Preliminary Ecological Risk Assessment of Butylparaben and Benzylparaben —2. Fate and Partitioning in Aquatic Environments

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Butylparaben and benzylparaben, used as preservatives mainly in cosmetic products, have recently been shown to be weakly estrogenic. Batch sunlight photolysis and river water biodegradation experiments were conducted to determine the persistence of these compounds in aquatic environments. As a result, benzylparaben was found to be moderately photodegradable whereas both *n*-butylparaben and *i*-butylparaben were highly stable against sunlight. Both benzylparaben and butylparabens were relatively biodegradable in the river water but the degradability was dependent on the sampling site and time. Batch sorption experiments were also conducted to determine the coefficients of sorption into river sediments and a model soil sample. The determined coefficients were slightly higher for benzylparaben than the two butylparabens and comparable to that of the natural estrogen 17β-estradiol. The coefficients were also higher for sediment/soil with a higher organic content and the organic-carbon-based sorption coefficient (log K_{oc}) shows a moderate linear correlation with the octanol-water partition coefficient (log K_{ow}). These results suggest that hydrophobic interaction plays a predominant role in sorption at neutral pH.

1. Introduction

Parabens (alkyl esters of *p*-hydroxybenzoic acid) are a class of antimicrobials, particularly useful against molds and yeasts, and are widely used as preservatives at below 1% in cosmetics.(1) As presented in our parallel paper,(2) a number of studies suggest the estrogenic activities of these compounds. Methyl-, ethyl-, *n*-propyl- and *n*-butylparabens were all found to be weakly estrogenic by rat estrogen receptor binding assay and a yeast-based estrogen assay, whereas *n*-butylparaben showed estrogenic activity in a rat uterotrophic assay.(3) Darbre *et al.*(4) also found an increase in rat uterine weight induced by benzylparaben. The estrogenic activity of benzylparaben is higher than those of linear alkyl parabens, and *i*-butylparaben shows the highest potency followed by *n*-butylparaben among seven parabens, as determined by the human estrogen receptor binding assay and yeast two-hybrid assay.(5)

As far as aquatic organisms are concerned, *n*-propylparaben was found to induce increases in plasma vitellogenin (VTG) concentration and up-regulate the gene expressions of VTG-1, VTG-2, choriogenin (CHG)-L, and CHG-H in male medaka.(6) Pedersen *et al.*(7) found that intraperitoneal injections of ethyl-, *n*-propyl- and *n*-butylparabens into rainbow trout caused estrogenic responses such as a significant VTG induction at doses of 100–300 mg kg^{-1}. Alslev *et al.*(8) reported that exposure of rainbow trout to

201 μg L^{-1} of *n*-butylparaben for 12 days also increased the plasma VTG concentration in the fish. In the first part of two consecutive papers of ours,(2) we reported that the maximum no-effect concentrations (NOECs) determined by male medaka VTG assay in *n*-butylparaben, *i*-butylparaben, and benzylparaben were 40, 20, and 20 μg L^{-1}, respectively, and the predicted no-effect concentrations (PNECs) determined on the basis of conventional acute/chronic tests using medaka, daphnia, and green algae for the three parabens were 8.0, 6.0, and 5.2 μg L^{-1}, respectively. The results of a medaka DNA microarray study of benzylparaben suggested a slight up-regulation of estrogen-related genes at the lowest concentration of 4 μg L^{-1}.

