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Modeling the Changes in the Concentration of Aromatic Hydrocarbons from an Oil-Coated Gravel Column

Jee-Hyun Jung[1], Hyun-Joong Kang[2], Moonkoo Kim[1], Un Hyuk Yim[1], Joon Geon An[1], Won Joon Shim[1], and Jung-Hwan Kwon[2]*

[1]*Oil and POPs Research Group, KIOST, Geoje 53201, Korea*
[2]*Division of Environmental Science & Ecological Engineering, College of Life Sciences & Biotechnology, Korea University, Seoul 02841, Korea*

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Abstract – The performance of a lab-scale flow-through exposure system designed for the evaluation of ecotoxicity due to oil spills was evaluated. The system simulates a spill event using an oil-coated gravel column through which filtered seawater is passed and flows into an aquarium containing fish embryos of olive flounder (*Paralichthys olivaceus*) and spotted sea bass (*Lateolabrax maculates*). The dissolved concentrations of individual polycyclic aromatic hydrocarbons (PAHs) in the column effluent were monitored and compared with theoretical solubilities predicted by Raoult's law. The effluent concentrations after 24 and 48 h were close to the theoretical predictions for the higher molecular weight PAHs, whereas the measured values for the lower molecular weight PAHs were lower than predicted. The ratios of the concentration of PAHs in flounder embryos to that in seawater were close to the lipid-water partition coefficients for the less hydrophobic PAHs, showing that equilibrium was attained between embryos and water. On the other hand, 48 h were insufficient to attain phase equilibrium for the more hydrophobic PAHs, indicating that the concentration in fish embryos may be lower than expected by equilibrium assumption. The results indicate that the equilibrium approach may be suitable for less hydrophobic PAHs, whereas it might overestimate the effects of more hydrophobic PAHs after oil spills because phase equilibrium in an oil-seawater-biota system is unlikely to be achieved. The ecotoxicological endpoints that were affected within a few days are likely to be influenced mainly by moderately hydrophobic components such as 3-ring PAHs.

Key words – oil spill, Raoult's law, bioconcentration, environmental exposure, embryo

*Corresponding author. E-mail: junghwankwon@korea.ac.kr

1. Introduction

Oil spills are one of the most serious environmental problems worldwide (Peterson et al. 2003; Yim et al. 2012). Spilled oil significantly disturbs the structure and function of the ecosystem and recovery takes decades (Peterson et al. 2003; Monson et al. 2011). For the qualitative and quantitative evaluations of the effects of oil spills on ecosystems, researchers have conducted both laboratory and field assessments at various biological levels (e.g. Peterson et al. 2003; Marigómez et al. 2006; Jung et al. 2011, 2012, 2013; Kim et al. 2013). The advantages of laboratory assessments over field surveys lie in that researchers can control the test conditions to obtain reproducible results. Many lab-scale studies on the assessment of the ecological impacts of oil spills have been conducted to quantify the harmful effects of oils or individual chemical species. Those studies investigated the effects of the water accommodated fraction of either crude or weathered oils (e.g. Bellas et al. 2013; Lee et al. 2013a) or the effects of single chemical species such as polycyclic aromatic hydrocarbons (PAHs) (e.g. Carson et al. 2002; Nacci et al. 2002; Barron et al. 2004; Incardona et al. 2004; Wang et al. 2009; Lee et al. 2013b) on various toxicological endpoints. However, the effect of time-course changes in the composition of the complex mixture of oils on the toxicological effects has rarely been investigated.

Petroleum oil is comprised of more than a hundred thousand chemical species including aliphatic- and aromatic-hydrocarbons and polar organic chemicals (Prince 1993). Unlike crude and refined oils, for which the chemical composition is relatively

well-characterized, changes in chemical composition caused by the weathering of spilled oils are still in need of elucidation. Spilled oils may undergo significant changes in their chemical composition. Important physical and chemical processes include volatilization, dissolution, sorption/desorption, and biotic/abiotic transformations. Volatilization into the air and dissolution from the non-aqueous oil phase into water would be the two most important processes that change the chemical composition of spilled oils during a short time after the spill. These dynamic changes may result in changes in the exposure concentrations and thus to toxicological effects on many indigenous marine species in the affected area. For example, the exposure concentration of a more volatile species with a high Henry's law constant and/or vapor pressure may decrease significantly shortly after the spill. The effects caused by these species would be due to short-term exposure. On the other hand, chemicals with low water solubility and low Henry's law constant would remain at concentrations close to their equilibrium concentrations as long as a non-aqueous residual oil phase exists after the spill. However, toxic effects have rarely been investigated under the conditions that mimic dynamic changes after oil spills.

