한국환경보건학회지, 제32권 제5호(2006)
Kor. J. Env. Hlth., Vol. 32, No. 5, pp 440~445(2006)

Ecotoxicological Risk Assessment for Acetaminophen in Kyongahn River

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(Received September 2, 2006/Accepted October 25, 2006)

요 약

통증경감을 위하여 광범위하게 사용되는 acetoaminophen은 치통, 두통에 주로 사용되는 의약물질이다. 의약품의 환경오염과 관련하여 수자원에 유입되는 acetaminophen의 경안천 오염정도를 파악하고 생태 위해성을 평가하기 위하여 본 연구를 수행하였다. 한강 수계이며 용인시와 광주시를 거쳐 팔당호로 흘러 들어가는 경안천(해실교, 수포교, 왕산교, 경안교, 지월교, 광동교, 팔당호)내 acetaminophen 잔류 농도와 분포를 조사하였다. 또한 Daphnia magna를 대상으로 48시간 급성독성평가를 실시하여 위해도를 평가하였다. 잔류의약품 검출을 위해 SPE 추출법을 선택 하였으며, liquid chromatography/mass spectrometry (LC/MS)를 이용하여 분석하였다. 경안천 내에서 acetaminophen 0.439 mg/*l*이 검출되었다. 독성평가를 실시한 결과, 48시간 LC_{50}은 16.9 mg/*l*로 나타났다. 경안천의 acetamionophen 농도와 독성평가를 바탕으로 위해성 평가를 실시한 결과 위해성은 1 이하였다. 하지만 국내에 의약품 발달과 함께 사용이 계속적으로 늘어나는 추세이고, 잔류의약품에 대한 대책이 마련되어있지 않은 이상 수환경은 의약품에 노출될 것이다. 그러므로 잔류의약품이 생태계에 악영향을 미치지 않도록 구체적인 방안이 강구되어야 할 것으로 사료된다.

Keywords: Kyongahn river, acetaminophen, *D. magna*, LC_{50}, hazard quotient(HQ)

I. Introduction

There are several drugs to suppress inflammation without the side effects different from steroids. Non-steroid anti-inflammatory drugs (NSAIDs) are commonly used to relieve the symptoms of arthritis, bursitis, gout, swelling, stiffness and joint pain. The pharmacological mode of action for NSAIDs is via inhibition of the synthesis of prostaglandins, leukotrienes, and other compounds that are involved in the inflammatory response. Many of these pharmaceuticals, and in particular those sold over the counter as non-prescription drug also have analgesic (pain killing) and/or antipyretic (fever reducing) activities. There are many different types of NSAIDs available over the counter, including acetaminophen, acetylsalicylic acid or aspirin, indomethacin, and ibuprofen in low doses. Drugs from this class sold by prescription include celebrex, diclofenac, ciflunisal, etodolac, ketorolac, fenoprofen, nabumetone, naproxen, oxaprozin, piroxicam, sulindac, tenoxicam, tiaprofenic acid, and tolmetin. Pharmaceutics are excreted unmetabolised or as active metabolites: they escape from degradation in waste treatment plants and enter the environment (Sorensen *et al.*, 1998, Ettore Zuccato *et al.*, 2000). Recently, major efforts to clarify the ecological occurrence of pharmaceuticals such as acetaminophen in the aquatic environment have been attempted in North American and European countries. Since the sophistication of analytical method has developed, so the range of so has the range of detection of xenobiotics in the environment has increased. Despite many studies, it remains unclear whether the exposure of aquatic biota to these pharmaceuticals have direct or indirect effects on the environments. Furthermore, no data is available within the country. Few new medical substances have been subjected to a complete risk assessment primarily due to the fact that in most instances the calculated environmental concentrations lie below the proposed cut-off values, making further ecotoxicological studies unnecessary.

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Table 1. Operating condition for LC/MS analysis of acetaminophen

	Activity	Condition
MS	· Type	SIR
	· Ion mode	ES+
	· Sourec Temperature	147~150(°C)
	· Desolvation Temperature	400~395(°C)
	· Cone Gas Flow	64(L/Hr)
	· Desolvation Gas Flow	419(L/Hr)
	· LM Resolution	17.4
	· HMResolution	15.5
	· Multiplier	650~648
	· Run Time	35 min
LC	· Column	Luna C8 Column (3 μm, 100×4.6 mm)
	· Flow	0.250 ml/min
	· Stop Time	40 min
	· Column Temperature	30(°C)
	· Sample Temperature	20(°C)

This study was conducted to monitor the extent of acetaminophen residues in Kyongahn river and to investigate the ecological risks of target compounds. Acute toxicity of acetaminophen was also evaluated for Daphnia magna. From these results, environmental risk assessment was performed for drug residues in Kyongahn river to predict their potential adverse effect.

