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Aquatic toxicity of acetaminophen, carbamazepine, cimetidine, diltiazem and six major sulfonamides, and their potential ecological risks in Korea

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Abstract

Pharmaceuticals are manufactured and used for specific biological functions in veterinary and human medicine. Their detection in the environment and their bioactivity have resulted in concern for potential adverse effects on non-target species. Notwithstanding recent attention for their occurrence in the environment, there are significant research gaps for existing pharmaceuticals with regard to their potential ecological consequences. In this study, the four most abundantly used pharmaceuticals in Korea, namely acetaminophen, carbamazepine, cimetidine, and diltiazem, and six sulfonamide related antibiotics, including sulfamethoxazole, sulfachlorpyridazine, sulfathiazole, sulfamethazine, sulfadimethoxine, and trimethoprim were examined for their acute aquatic toxicity employing a marine bacterium (*Vibrio fischeri*), a freshwater invertebrate (*Daphnia magna*), and the Japanese medaka fish (*Oryzias latipes*). In general, *Daphnia* was the most susceptible among the test organisms. The most acutely toxic among the chemicals tested in this study was diltiazem, with a median lethal concentration of 8.2 mg/L for *D. magna*. The resulting acute toxicity of these pharmaceuticals was reasonably predicted by physicochemical descriptors such as pH-dependent distribution coefficient and $E_{HOMO} - E_{LUMO}$ gap. Predicted environmental concentrations (PECs) derived for the test pharmaceuticals in Korea ranged between 0.14 and 16.5 μg/L. Hazard quotients derived from PECs and predicted no effect concentrations (PNECs) for sulfamethoxazole and acetaminophen were 6.3 and 1.8, respectively, suggesting potential environmental concerns and a need for further investigation.

Keywords: Pharmaceutical; Environment; Sulfonamide; Toxicity; Hazard quotient

1. Introduction

Pharmaceuticals are developed and used for specific biological effects, being administered for human and animal health care, and livestock farming. Because of their physicochemical and biological properties, there are concerns about the potential for their impacts to non-target species (Park, 2005). Although a variety of pharmaceutical compounds have been detected in the environment, their potential ecological significance remains largely unknown (Sanderson et al., 2004).

Due to the high human population density and more concentrated animal feeding operations, the nation's water is expected to be at high risk for potential contamination with various pharmaceutical products. Han et al. (2006) reported the occurrence of several pharmaceuticals from the effluent of wastewater treatment plants in Korea. Detected compounds included acetaminophen, carbamazepine, diclofenac, ibuprofen, and salicylic acid.

The present study was conducted to estimate the environmental risk of frequently used pharmaceuticals in Korea, and for those that have been frequently reported in aquatic environments in other countries: Acetaminophen, carbamazepine, cimetidine, and diltiazem are frequently used pharmaceuticals and have been detected worldwide (Kolpin et al., 2002; Metcalfe et al., 2004; Ternes, 1998; Ternes et al., 2001). Sulfonamides are the second largest group in Korea as well as in many European countries among the administered antibiotic compound classes (National Veterinary Research and Quarantine Service, 2005; Thiele-Bruhn, 2003). Many sulfonamides

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such as sulfamethoxazole, sulfadimethoxine, and trimethoprim have been detected frequently in surface waters worldwide (Park, 2005).

In order to estimate ecological risks for these compounds, their predicted environmental concentrations (PECs) were calculated following European Union (1994) based on a series of conservative assumptions. In addition, we investigated their acute aquatic toxicity using indicator species that represent different trophic levels, i.e., a marine bacterium *Vibrio fischeri*, a freshwater invertebrate *Daphnia magna*, and a fish Japanese medaka (*Oryzias latipes*). We present an environmental risk assessment of these pharmaceuticals in order to aid in future risk management decisions for these emerging water contaminants.

2. Materials and methods

2.1. Test chemicals

Tested pharmaceuticals were acetaminophen (analgesic), carbamazepine (antiepileptic), cimetidine (H2 blocker) and diltiazem (antihypertensive), and five sulfonamide antibiotics, i.e., sulfamethoxazole, sulfachlorpyridazine, sulfathiazole, sulfamethazine, and sulfadimethoxine. Trimethoprim was also added because this antibiotic is commonly used with other sulfonamide antibiotics (López-Martínez et al., 2002).