In order to examine the changes in chemical composition of spilled oil and to assess the ecotoxic effects under the dynamic conditions relevant to an oil spill accident on a laboratory scale, an exposure system was built and applied to investigate what effect the increase or change in toxicity levels had on fish embryos. Embryos of olive flounder (*Paralichthys olivaceus*) and spotted sea bass (*Lateolabrax maculates*) were exposed to the effluent from a column containing gravel coated with oil. Iranian heavy crude oil (IHCO), which was the major component of the Hebei Spirit Oil Spill in Korea, 2007, was used as the model oil. Aromatic hydrocarbons were chosen as model chemicals. The concentrations of aromatic hydrocarbons were monitored both in the effluent and in the fish embryos at 24 and 48 h after exposure. The measured concentrations in the effluent were compared with the theoretical solubilities using Raoult's law, assuming an ideal liquid mixture. In addition, the lipid-normalized concentration ratio of chemicals between the fish embryo and the water was compared with the lipid-water partition coefficient to evaluate the degree of bioconcentration in the system.

2. Material and Methods

Chemicals

Seawater was filtered through a 0.7 mm GF/C membrane for the experiments. The water quality variables, including pH, salinity and dissolved oxygen, were monitored daily using a 555 MPS system (YSI Inc., Yellow Springs, OH, USA).

GC2-grade dichloromethane and *n*-hexane were purchased from Burdick & Jackson (Morristown, NJ, USA). ACS-grade sodium sulfate and silica gel were purchased from Fisher Scientific (Seoul, Republic of Korea). PAH surrogate standards (naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{12}, chrysene-d_{12}, perylene-d_{12}) and an internal standard (*p*-terphenyl-d_{14}) were purchased from Supelco (Bellefonte, PA, USA). The IHCO was obtained from SGS Korea Co. (Seoul, Republic of Korea) and was collected on-board before harbor unloading to prevent mixing with other crude oils. Fresh IHCO (FIHCO) was evaporated to simulate the effects of weathering (mainly volatilization) right after a spill, as described by Jokuty et al. (1999). In short, 200 mL of fresh IHCO in a 10 L flask was placed in a rotary evaporator with a water bath filled with distilled water at 80 ± 5°C. The flask was rotated at 135 rpm with an air flow of approximately 13 L min^{-1} through the flask. The oil was evaporated for 48 h to reach the relatively constant total weight loss of approximately 30%. As shown in Table 1, concentrations of individual PAHs in EIHCO did not differ greatly from those in FIHCO implying that more volatile substances were mainly lost during this laboratory weathering.

Preparation of an oil-coated gravel column and the embryo tests

Embryos of *P. olivaceus* and *L. maculates* were exposed to the effluent seawater from gravel columns containing gravel soaked with fresh or artificially evaporated IHCO (EIHCO). Those two fish species were chosen because they are of commercial value and their breeding seasons overlap with the time-period in which the Hebei Spirit oil spill occurred. The schematic diagram of the experimental setup is described in Figure 1. Approximately 30,000 embryos of each species (flounder: 1.00 mm in diameter, 0.36 mg in weight, spotted sea bass: 1.35 mm in diameter, 1.5 mg in weight) were placed in a 40 L exposure tank, receiving seawater overflow at a rate of 0.6 L h^{-1}. Artificially fertilized embryos were obtained from the Ihwasangrok and Geungyang fishery stations. The measured lipid contents of the embryos were 0.051 and 0.227 $g_{\text{lipid}}\ g^{-1}_{\text{dry weight}}$ for *P. olivaceus* and *L. maculates*, respectively, using the Bligh and Dyer method (Bligh and Dyer 1959). Gravel was coated with FIHCO or EIHCO as described earlier (Jung et al. 2013). In short, 3 g of FIHCO or EIHCO was loaded onto 2 kg of gravel (4−10 mm) by