II. Materials and Method

1. Materials

Acetaminophen was purchased from Sigma-Aldrich. Disdium ethylenediaminetetraacetate (Na_2EDTA) and ammonium formate were purchased from Junsei Chemical and Sigma-Aldrich. Standard stock solutions with concentrations of 1000 ng/μl in methanol was prepared and stored in the dark at below −10°C for up to 1 month. Working standard solutions were used in calibration. Methanol were obtained from Merk (Germany).

2. Sampling

Surface water samples were collected from 7 sites (Heasilgyo, Soopogyo, Wangsangyo, Kyonahngyo, Jiwolgyo, Kwangdonggyo, and Paldang) along Kyonahn river in Gyunggido between June and August 2005 according to the Standard Sampling Mehtod of Ministry of Environment (see Fig. 1). To minimize sample contamination, use of personal, use of personal care items (i.e. insect repellents, colognes, perfumes), caffeinated products, and tobacco was discouraged during sample collection and processing. Water samples were stored in an icebox for their return to the laboratory, stored in the dark at 4°C until analysis when they were not immediately extracted after arriving to the laboratory.

※●Sampling site ■Publicly Owned Treatment Works

Fig. 1. Sampling sites at Kyongahn river.

3. Preparation Samples

All samples were filtered through filter paper (5C, 110 mm). Water samples were prepared extracted by adding Atrazine-d5 ($C_8H_{14}CLN_5$-D5) and 10 g of Na_2EDTA. Analytes were extracted using the 1 g hydrophilic-lipophilic balance (HLB) cartridge from Waters (Millford, MA). The 1 g HLB cartridge was chosen as the best overall. Cartridges were preconditioned with 6 ml methanol, 6 ml 0.5 N HCl, and 6 ml distilled water. Water sample then were passed through the cartridges at 10 ml/min. After isolation, cartridges were rinsed with 10 ml distilled water to remove excess Na_2EDTA. The analytes were diluted using 10 ml methanol. The extracts then were concen-

trated under a flow of N_2 to approximate volume of 500 μl. To this, 750 μl of mobile phase A was added. The resulting solutions were transferred to 2 ml amber autosampler vials.

4. Analytical Procedure

Samples were analysed with a LC/MSD system consisting of 2690 HPLC (Waters) and Mass Selective ZQ 2000 (Micro mass), a Waters 2690 HPLC auto injector and controller. LC/MSD method was applied for LC/MS-ESI positive ion mode. A binary gradient with a flow rate of 0.60 ml/min was used. Mobile phase A contained 10 mM ammonium formate in the ratio of 90/10 for water/methanol with 0.3% formic acid. Mobile phase B contained 10 mM ammonium formate with 0.5% formic acid in methanol. The gradient was as follows: B = 9% for the first 5 min, increased to 42% by 15 min, and finally increased to 100% by 20 min. LC/MS condition is summarized in Table 1.

To determine of acetaminophen, we used 152 m/z pecursor ion, 110 m/z product ion and 18V cone voltage. Finally the method detection limit (MDL) for the determination of acetaminophen was 5 ng/l. The recovery efficiency for sample extraction was ranged from 78.6 to 92.4%.

5. Test Organisms

Daphnia magna cultures consisted of 1 l glass beaker containing 800 ml~1 l of culture medium and 20 of daphnids. Culture medium was renewed and offspring produced discarded twice a week. Brood daphnids were discarded after 5 weeds in culture and replaced with neonatal organisms. Cultures were maintained under the conditon of 21 ± 0.5°C and 16-h light : 8-h dark photoperiod. Cultured daphnids were fed with a suspension of the unicellular Mesangi (*Capsosiphon fulvecense*) once a day. The Mesangi, the culture of the food organism, was obtained from SamDoek Company. The feeding rate was about 2×10^5 cells/ml/d.

6. Acute Toxicity Test

Daphnia tests were conducted following the USEPA Guideline (1993). Daphnids were bred in culture medium imitating natural fresh water. Neonates (<24 h old) from 2-3-weed-old mother were placed in a 50 ml glass beaker containing 40 ml of a test solution. All experiments for exposure and control without chemicals were made in five replicates and performed at 21 ± 0.3°C under 16 h light: 8 h dark photoperiod. Morality was recorded 24 h and 48 h later. The number of dead daphnids was recorded to determine the concentration for LC_{50}. LC_{50} values were calculated by Probit analyses (USEPA 1993), based on nominal concentrations. Mortality data were used to calculate the 24 h and 48 h LC_{50} by US EPA probit analysis or moving-average, using a statistical package.