Despite several reports of estrogenic activity and the wide use of parabens in cosmetic, pharmaceutical, and food products, few studies have been conducted to clarify the fate of these compounds in aquatic environments. Ethylparaben was detected to be as high as 3.3 μg kg^{-1} in Japanese river sediments from only two sampling sites, but the concentrations of methyl-, propyl-, *n*-butyl-, and *i*-butylparaben were below the detection limit for other river water and sediment samples.(9) Benzyl-, ethyl-, and *n*-propylparaben were detected to be as high as 1 μg L^{-1} in the effluent of Swedish wastewater treatment plants (WWTPs).(10) *n*-Butylparaben was detected at 0.01–0.26 μg L^{-1} in the effluent of Canadian(11) and Spanish(12) wastewater treatment plants. In the comparison between the concentrations of parabens in the influent and effluent of WWTPs, the removal efficiencies in the conventional WWTP were as high as 96, 99, 96, and 99% for *n*-butyl-, propyl-, ethyl-, and methylparaben, respectively.(11) As for chlorination, in our parallel paper,(2) we reported that the addition of 2.0 mg L^{-1} chlorine as sodium hypochlorite into *n*-butylparaben, *i*-butylparaben, and benzylparaben water for 15 min resulted in 67, 72 and 71% removal, respectively. However, not much has been investigated regarding the persistence and partitioning of parabens in the aquatic environment such as photodegradability, biodegradability, and sorption by sediments.

In this study, we selected three parabens with higher estrogenic potencies than other parabens, namely, *n*-butylparaben, *i*-butylparaben, and benzylparaben, and attempted to clarify the fate and partitioning of these compounds in aquatic environments. Therefore, the objectives of this second part of our two consecutive studies were to clarify their (1) photodegradability by sunlight, (2) biodegradability in river water, and (3) coefficients of sorption into river sediment/soil.

2. Materials and Methods

2.1 *Materials*

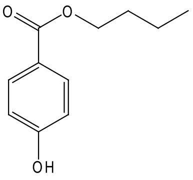
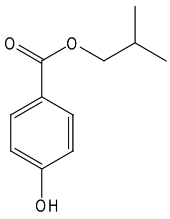
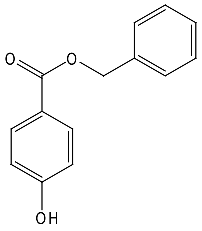
n-Butyl-*p*-hydroxybenzoate (*n*-butylparaben) of at least 98% purity was purchased from Wako Pure Chemical Industries (Osaka, Japan). *i*-Butyl-*p*-hydroxybenzoate (*i*-butylparaben) was purchased from Tokyo Kasei Co. (Tokyo, Japan). Benzyl-*p*-hydroxybenzoate (benzylparaben) of at least 99% purity, *p*-nitroacetophenone, and pyridine (ultrapure grade) were purchased from Wako Pure Chemical Industries (Osaka, Japan). 17β-estradiol of at least 97% purity and pyrene of at least 98% purity were purchased from Sigma-Aldrich Chemical (Milawaukee, WI, USA).

The chemical structures, acidity constant (pK_a) values and octanol-water distribution constant ($\log D_{ow}$) values are shown in Table 1. The excretion ratio of the unaltered form was from the report of Ye *et al.*;(13) $\log D_{ow}$ and pK_a values were estimated using the ACD software LogD Suite. The annual consumption in Japan was estimated, as shown in our parallel paper.(2)

2.2 *Procedure of photolysis experiments*

Experiments on the photodegradation of selected parabens by sunlight were conducted in accordance with US Environmental Protection Agency (USEPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) harmonized test guideline

Table 1
Parabens selected in this study.

Parabens	*n*-Butylparaben	*i*-Butylparaben	Benzylparaben
Estimated Domestic Consumption as Cosmetic Products or Topical Medicine[a)]	168 t	3.0 t	25 t
Estimated Domestic Consumption as Food Products[a)]	12 t	1.5 t	none
Excretion Ratio of Unaltered Form[b)]	17%	unknown	< 1%
Chemical Structure			
log D_{ow}[c)] (at pH 7)	3.43	3.27	3.61
pK_a[c)]	8.22	8.17	8.18

[a)]Procedure of estimation is described in our parallel paper,(2) [b)]Cited from Ye *et al.*,(13) [c)]Predicted by log D Suite (ACD software).