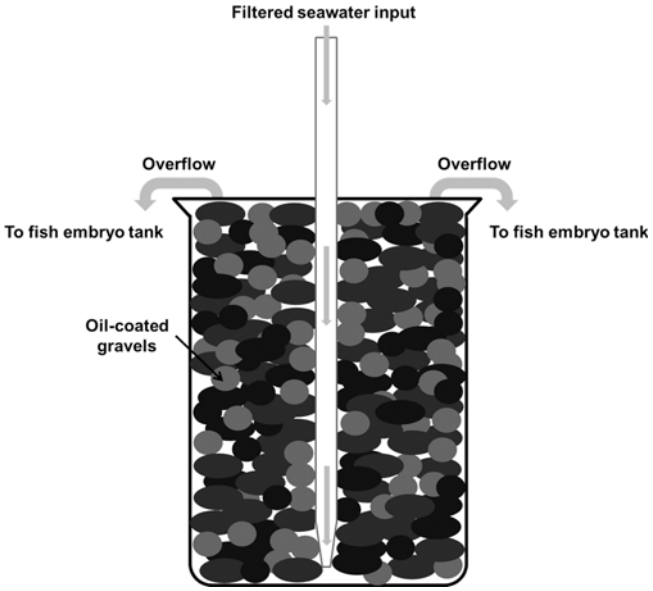

Fig. 1. Schematic diagram of the flow-through exposure system used in this study

manually shaking them together in a stainless steel container for 5 min. The generator column was filled with the oil-soaked gravel after it had been air-dried for 24 h to give it a thin oil film. The porosity was calculated by measuring pore volume of water and was 0.40. The exposure experiments were conducted in three groups: control (no oil-treated gravel column), FIHCO, and EIHCO.

Modeling the changes in concentration in the seawater and fish embryos

Dissolution of individual PAHs from the IHCO coating on the gravel surfaces can be explained by Raoult's law. Because water passed through the gravel column at a sufficiently slow velocity (0.11 m h^{-1}) relative to the surface contact area, it was assumed that the effluent water was in equilibrium with the oil coating on the gravel surfaces. Thus, the concentration of chemical species *i* in the effluent ($C_{w,i}$) can be obtained from

$$C_{w,i} = x_i S^*_{w,i} \quad (1)$$

where x_i is the mole fraction of *i* in the oil coating (that may change with time), and $S^*_{w,i}$ is the subcooled liquid solubility of *i* in seawater. The subcooled liquid solubility in seawater was estimated using Trouton's rule and the Setschenow equation as described in the literature (Yalkowsky 1979; Kang et al. 2014) for FIHCO and EIHCO. Although the fish embryo tests were conducted at 16°C, solubilities and all other thermodynamic data at 25°C were used because of the availability of data. The detailed values for all the selected chemicals are listed in Table 1. Because the number average molecular weight (MW_n) for FIHCO and EIHCO was not known, the MW_n for West Texas crude oil (estimated to be 375 g mol^{-1}) was used, as in previous studies (Page et al. 2000; Kang et al. 2014). Although MW_n would increase after evaporation because lighter components tend to volatilize more easily, the same value of MW_n was used because the effects of increased MW_n do not result in large differences in the mole fraction.

Using a generic one-compartment model, the concentration of the chemical species *i* in the fish embryo ($C_{embryo,i}$) is represented during the uptake phase by

$$C_{embryo,i} = \frac{k_u}{k_e}(1 - \exp(-k_e t))C_{w,i} \quad (2)$$

where k_u and k_e are the uptake and elimination rate constants. Without the existence of metabolic transformation of PAHs in the embryos, the value of k_u/k_e should be close to the lipid-water partition coefficient (K_{lipw}) when the rate constants are normalized by the lipid content. For the hydrophobic organic chemicals such as the PAHs used in this study, the uptake rate constant is almost constant regardless of the type of chemical because the overall mass transport to aquatic organisms including fish embryos is limited by diffusion in the water boundary layer (Gobas et al. 1986; Sijm and van der Linde 1995; Kwon et al. 2006). Thus, the factor that determines the overall equilibration between the water and the embryos is the elimination rate constant, which depends on the size of the organism and the hydrophobicity of the chemicals. Because the embryo size of each species is considered to be the same, a longer equilibration time is expected with increasing hydrophobicity (i.e. K_{lipw}) of the PAHs.