All the test pharmaceuticals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Table 1 summarizes physicochemical characteristics of the test pharmaceuticals. Stock solutions were prepared with dimethyl sulfoxide, from which test solutions were prepared with appropriate dilution medium for each test species. The solvent, dimethyl sulfoxide, was not used in excess of 0.5% in the final exposure solution. Solvent controls were established throughout this study, and no adverse effect was observed at this level of the solvent. Stock solutions that were not used immediately were stored in glass containers, for no longer than 2 d before use, wrapped with aluminum foil to minimize potential photo-degradation.

2.2. Test organisms and maintenance

Standardized single species toxicity test were conducted using *V. fischeri*, *D. magna*, and *O. latipes* to measure the aquatic toxicities of the test pharmaceuticals. Moderately hard water (MHW) manufactured following US EPA guideline (2002), and dechlorinated tap water prepared with three-way filtration were used for culture and maintenance of *Daphnia* and the fish, respectively. Water quality parameters including hardness, alkalinity, pH, conductivity, and dissolved oxygen were routinely monitored following the standard methods by American Public Health Association, American Water Works Association, and Water Pollution Control Federation (1992).

Daphnia cultures were maintained at 20±1 °C in 6 L glass jars. Fish cultures were maintained at 25±1 °C in 30 L aquaria. Photoperiod of 16L:8D was maintained. The culture water was renewed twice a week. Daphnids were fed with YCT (Yeast:Ceropyl®:Tetramin®) and algae (*Selenastrum capricornutum*) daily. Japanese medaka was fed with freshly hatched *Artemia* nauplii and flake food (Tetramin®) every day.

2.3. Toxicity tests

A microbial bioassay with *V. fischeri* was performed using the Microtox® Toxicity Analyzer (Microbics now SDI, Newark, DE, USA) following the manufacturer's instructions. The '81.9% Basic Test' procedure was followed. Alteration in light output was measured after 5 and 15 min of exposures.

The 96 h toxicity tests with *D. magna* were conducted in accordance with the recommended procedure outlined in U.S. EPA (2002). Endpoint was immobility of test organisms. Preliminary range finding tests were conducted to determine the concentration range to be used in the definitive tests. Reference tests using sodium chloride as a standard toxicant were carried out on a monthly basis to confirm the comparable sensitivity of the test organisms over time (data not shown).

Acute fish toxicity tests were carried out with *O. latipes* (length, 2.0± 1.0 cm) in accordance with the Organization for Economic Co-operation and Development guideline TG 203 (OECD, 1992). Seven or more fish were exposed to each test concentrations at 25±1 °C for 96 h. Mortality was checked every 24 h. Freshly manufactured test concentrations were renewed after 48 h of the exposure. Fish were not fed throughout the acute exposure duration. Dissolved oxygen concentration greater than 80% of the saturation limit was ascertained during the exposure.

For acute tests with the cladoceran and the fish, the median effective and lethal concentrations were calculated using ToxStat (version 3.5, West Inc., Cheyenne, WY, USA). For Microtox test, a vendor provided software was employed for estimating the median inhibitory concentrations of each chemical. Nominal concentrations were used throughout the study.

2.4. Determination of physicochemical descriptors

Physicochemical descriptors were calculated to help explain the observed toxicity, e.g., energy of the highest occupied molecular orbital (E_{HOMO}),

Table 1
Physicochemical characteristics of the test pharmaceuticals

	ATP	CBZ	CTD	DTZ	SMX	TMP	SCP	STZ	SMZ	SDM
CAS No.	103-90-2	298-46-4	51481-61-9	42399-41-7	723-46-6	738-70-5	80-32-0	72-14-0	57-68-1	122-11-2
Molecular weight (g/mol)	151.2	236.3	252.3	414.5	253.3	290.3	284.7	255.3	277.3	310.3
Formula	$C_8H_9NO_2$	$C_{15}H_{12}N_2O$	$C_{10}H_{16}N_6S$	$C_{22}H_{26}N_2O_4S$	$C_{10}H_{11}N_3O_3S$	$C_{14}H_{18}N_4O_3$	$C_{10}H_9ClN_4O_2S$	$C_9H_9N_3O_2S_2$	$C_{13}H_{15}N_3O_2S$	$C_{12}H_{14}N_4O_4S$
p*K*a	9.38	7.00	6.80	8.90	5.70	7.12	1.40	7.29	7.59	5.54
logK_{ow}	0.46	2.45	0.40	2.79	0.89	0.91	0.31	0.89	0.89	0.63
logD_{ow}	0.41	1.13	−1.41	2.73	0.19	−0.68	N.A.	−0.54	0.47	0.25
E_{HOMO} (eV)	−6.30	−5.81	−5.95	−5.76	−5.94	−5.44	−6.40	−5.91	−6.15	−6.15
E_{LUMO} (eV)	−0.54	−1.37	0.25	−0.75	−0.51	−0.07	−1.62	−1.16	−0.80	−0.77
E_{HOMO}-E_{LUMO} gap, ΔE (eV)	5.76	4.44	6.20	5.01	5.42	5.38	4.78	4.75	5.49	5.37