7. Risk Assessment

Ecological risk assessment was performed to characterize the degree of contamination and to evaluate the adverse effects of these chemicals in aquatic environments. In this study, results from the *Daphnia magna* toxicity tests were compared to those from the LC-MS analyses of selected compounds in the surface water. The hazard quotient (HQ) was calculated to evaluate the degree of risk for each target PhAC : HQ = EC (Exposure concentration)/LC_{50}. A value less than 1 indicates an insignificant risk. This study employed a quite moderate assessment factor of 1,000.

III. Result and Discussion

1. Targeted Monitoring

The highest concentration of acetaminophen (0.439 μg/l) was measured in Soopogyo site. The low concentration of acetaminophen was shown in Kyonahngyo and Jiwolgyo, Paldang (Fig. 2). The

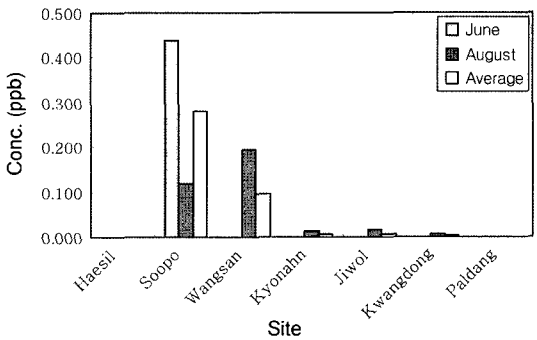

Fig. 2. Concentration distribution of acetaminophen in Kyongahn river.

mean concentration of Soopogyo site was the highest value at Kyonahn river. Acetaminophen concentration was declined as the current was close the the reservior Paldang. Studies in the US were reported acetaminophen to be present in certain rivers at maximum concentrations of up to 9.561 μg/*l* (Kolpin *et al.*, 2002).

2. Acute Daphnia Toxicity Tests

Acute test was performed on *Daphnia magna*. The test was made in accordance with standard protocol. In the acute tests with neonates, no control mortaity occurred. The O_2 content was not reduced and pH did not change during the test. LC_{50} were compared for the measured data and predicted one using the Ecological Structure-Activity Relationships (ECOSTAR) model from the websites of the US EPA (Sanderson *et al.*, 2003). To investigate the relation between toxicity and hydrophilicity, the LC_{50} versus log K_{ow} values were plotted (Fig. 3). The result for acetaminophen was exceptional, showing high toxicity for an extremely low log K_{ow} of 0.46 (Table 2).

Table 2. *Daphnia magna* acute test: LC values (with 95% probability confidence limits) for acetaminophen

Log K_{ow}[a]	*D. magna* LC_{50} (mg/*l*)	
	24-h	48-h
0.46	60.1 (48.1-81.5)	16.9 (14.4-19.3)

[a]Sangster 1994.

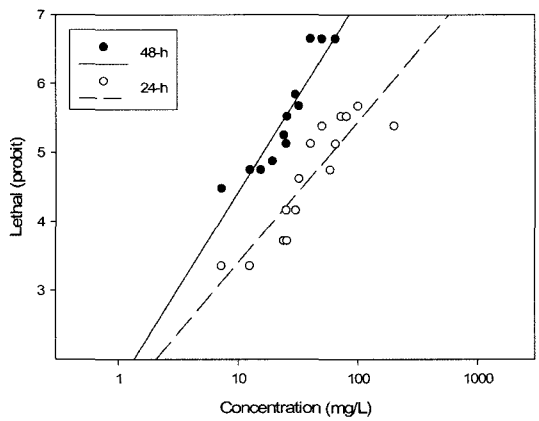

Fig. 3. LC_{50} of *Daphnia magna* acute toxicity test with acetaminophen.

3. Risk Assessment

The toxicity index was calculated by comparing the maximum measured concentrations of field data collected from the Kyongahn river with the acute toxicity value to *D. magna*. Table 3. illustrates that acute risks are probably not significant, as there is no overlap between the distribution of the ECs and the LC_{50} values. Any of the HQ values of acetaminophen tested in this study did not exceeded 1. LC_{99} of the target compounds are less than 1 without the application of an assessment factor. These results indicated that acetaminophen had no significant ecotoxicological impact on short-term acute exposure.