835.2210.(14) Briefly, the initial concentration of each paraben solution was placed at 100 µg L^{-1} and the solution was placed in a 30 mL quartz test tube. The blank samples were completely covered with aluminum foil to prevent exposure to sunlight. *p*-Nitroacetophenone (PNAP) – pyridine solutions with similar photolysis rates were used as actinometers. All the test tubes were exposed to sunlight from 8 a.m. to 6 p.m. at the flat roof of the Number Three Building of the Faculty of Integrated Arts and Sciences, The University of Tokushima, Japan (approximately 34 degrees north latitude). The experiments were conducted twice, in September 2006 and July 2007. The aqueous concentration was determined using an HPLC (LC-10AD VP series, Shimadzu, Kyoto, Japan) system equipped with a 3 µm ODS column (Cadenza CD-C18, Imtakt, Kyoto, Japan) and a UV/visible absorbance (SPD-10A VP, Shimadzu, Kyoto, Japan) detector. Molar absorptivities in wavelengths between 297.5 and 400 nm were measured using a UV-visible spectrophotometer (U-1500, Hitachi, Japan) to determine quantum yield.

2.3 *Procedure of biodegradation tests*

Biodegradation tests were conducted with reference to the DOC die-away test, "OECD Test Guideline for Testing of Chemicals No. 301A,"(15) modified for individual chemicals by the authors. Briefly, samples were collected from Tamiya River (October 2006) and Tsumeta River (September and November 2007) of Tokushima City (without tidal effects) and filtered through 3 µm pore size membrane filters (Millipore). The initial concentration of each paraben solution was 100 µg L^{-1} and the solution was placed in a 100 mL Erlenmeyer flask. The blank samples were prepared from sterilized milli Q water. The aqueous concentration of each paraben was determined using the HPLC system described above. The qualities of the river water samples were as follows: BOD, 3.4 mg L^{-1} and TOC, 2.4 mg L^{-1} in October 2006; BOD, 2.3 mg L^{-1} and TOC, 9.2 mg L^{-1} in September 2007; and BOD, 2.6 mg L^{-1} and TOC, 3.2 mg L^{-1} in November 2007.

2.4 *Procedure of experiments on sorption into river sediments/soil*(16)

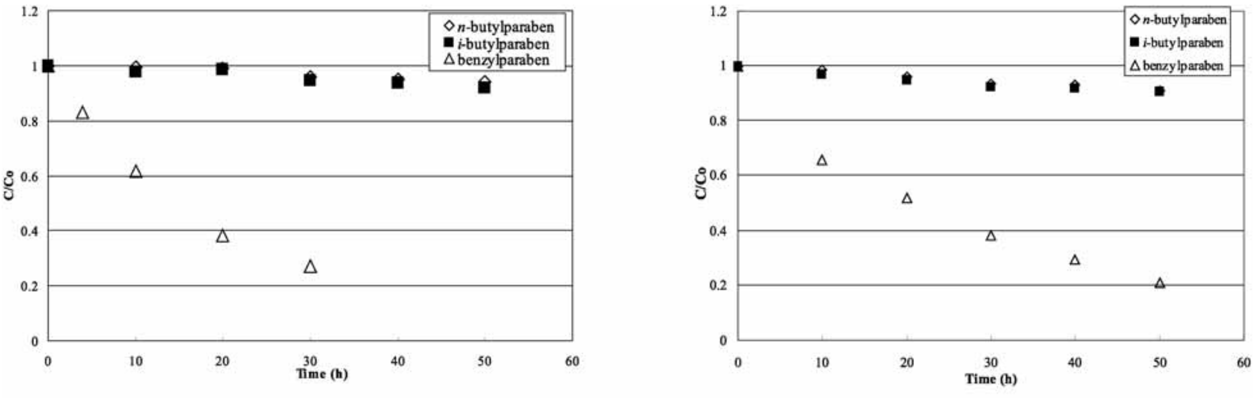
Experiments on sorption of the selected parabens into river sediments/soil were conducted in accordance with OECD Test Guideline 106.(17) The coefficients of sorption into sediment samples (K_d) were determined by simply measuring the decrease in the aquatic concentration of these parabens in 1 h for at least five different solid concentrations (0 to 10,000 mg L^{-1}) in 10 mL amber glass centrifuge tubes. The initial concentration of paraben was 100 µg L^{-1}, the pH was adjusted to 7 with phosphate buffer, and biocide (0.02 M sodium azide) was added to minimize microbial activity. Since the recoveries of the parabens in 1 h were above 90%, the effect of biodegradation was neglected. The final aqueous concentrations of the parabens were determined using the HPLC system described above.