Molecular weight, formula, p*K*a, LogK_{ow}, and LogD_{ow} of test pharmaceuticals were gleaned from available literatures. Molecular orbital energy descriptors were calculated with the Gaussian 98 suite of programs. Where applicable, units are shown in parenthesis.
Abbreviations — ATP: acetaminophen, CBZ: carbamazepine, CTD: cimetidine, DTZ: diltiazem, SMX: sulfamethoxazole, TMP: trimethoprim, SCP: sulfachlorpyridazine, STZ: sulfathiazole, SMZ: sulfamethazine, SDM: sulfadimethoxine, N.A.: not available.

Table 2
Acute median effective concentrations (E/LC_{50}s) of the test pharmaceuticals

Chemicals	This study						Literature			
	V. fischeri		*D. magna*		*O. latipes*		*V. fischeri*	*D. magna*	*Selenastrum capricornutum*	*Cyclotella meneghiniana*
	5 min	15 min	48 h	96 h	48 h	96 h	5 min	48 h	96 h	96 h
ATP	549.7 (534.0–565.9)	567.5 (358.6–898.1)	30.1 (23.2–39.0)	26.6 (19.6–33.6)	>160	>160	–	9.2–50[a,b]	–	–
CBZ	52.5 (49.2–56.1)	52.2 (45.8–59.5)	>100	76.3 (64.4–88.1)	35.4 (NA)	35.4 (NA)	>81[c]	>100[d]	–	31.6[c]
CTD	458.9 (382.1–551.0)	375.9 (306.6–460.7)	379.7 (310.0–449.5)	271.3 (230.6–312.0)	>100	>100	–	740[e]	–	–
DTZ	407.4 (381.0–435.7)	263.7 (234.9–296.1)	28.0 (23.3–32.7)	8.2 (6.0–10.4)	25.6 (21.4–30.7)	15.0 (NA)	–	–	–	–
SMX	74.2 (46.4–118.7)	78.1 (24.0–25.4)	189.2 (155.9–229.5)	177.3 (145.6–208.9)	>750	562.5 (NA)	>84[c]	>100[c]	0.146[c]	2.4[c]
TMP	165.1 (149.1–182.9)	176.7 (158.8–196.6)	167.4 (138.4–196.3)	120.7 (98.9–142.4)	>100	>100	–	123[f]	–	–
SCP	53.7 (47.4–60.9)	26.4 (19.8–35.4)	375.3 (325.9–424.7)	233.5 (202.3–264.7)	589.3 (451.8–726.7)	535.7 (398.2–673.2)	–	–	–	–
STZ	>1000	>1000	149.3 (115.8–192.5)	85.4 (74.0–98.5)	>500	>500	–	–	–	–
SMZ	303.0 (289.2–317.7)	344.7 (326.7–363.3)	174.4 (158.4–191.9)	158.8 (133.0–184.4)	>100	>100	–	–	–	–
SDM	>500	>500	248.0 (199.2–296.8)	204.5 (156.9–252.2)	>100	>100	–	–	–	–

Units in mg/L. Abbreviations — ATP: acetaminophen, CBZ: carbamazepine, CTD: cimetidine, DTZ: diltiazem, SMX: sulfamethoxazole, TMP: trimethoprim, SCP: sulfachlorpyridazine, STZ: sulfathiazole, SMZ: sulfamethazine, SDM: sulfadimethoxine
[a,b]Kühn et al. (1989), Henschel et al. (1997). [c]Ferrari et al. (2004). [d]*Daphnia* spp., Cleuvers (2002). [e]US FDA-CDER (1996). [f]Stuer-Lauridsen et al. (2000).