Chemical analyses

All of the 16 priority PAHs listed by the US EPA, except for naphthalene, as well as selected alkyl-substituted PAHs were analyzed. They were (C1–C4) naphthalene, acenaphthylene, acenaphthene, (C0–C3) fluorene, (C0–C4) phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, (C0–C3) chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo [*a*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenz[*a*,*h*]anthracene, benzo[*ghi*]perylene, and (C0–C3) dibenzothiophene. The concentrations of these chemicals in FIHCO and EIHCO were determined using the extraction and fractionation method

Table 1. Physical and chemical properties of selected hydrocarbons used for the evaluation of dissolution and uptake by fish embryos in this study

Compound	Molar mass (g mol^{-1})	T_m (°C)[a]	ΔS_m (J mol^{-1} K^{-1})[b]	K^s (L mol^{-1})[c]	Aqueous solubility (mg L^{-1}, at 25°C)			log K_{ow}	log K_{lipw}	Concentration in FIHCO (μg g^{-1})	Mole fraction in FIHCO	Predicted concentration in effluent for FIHCO (ng L^{-1})	Concentration in EIHCO (μg g^{-1})	Mole fraction in EIHCO	Predicted concentration in effluent for EIHCO (ng L^{-1})
					S	S^L	$S_{sw}{}^L$								
Acenaphthylene	152.19	92	42.4	0.35	16[d]	50	34	3.67[e]	3.83[f]	0.96	2.4×10^{-6}	80	0.81	2.0×10^{-6}	68
Acenaphthene	154.21	93	58.6	0.24[g]	4.2[d]	21	16	3.92[h]	4.08[f]	6.1	1.5×10^{-5}	240	7.1	1.7×10^{-5}	280
Fluorene	166.22	115	50.5	0.27[g]	1.6[i]	9.8	7.2	4.18[h]	4.34[f]	49	1.1×10^{-4}	800	48	1.1×10^{-4}	780
Phenanthrene	178.23	99	44.8	0.38	0.82[i]	3.1	2.0	4.52[h]	5.05[j]	90	1.9×10^{-4}	390	120	2.5×10^{-4}	500
Anthracene	178.23	216	60.1	0.35	0.044[i]	4.5	3.0	4.50[h]	5.28[j]	8.6	1.8×10^{-5}	54	11	2.3×10^{-5}	68
Fluoranthene	202.25	110	48.9	0.36	0.20[d]	1.1	0.70	5.20[h]	5.62[j]	0.66	1.2×10^{-6}	0.86	0.82	1.5×10^{-6}	1.1
Pyrene	202.25	151	43.4	0.35	0.086[i]	0.77	0.52	5.00[h]	5.74[j]	8.0	1.5×10^{-5}	7.7	6.6	1.2×10^{-5}	6.3
Benz[*a*]anthracene	228.29	161	49.2	0.36	0.017[d]	0.25	0.16	5.91[h]	6.46[j]	4.0	6.6×10^{-6}	1.1	5.1	8.4×10^{-6}	1.4
Chrysene	228.29	256	55.5	0.37	0.00070[i]	0.12	0.080	5.86[h]	6.46[j]	17	2.8×10^{-5}	2.2	22	3.7×10^{-5}	2.9
Benzo[*b*]fluoranthene	252.31	168	56.5[e]	0.35	0.0015[k]	0.039	0.026	5.78[h]	7.21[j]	1.6	2.3×10^{-6}	0.060	2.3	3.5×10^{-6}	0.091