3. Results

3.1 *Photodegradation of the selected parabens by sunlight*

The time profiles of the three parabens obtained by the two sunlight photolysis tests conducted in September 2006 and July 2007 are shown in Fig. 1, where C is the aqueous concentration of the parabens at the sampling time whereas C_o is the initial concentration. The estimated half-life, first-order reaction constant, and quantum yield are summarized in Table 2.

As can be noted from Fig. 1, the aqueous concentration of benzylparaben decreased rapidly and became less than 10% of the initial concentration at 30 h whereas those of *n*-butylparaben and *i*-butylparaben moderately decreased and remained as high as 90% of the initial concentration at 50 h for both times of collection. Therefore, the estimated half-lives of *n*-butylparaben and *i*-butylparaben were significantly longer than those of benzylparaben at 11 and 15 h for September 2006 and July 2007, respectively. The decreases in paraben concentrations were slightly faster for



(a) (b)

Fig. 1. Time profiles of paraben concentrations in the photolysis experiments conducted in (a) September 2006 and (b) July 2007.

Table 2
Results of photolysis experiments.

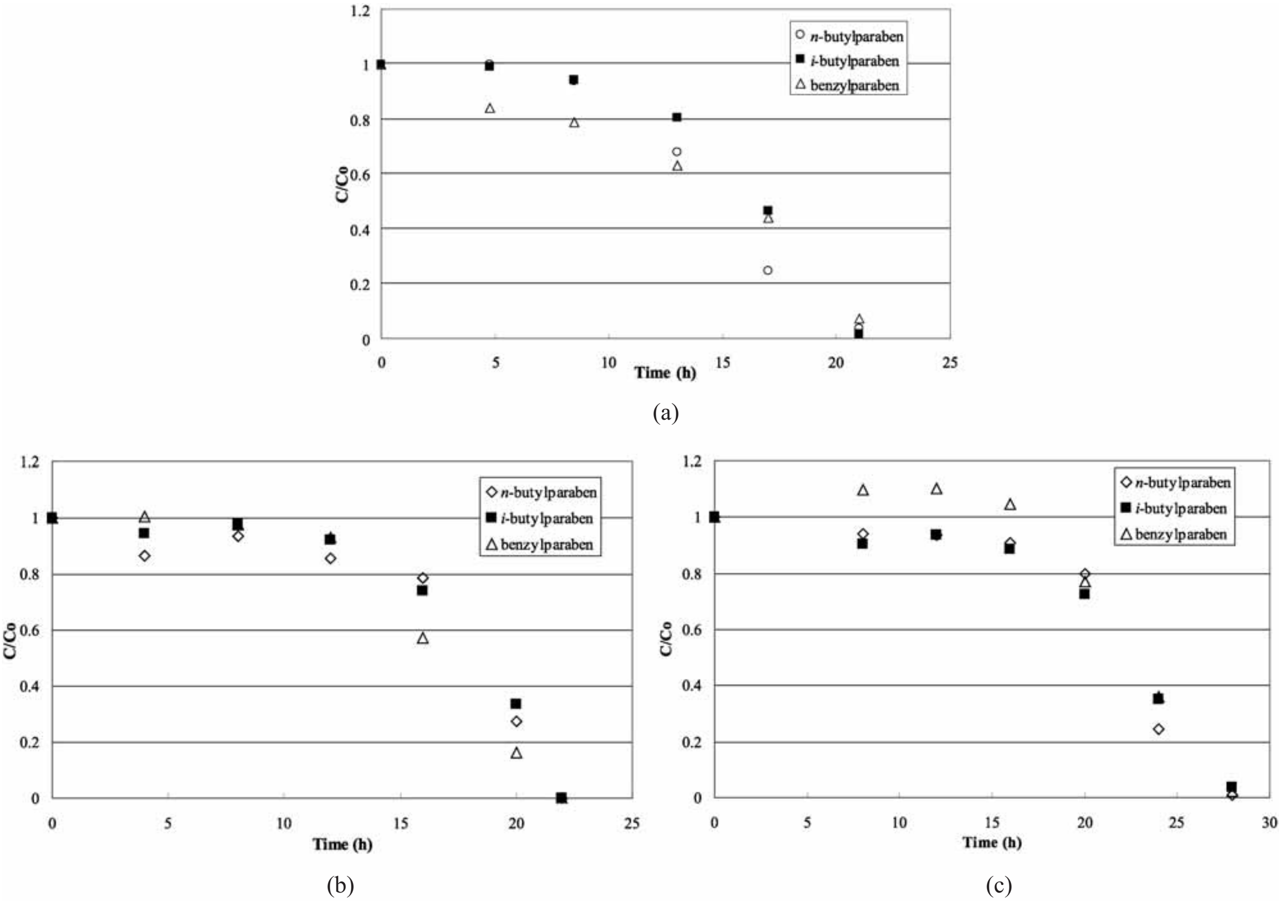
	September 2006			July 2007		
	Reaction constant (1/h)	Half-life (h)	Quantum yield	Reaction constant (1/h)	Half-life (h)	Quantum yield
n-Butylparaben	1.7×10^{-3}	410	7.6×10^{-6}	2.0×10^{-3}	350	8.6×10^{-6}
i-Butylparaben	1.2×10^{-3}	580	2.4×10^{-5}	2.0×10^{-3}	350	2.3×10^{-5}
Benzylparaben	4.0×10^{-2}	15	3.9×10^{-4}	6.5×10^{-2}	11	6.2×10^{-4}