energy of the lowest unoccupied molecular orbital (E_{LUMO}), and $E_{HOMO}-E_{LUMO}$ gap (ΔE) using the Gaussian 98 suite of programs in the restricted B3LYP level with 3-21G basis set. The D_{ow}, which qualifies the partitioning of the un-dissociated species into the 1-octanol, was derived from the following Eq. (1).

$$D_{ow} = K_{ow}/(1 + 10^{pH-pKa}) \quad (1)$$

Where, K_{ow} is the octanol–water partition coefficient, pH is that of the highest concentration tested, and p*K*a is the acidity dissociation constant in logarithmic form.

2.5. Estimation of hazard quotients

Hazard quotients of each test pharmaceutical were calculated from PEC divided by predicted no effect concentration (PNEC) of each pharmaceutical. The PECs of test pharmaceuticals were estimated from Eq. (2) based on several conservative assumptions (European Union, 1994). To determine the amount of a pharmaceutical substance manufactured per year in Korea, the first step was to search for the commercial medicinal product names for each substance using the online Korean Index of Medicinal Specialties (KIMS) (http://kimsonline.co.kr). The second step was to search for production data for each pharmaceutical product at the Korean Pharmaceuticals Manufacturers Association (KPMA). The amount for domestic consumption only was calculated by subtracting exported amount from the total amount of the drug manufactured per year in Korea. Among the ten test pharmaceuticals, only six have necessary information, and these pharmaceuticals were acetaminophen, carbamazepine, cimetidine, diltiazem, sulfamethoxazole, and trimethoprim. Therefore PECs were estimated for these six pharmaceuticals only. For the estimation of PECs, the following assumptions were made: (1) all manufactured amount of pharmaceuticals (except for exports) was sold and used in the same year; (2) all the pharmaceuticals were released through the sewer system; (3) there was no significant metabolite formation in humans as well as negligible elimination in the sewage treatment systems, and (4) the use pattern was evenly distributed temporally and spatially. The PEC of the test pharmaceutical was calculated by following the Eq. (2).

$$PEC_w = \frac{A \times (100-R)}{365 \times P \times V \times D \times 100} \times 10^9 \quad (2)$$

Where,

- A amount of substance manufactured per year (kg/y)
- R removal rate in percent in sewage treatment (set to zero)
- P population of Korea (~48,000,000 in 2005 according to Korea National Statistical Office)
- V volume of wastewater produced per day per capita (370 L in 2003). Wastewater generation volume per capita was calculated using the annual reporting data of wastewater treatment capacity of sewage plants by the Korea Ministry of Environment (2001)
- D dilution factor in the environment. A default value of 10 is applied, i.e., the effluent of sewage treatment plant is diluted by a factor of 10 in receiving waters.

Based on the acute toxicity data obtained from the present study, and also from the available literature as shown on Table 2, PNECs for each test pharmaceuticals were derived by dividing the lowest median effective concentration (EC50) value obtained with an assessment factor of 1000 as recommended in European Commission (2003).

3. Results and discussion

3.1. Toxicity to aquatic organisms

The acute toxicity of each pharmaceutical varied by test organisms. None of the chemicals however showed a high level of acute lethal

toxicity. Table 2 shows the summary of acute median effective/lethal concentrations (E/LC50s) obtained for the test pharmaceuticals, and also toxicity values reported elsewhere. Among the test organisms, *D. magna* was in general the most sensitive to the most of pharmaceuticals tested in the present study. Among the test pharmaceuticals, diltiazem was the most toxic, as evidenced by the resulting *Daphnia* 96 h EC50 value at 8.2 mg/L. For acetaminophen, the 48 h *D. magna* EC50 value was 30.1 mg/L, which was within the literature reported values of 9.2 mg/L (Kühn et al., 1989) and 50 mg/L (Henschel et al., 1997). The EC50 values of acetaminophen obtained from *V. fischeri* and *O. latipes* were 549.7 and >160 mg/L, respectively. For carbamazepine, the median effective concentrations for *V. fischeri* and *D. magna* were 52.5 mg/L (5 min exposure) and 76.3 mg/L (96 h exposure), respectively. These acute toxicity values reported in the present study are consistent with those reported elsewhere in the literature (Jos et al., 2003).

