceuticals. Risk quotients for acetaminophene and cimitidine were relatively high at 0.14 ~ 0.25, and no test pharmaceuticals exhibited risk quotients >1, which suggest no serious ecological concerns associated. (Fig. 2)

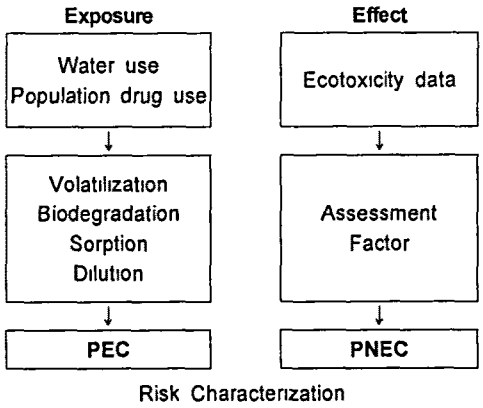

Fig. 1. Schematic diagram showing derivation of predicted environmental concentration (PEC) and predicted no effect concentration (PNEC) of the test pharmaceuticals[2].

Table 2. Potential ecological risks derived from PECs and PNECs of the test pharmaceuticals

	U.K PEC[a] (μg/L)	PNEC[b] (μg/L)	Risk Quotient
Acetaminophen	11.96	47.40	0.250
Carbamazepine	1.230	35.40	0.035
Cimetidine	1.090	13.90	0.140
Diltiazem	0.670	7.600	0.090
Metformin	6.300	66.00	0.090

a: Aquatic environmental assessment of the top 25 English prescription pharmaceuticals. (Jones et al. 2002)
b: EC50 value from the most sensitive species was used

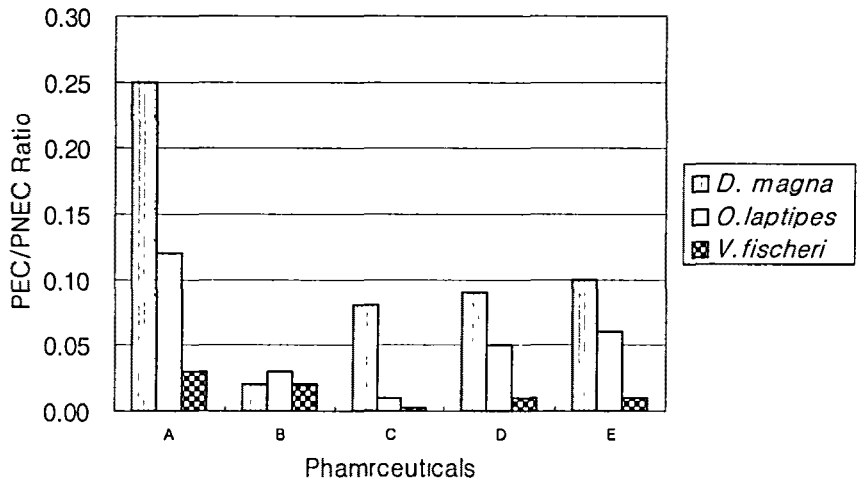

Fig. 2. Risk quotients of five tested pharmaceuticals. (A: Acetaminophen, B: Carbamazepine, C: Cimetidine. D: Diltiazem, E: Metformin)

Conclusion

Acute toxicities of five major human pharmaceuticals were evaluated with *V. fischeri, D. magna,* and Japanese medaka. Using PNECs derived from this study and PECs estimated for the U.K. waterway, we could calculate PEC/PNEC ratios, the risk quotients of five pharmaceuticals.

The results of this assessment indicate that all compounds have risk quotients less than 1, which means that none of these pharmaceuticals can be considered as potential environmental concern. However, uncertainties related to derivation of PECs should not be ignored. To better assess environmental risk of these pharmaceuticals in Korea, the refinement of PEC estimation is warranted: i.e., the annual consumption data of each pharmaceuticals, and their degradtion in the environment may need to be incorporated.

Test endpoints should be diversified and inclusive of more subtle but permanent responses. Standard acute bioassays with their focus on immediate endpoint such as lethality may not be the

most appropriate basis for risk assessment given the intended narrow scope of biological activity/effect and general potency of pharmaceuticals: Chronic bioassays performed over the life-cycle of various organisms from different trophic levels may be more appropriate.

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