NA: Not Available

the July test, which has a higher solar energy than September, whereas the calculated quantum yields were similar to each other. The photodegradability of benzylparaben is comparable to that of the highly photodegradable pharmaceutical propranolol.(18)

3.2 *Biodegradability of the selected parabens in river water*

The time profiles of the three parabens obtained by biodegradation tests are shown in Fig. 2, where C is the aqueous concentration of the parabens at the sampling time whereas C_o is the initial concentration as with Fig. 1. The estimated half-lives and first-order reaction constants are shown in Table 3.



(a)

(b)

(c)

Fig. 2. Time profiles of paraben concentrations in the biodegradation experiments conducted in (a) October 2006, (b) September 2007 and (c) November 2007.

Table 3
Results of biodegradation experiments.

	October 2006		September 2007		November 2007	
	Reaction constant (1/h)	Half-life (h)	Reaction constant (1/h)	Half-life (h)	Reaction constant (1/h)	Half-life (h)
n-Butylparaben	0.14	9.5 (14)	0.061	16 (17)	0.090	15 (21)
i-Butylparaben	0.15	9.6 (15)	0.045	20 (18)	0.092	15 (22)
Benzylparaben	0.10	10 (15)	0.077	14 (15)	0.069	19 (22)

Within parentheses: virtual half-lives from Fig. 2.

As can be noted from Fig. 2, it took approximately 10 h for the paraben concentration to start to decrease in all the three tests using different river waters (i.e., Tamiya River and Tsumeta River) and at different temperatures (20°C and 28°C). These time profiles apparently do not indicate a first-order reaction. The decrease was slightly faster for those exposed to a higher temperature of 28°C than those exposed to lower temperature of 20°C but the difference was not significant.

3.3 *Sorption of the selected parabens into river sediments/soil*

The coefficients of sorption of the selected parabens into the two samples of river sediments and a model soil sample are summarized and shown in Table 4. The physicochemical characteristics such as organic carbon content and cation exchange capacity (CEC) of the river sediment samples and a model soil sample are shown in Table 5.

As shown in Table 4, the sorption coefficients of benzylparaben were higher than those of the two butylparabens, and those of *n*-butylparaben and *i*-butylparaben were similar. The highest sorption coefficients were found for ESLS for all three parabens, followed by Tamiya River sediment and Akui River sediment. ESLS contained the highest organic carbon and the finest particles followed by Tamiya River sediment and Akui River sediment.