With *V. fischeri*, EC50s were found at >100 mg/L for all test pharmaceuticals except for carbamazepine, sulfachlorpyridazine, and sulfamethoxazole. In general, sulfonamides were not very acutely toxic to Japanese medaka (LC50s>100 mg/L). However, for carbamazepine and diltiazem, LC50 test results for the medaka fish ranged from 15.0 to 35.4 mg/L.

3.2. Quantitative structure–activity relationships of test pharmaceuticals

The observed acute toxicity was correlated with two classes of physicochemical descriptors, i.e., penetration and bio-reactivity, which are shown in Table 1. Because many pharmaceuticals have both lipophilic and hydrophilic characteristics, the penetration of pharmaceuticals through cell membrane may not be explained by LogK_{ow} (Williams, 2005). In the present study, a backward stepwise regression analysis was carried out for toxicity values obtained from *V. fischeri* and *D. magna* using a variety of physicochemical descriptors. As shown in Eq. (3), with *V. fischeri* a negative correlation was found with ΔE ($p<0.05$). The ΔE serves as a measure of the excitability of the molecule (Mekenyan et al., 1994). Chemicals with lower ΔE are more likely to be excited, and therefore more prone to interact with other molecules, potentially resulting in greater toxicity. LogD_{ow} is a distribution coefficient adjusted for the pH of medium and p*K*a of a given chemical. Huang et al. (2003) correlated LogD_{ow} values with the observed toxicity of benzene derivatives with tadpole *Rana japonica*, which is in line with the observation with *D. magna* in the present study ($p<0.05$, Eq. (4)). In the quantitative structure–activity relationship equation derived for *V. fischeri* 15 min exposure (Eq. (3)), however, the correlation with LogD_{ow} was not evident: the 95% confidence interval calculated for the coefficient of LogD_{ow} was between −0.233 and 0.017, indicating neither positive nor negative correlation with these prokaryotic organisms. Fig. 1 shows the relationships between experimental values and estimated values of the test pharmaceuticals.

$$\text{15 min } \textit{V. fischeri}\ \text{Log}(1/\text{EC50}) = -0.108\ \text{Log}\ D_{ow} - 0.729\ \Delta E + 4.070\ n = 8, r = 0.893, \text{SE} = 0.271, F = 9.867, p = 0.018 \quad (3)$$

$$\text{48 h}\ \textit{D. magna}\ \text{Log}(1/\text{EC50}) = 0.165\ \text{Log}\ D_{ow} + 0.329\ n = 9, r = 0.761, \text{SE} = 0.290, F = 9.623, p = 0.017 \quad (4)$$

3.3. Hazard quotients

Although one cannot estimate the exact probability that adverse effects will occur, the hazard quotient is a useful measure that can be employed to characterize potential ecological risk of a stressor