The importance of identifying emergent risks such as pharmaceuticals in the environment is reflected in the fact that pharmaceuticals are one of the top five goals of the strategic plan 2000 for the US environmental Protection Agenc's Office of Research and Development. The current US regulatory guidance requires new pharmaceuticals to undergo standard acute toxicity tests (algae, *Daphnia magna* and fish) if the predicted or measured environmental concentration (PEC/MEC) of the active ingredient is >1 μg/*l*. In the EU the cut-off PEC value is 0.01 μg/*l*, and no environmental concerns are apparent, therefore, no further testing is deemed necessary. In the second tier a crude predicted no-effect concentration (PNEC) for the aquatic compartment is to be extrapolated by dividing the lowest $E(L)C_{50}$ from standard tests by an assessment factor of up to 1000 in the EU. If the PEC/PNEC is below 1, no further assessment is necessary. The third tier is a case-by-case study. Regulations may result in labeling or restricted use (e.g. in hospitals, in surgery, etc.) Due to the scarcity of ecotoxicogical data and the presence of pharmaceuticals in water, the primary question is whether medical substances at low environmentally realistic concentrations will have any effect at all on different trophic levels and/or ecosystems.

Table 3. Illustration of the calculated hazard quotients (HQ) for acetaminophen in the Kyonahn river

Water concentration (Max: μg/*l*)	LC_{50} (48 h: mg/*l*)	HQ
0.439	16.9	2.6×10^{-2}

The overall ecotoxicological effects of PhACs detected in Kyonahn river were investigated by biological and chemical analysis. Acetaminophen was detected at Kyonahn river. The concentration in aquatic environment was detected 0.439 μg/*l*. Flux in August was presented higher than flux in June. Pharmaceutical concentration was decreased with increasing flux of Kyongahn river. The concentration of pharmaceuticals in Kyongahn river were compared to those from other countries (U.S., Canada, Germany, and Italy). The PhACs concentration detected in Kyongahn river was lower than those of the surface water surface water collected in U.S and Germany, Canada etc.

The bioassay results showed LC_{50} ranged from several mg/*l* to several tens of ppm in the acute tests. respectively, for the selected PhACs. The LC_{50} was observed for acetaminophen, in the range of 14.4 to 81.5 mg/*l*. Generally speaking the LC_{50} was seen to decrease with an increasing log K_{ow} of pharmaceuticals, but overall toxicity was seen to increase. However, the result for acetaminophen was exceptional. These bioassay results would be valuable in risk assessments with respect to pharmaceutical residues in surface waters, taking into account the insufficient toxicological data regarding pharmaceuticals. Finally, the potential risk from the pharmaceuticals detected in the surface waters from Kyongahn river indicated a low biological risk (HQ < 1) to aquatic ecosystems when a risk assessment was applied to the tests on *D. magna*. The monitored surface water concentrations did not reach critically harmful levels. However, the potential risk of pharmaceuticals should be monitored carefully with more bioassay data, although no risk was found in the surface waters from Kyonahn river. In 1970, studies on overseas of environmental pollution by pharmaceutical were reported. From the mid 1990s, the pollution by pharmaceutical began to draw people's attention on a full scale. At present, there are various studies in process, which are not only of the actual condition research over residue pharmaceutical but also study for pharmaceutical treatment inside STP (Thomas, 2002). There are a few studies and research of pharmaceutical affecting the environment (Kang *et al.*, 2005, Kang, 2006) done within Korea, but they are still deficient. What is more, effect of pharmaceutical exposure in domestic environment has almost never been measured properly. As the case stands, neither any regulations nor counterplans for domestic exposure in environment exist at the moment. The possibility of pharmaceutical exposure in domestic environment will continue to grow. It is an issue which demands more further attention and requires study in detail.

Table 4. Concentrations of acetaminophen (μg/*l*) found in Korea and United States of America

Chemical/ Concentraitons	This study	USA[a]
	Mean(Max)	Median(Max)
Acetaminophen	0.014(0.056)	0.11(10.00)

※a: Kolpin *et al.*, 2002.

IV. Summary

Acetaminophen (paracetamol), generally used as a pain reducing agent, has good analgesic efficacy in toothaches and headaches, but is of little use in inflammatory and visceral pain. This study was performed to analyze the level of acetaminophen in the Kyongahn river and to investigate the ecological risks of target compounds. Sampling sites were Haesil, Soopyo, Wangsan, Kyongahn, Jiwol, Kwangdong, Paldang and they were analyzed in June and August, 2005. Acute toxicity of acetaminophen wwas evaluated for Daphnia magna. From the ecotoxicological results, environmental risk assessments were performed for acetaminophen residues in Kyongahn river to predict their potential adverse effect. Acetaminophen was detected at Kyonahn river, 0.439 μg/*l*. The toxic concentration of acetaminophen calculated with 48-h LC_{50} values as 16.9 mg/*l*. These results indicated that acetaminophen had no significant ecotoxicological impact on short-term acute exposure.

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