4. Discussion

The results of photolysis experiments suggest a slight photodegradation of butylparabens and a substantial photodegradation of benzylparaben. We found a relatively high molar absorptivity in the wavelengths between 297.5 and 400 nm and a high quantum yield for benzylparaben compared with *n*-butylparaben and *i*-butylparaben. The possible reason is the second benzene ring of the benzylparaben having no electron-donating or electron-accepting group, which possesses extra π-electrons that are easily excited by sunlight.(19) In our experiments, we used small quartz test tubes that were exposed to sunlight from every direction, which may overestimate the photolysis rate in natural environments such as a river. The effects of night time, cloudiness, and season should also be further investigated.

Table 4
Sorption coefficients (and organic-carbon-based sorption coefficients) of the selected parabens (±standard deviation).

	River Sediment		Elliot Silt Loam Soil
	Akui River	Tamiya River	(ESLS)
n-Butylparaben	0.19 ± 0.07 ($2.5 \pm 0.9 \times 10^2$)	3.4 ± 1.1 ($3.9 \pm 1.2 \times 10^2$)	34 ± 3 ($1.5 \pm 0.1 \times 10^3$)
i-Butylparaben	0.17 ± 0.07 ($2.3 \pm 0.9 \times 10^2$)	2.1 ± 0.5 ($2.4 \pm 0.5 \times 10^2$)	29 ± 1 ($1.3 \pm 0.0 \times 10^3$)
Benzylparaben	1.4 ± 0.9 ($1.9 \pm 1.2 \times 10^3$)	13 ± 1 ($1.5 \pm 0.2 \times 10^3$)	92 ± 9 ($4.2 \pm 0.4 \times 10^3$)
17β-Estradiol	1.7 ± 1.1 ($2.3 \pm 1.3 \times 10^3$)	11 ± 3 ($1.3 \pm 0.3 \times 10^3$)	93 ± 5 ($4.2 \pm 0.2 \times 10^3$)
Pyrene(16)	NA	$7.5 \pm 0.4 \times 10^2$ ($9.5 \pm 0.5 \times 10^4$)	$1.7 \pm 0.0 \times 10^3$ ($5.7 \pm 0.1 \times 10^4$)

Unit: L/kg (L/kgC)

Table 5
Physicochemical properties of the river sediments and soil samples used in this study.

	River Sediment		Elliot Silt Loam Soil
	Akui River	Tamiya River	(ESLS)
Organic carbon content (%)	0.075	0.87	2.2
CEC (meq/100 mL)	2	11	28
pH	6.7	6.6	6.6
Mean diameter (mm)	0.94	0.46	0.10

A lag time of approximately 10 h for the decreases in paraben concentrations to start suggests the existence of an acclimation period for microbes to the experimental conditions to metabolize or cometabolize the parabens. The effects of temperature were not clear between the September (28°C) and November (20°C) experiments. The effects of cell count, microbial species, and/or water qualities such as dissolved oxygen, carbon, nitrogen, and phosphorus levels may offset the effects of temperature. Further investigation is necessary to identify the predominant factors that control the biodegradation of parabens in river water.

In this study, we did not identify the metabolite of the three parabens, which is likely to be *p*-hydroxy benzoic acid.(20) We should attempt to track the mass balance of the system by measuring the metabolite or using radiolabeled compounds in future studies. Compared with the activated sludge process that completely removed the three parabens in 6 h,(2,11) the biodegradability of the parabens may not be very high in river water. This difference was possibly attributed to the differences in cell count, microbial species, and other water quality parameters such as concentrations of nutrients.

The half-lives of the selected three parabens in both photolysis and biodegradation experiments were higher than 10 h, which suggests the moderate persistence of these compounds in aquatic environments. For areas with no sewage service coverage, household effluents containing a relatively large amount of parabens can be released into aquatic environments without any treatment, and the parabens remain intact for as long as 10 h in these rivers and streams. A large-scale monitoring of parabens in urban rivers and streams without sewage service coverage such as the Tamiya River and Tsumeta River in Tokushima City is absolutely necessary to more accurately evaluate their fate in the environment and their ecological risk.