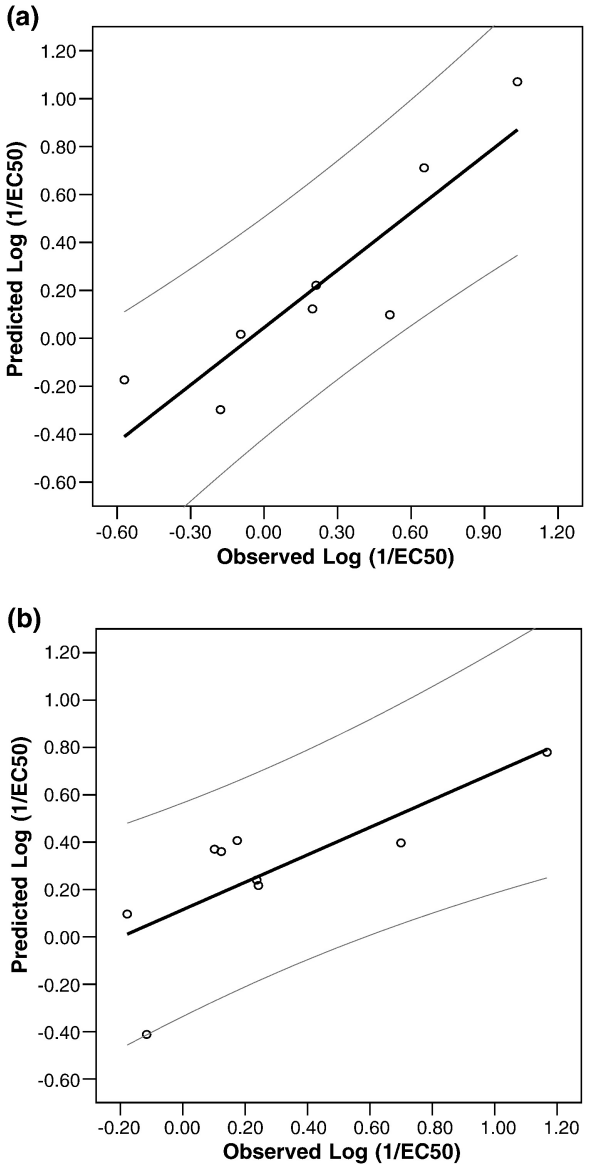

Fig. 1. Comparative plots of predicted versus observed values of EC50s. (a) *V. fischeri* 15 min EC50s, and (b) *D. magna* 48 h EC50s of test pharmaceuticals. Red curves indicate corresponding 95% confidence interval.

(Solomon et al., 1996). The PECs of the six pharmaceuticals were calculated based on Eq. (2), and they ranged from 0.14 to 16.5 μg/L. The highest PEC was expected with acetaminophen, very popular antipyretic, which was also the highest volume production pharmaceutical in Korea with 1,068,921 kg in 2003. Acetaminophen is reported as one of the most frequently detected pharmaceuticals in sewage treatment plant effluents, drinking water, or surface water. This compound was detected in 24% of the US stream water samples with a maximum detection level of 10 μg/L (Kolpin et al., 2002), which is comparable with the PEC value of acetaminophen calculated in this study, i.e., 16.5 μg/L. Stuer-Lauridsen et al. (2000) predicted the environmental concentration of acetaminophen to be 65.4 μg/L in EU using the same equation (Eq. (2)) employed in the current study. However the measured levels of acetaminophen in surface water were generally less than the predicted levels: Kolpin et al. (2004) reported the maximum acetaminophen level of 1.95 μg/L in streams of Iowa, USA in low flow season. In river Elbe, Wiegel et al. (2004) detected

acetaminophen in 13 out of 24 samples with the maximum detection level of 0.066 μg/L. Bound and Voulvoulis (2006) reported the maximum acetaminophen concentrations of 0.264 μg/L and 0.165 μg/L in upstream and downstream Thames, UK, respectively. Boyd et al. (2003) could not detect acetaminophen in surface water samples collected from Mississippi River and Pontchartrain Lake, in Louisiana, USA. In general, measured levels of acetaminophen were much lower than the predicted values, as shown by Bound and Voulvoulis (2006) in UK surface water. This observation may reflect high metabolic rate (Sweetman, 2002) and rapid degradation characteristics (Richardson and Bowron, 1985) of acetaminophen. Among the test pharmaceuticals, cimetidine followed the next to acetaminophen in annual production amount with 132,809 kg, and resulted in the second highest PEC of 2.05 μg/L. The PEC of carbamazepine was estimated at 0.14 μg/L, and this level is comparable to the measured levels from sewage treatment plant effluents (0.11–0.32 μg/L) in US (Skadsen et al., 2004), Canada (Metcalfe et al., 2004) and Italy (Zuccato et al., 2004). Among sulfonamides, sulfamethoxazole showed the highest PEC value of 0.95 μg/L. In fact, sulfamethoxazole is one of the most frequently detected antibiotics. Kolpin et al. (2002) reported that this sulfonamide was detected from 19% of stream samples at varying concentrations up to 1.9 μg/L. Trimethoprim, an antibiotic commonly used in combination with other sulfonamides, was estimated to be present in the water at 0.21 μg/L. This compound was detected from 27.4% of stream water samples at an average of 0.15 μg/L (Kolpin et al., 2002).

The lowest PNEC derived from the lowest E/LC50s of test pharmaceuticals was found with sulfamethoxazole at 0.15 μg/L, followed by diltiazem and acetaminophen at 8.2, and 9.2 μg/L, respectively. Hazard quotients calculated for sulfamethoxazole and acetaminophen were 6.3 and 1.8, respectively, suggesting potential adverse ecological consequences. It should be noted that only the amount used for human medicine was considered for deriving the PECs of sulfonamides. Considering sulfonamides are the second ranked high volume veterinary antibiotics in Korea, the PECs calculated in this study for sulfonamides might be underestimated. It is estimated that in Korea 14,791 kg of sulfamethoxazole and 7575 kg of trimethoprim were sold for livestock farming in 2004 (National Veterinary Research and Quarantine Service, 2005). Ferrari et al. (2004) calculated the hazard quotients of sulfamethoxazole to be 11.4 and 59.3 based on its PECs in France and Germany, respectively, which are greater than the hazard quotient of 6.3 calculated in this study. It should be noted that Ferrari et al. also considered the amount used in human medicine only for calculating the PECs of sulfamethoxazole of both countries. For acetaminophen, the hazard quotient of 1.8 was derived in this study, and this is much lower than the hazard quotient of 7.1 calculated for EU based on PEC value (Stuer-Lauridsen et al., 2000). However hazard quotients that were derived from the measured environmental concentrations of acetaminophen reported in the literature (Kolpin et al., 2004; Wiegel et al., 2004; Bound and Voulvoulis, 2006; Boyd et al., 2003) were generally much lower than one, suggesting no significant environmental concern. Refinement of PEC derivation model by incorporating the fate characteristics in body and wastewater treatment plant would result in more realistic estimates of the environmental concentrations (Bound and Voulvoulis, 2006). The rest of the pharmaceuticals exhibited hazard quotients less than one, suggesting the risk to the environment be considered low. More detailed exposure assessments including environmental fate modeling, as well as more definitive effect studies with subtle but ecologically meaningful endpoints such as reproduction, endocrine disruption, behavioral changes, and effects on multiple levels of biological organization, need to be investigated to further increase the scientific understanding of the potential impacts of these pharmaceuticals in the environment.

Table 3
Derivation of hazard quotients of the test pharmaceuticals

Substances	Amount manufactured (kg)	PEC (μg/L)	Lowest EC50 (μg/L)	PNEC (μg/L)	Hazard quotient
Acetaminophen	1,068,921	16.5	9200 [a]	9.2	1.8
Carbamazepine	9155	0.14	31,600 [b]	31.6	0.0044
Cimetidine	132,809	2.05	271,300	271.3	0.0076
Diltiazem	9071	0.14	8200	8.2	0.017
Sulfamethoxazole	61,272	0.95	146 [c]	0.146	6.3
Trimethoprim	13,553	0.21	120,700	120.7	0.0017

Amounts of pharmaceuticals manufactured are as of 2003, and were calculated using data obtained from online Korean Index of Medicinal Specialites, and Korean Pharmaceuticals Manufacturers Association. Since Production and export amount information was available for acetaminophen, carbamazepine, cimetidine, diltiazem, sulfamethoxazole, and trimethoprim, PECs were calculated only for these six test pharmaceuticals. PNEC values were derived from the lowest acute L/EC50 values obtained either from the present study or from the literature.

[a] Based on the lowest *D. magna* 48 h EC50 in the literature (Kühn et al., 1989).

[b] Based on the lowest *Cyclotella meneghiniana* 96 h growth EC50 in the literature (Ferrari et al., 2004).

[c] Based on the lowest *Selenastrum capricornutum* 96 h growth EC50 in the literature (Ferrari et al., 2004).

4. Conclusion

In the present study, we evaluated the acute aquatic toxicity of several widely used pharmaceuticals including acetaminophen, carbamazepine, cimetidine, diltiazem, and six sulfonamides, using representative model species of *V. fischeri*, *D. magna*, and Japanese medaka. The acute toxicity results were reasonably predicted by a quantitative structure activity relationship model using pH-dependent distribution coefficient and molecular orbital energy parameters of the test pharmaceuticals. Utilizing a series of conservative assumptions, the PECs of the pharmaceuticals tested were calculated, and their hazard quotients were derived. Hazard quotients were greater than unity for acetaminophen and sulfamethoxazole, suggesting potential ecological risks and the need for further investigation. The ecotoxicological assessment of environmentally relevant levels of pharmaceuticals for long-term exposure is needed to more realistically characterize the ecological significance of pharmaceutical contamination in the environment.

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