As partially presented above, the coefficients of sorption of the parabens into river sediments/soil were highly dependent on the organic content of sediment/soil and the hydrophobicity of the parabens (i.e., log K_{ow}), which suggests the predominant contribution of hydrophobic interaction. A linear relationship between log K_{ow} and log K_{oc} values of nonionic organic compounds has been found by several researchers since the late 1970s.(21,22) The relationship between log K_{ow} and log K_{oc} is plotted and the classical Karickhoff's empirical formula (log K_{oc} = log K_{ow}–0.21)(21) and the Chiou's empirical formula (log K_{oc} = 0.90 log K_{ow}–0.78)(22) both derived from data of polycyclic aromatic hydrocarbons and other highly hydrophobic compounds are drawn in the plot. As can be noted in Fig. 3, all the compounds were in the vicinity of both lines, and again, the hydrophobic interactions between the organic contents of sediments/soil and parabens are considered as the predominant mechanism of sorption.

Because the acidic constants (pK_a) of the three parabens are near 8.2, the pH of the water sample significantly affects the sorption at alkaline pH. If the pH of the water sample is higher than 8.2, a significant amount of ionized species decreases the hydrophobicity, and further decreases the sorption coefficients.

Several important parameters were determined to affect the fate and partitioning of benzylparaben and butylparaben in the environment. Once these parameters are determined, the next step is the construction of a mathematical model that accurately predicts the environmental concentrations of the parabens. This model should be verified by measuring of the concentrations of the parabens in the rivers and streams. Indeed, a more detailed ecological risk assessment based on the ecotoxicological data and parameters determined in this study is necessary. The extension of this work to more frequently used parabens such as methylparaben and the other personal care products should also be performed to assess the total ecological risk of parabens.

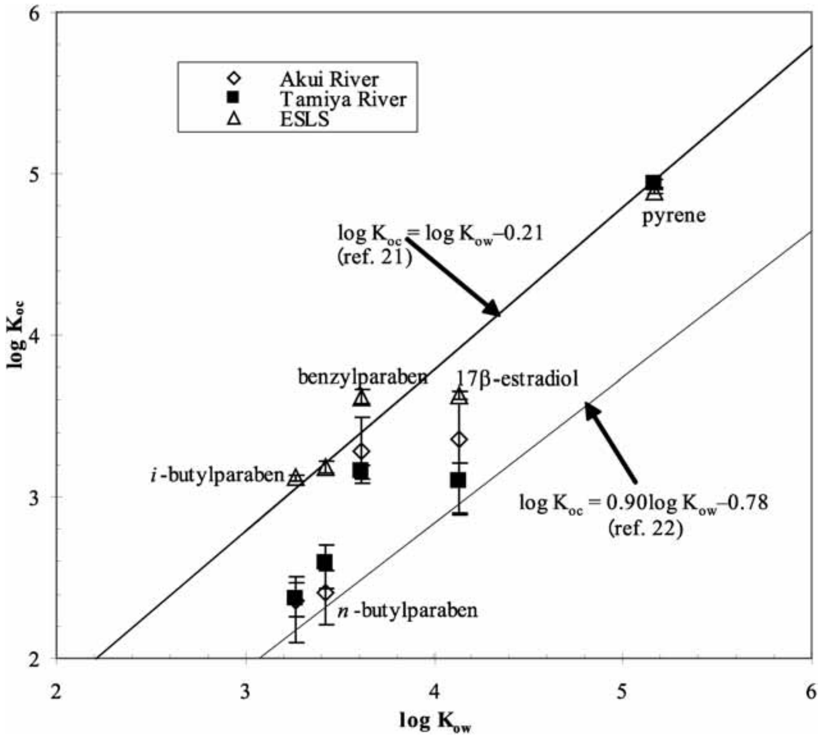


Fig. 3. Relationship between log K_{ow} and log K_{oc}.

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