Benzo[*k*]fluoranthene	252.31	217	56.6[e]	0.35	0.00080[k]	0.064	0.043	6.11[h]	7.22[j]	0.29	4.3×10^{-7}	0.018	0.46	6.9×10^{-7}	0.030
Benzo[*a*]pyrene	252.31	181	42.4	0.35	0.0015[i]	0.021	0.014	6.35[h]	7.35[j]	1.7	2.5×10^{-6}	0.036	3.2	4.8×10^{-6}	0.067
Indeno[*1,2,3-cd*]pyrene	276.33	162	49.4	0.35	0.00019[k]	0.0029	0.0020	6.72[h]	7.95[j]	0.76	1.0×10^{-6}	0.0020	0.58	7.8×10^{-7}	0.0015
Dibenz[*a,h*]anthracene	278.35	270	58.3	0.34	0.0025[l]	0.78	0.53	6.75[h]	7.77[j]	1.0	1.4×10^{-6}	0.75	0.84	1.1×10^{-6}	0.60
Benzo[*ghi*]perylene	276.33	273	31.3	0.29	0.00014[k]	0.0032	0.0023	6.90[h]	7.97[j]	1.5	2.0×10^{-6}	0.0046	1.2	1.6×10^{-6}	0.0036
Dibenzothiophene	184.26	98	56.4[m]	0.35	1.5[l]	7.8	5.2	4.38[h]	4.54[f]	210	4.2×10^{-4}	2200	260	5.4×10^{-4}	2800
1-Methylnaphthalene	142.20	-30	49.3	0.44[g]	32[n]	32	19	3.87[h]	4.03[f]	720	1.9×10^{-3}	36000	390	1.0×10^{-3}	20000
2-Methylnaphthalene	142.20	35	58.9	0.35	22[i]	27	18	4.00[h]	4.16[f]	890	2.3×10^{-3}	42000	420	1.1×10^{-3}	20000
C2-Naphthalene	156.22	112	65.4	0.35	1.0[o]	9.9	6.6	4.31[h]	4.47[f]	2200	5.3×10^{-3}	35000	2100	5.0×10^{-3}	33000
C3-Naphthalene	170.25	63	56.5[p]	0.35	2.1[q]	5.0	3.3	4.90[h]	5.07[f]	2400	5.2×10^{-3}	17000	2500	5.5×10^{-3}	18000
C4-Naphthalene	184.28	72[r]	56.5[p]	0.35	1.4[s]	4.1	2.7	4.74[t]	4.91[f]	1100	2.2×10^{-3}	6000	1100	2.3×10^{-3}	6200
C1-Fluorene	180.25	87	56.5[p]	0.35	1.1[u]	4.5	3.0	4.97[h]	5.14[f]	140	3.0×10^{-4}	890	150	3.0×10^{-4}	900
C2-Fluorene	194.27	101[r]	56.5[p]	0.35	0.15[s]	0.83	0.56	5.13[v]	5.30[f]	230	4.5×10^{-4}	250	240	4.6×10^{-4}	260
C3-Fluorene	208.30	94[r]	56.5[p]	0.35	0.10[s]	0.50	0.33	5.61[v]	5.79[f]	330	5.9×10^{-4}	200	340	6.1×10^{-4}	200
1-Methylphenanthrene	192.26	123	56.5[p]	0.35	0.27[w]	2.5	1.7	5.08[h]	5.25[f]	73	1.4×10^{-4}	240	90	1.8×10^{-4}	300
2-Methylphenanthrene	192.26	94[r]	56.5[p]	0.35	0.28[x]	1.3	0.89	5.24[h]	5.41[f]	83	1.6×10^{-4}	140	100	2.0×10^{-4}	180
3-Methylphenanthrene	192.26	65	56.5[p]	0.35	0.28[x]	0.70	0.47	5.15[h]	5.32[f]	69	1.4×10^{-4}	63	89	1.7×10^{-4}	81
4/9-Methylphenanthrene	192.26	54	56.5[p]	0.35	0.27[y]	0.52	0.34	5.08[v]	5.25[f]	120	2.4×10^{-4}	83	150	2.8×10^{-4}	98
C2-Phenanthrene	206.28	109[r]	56.5[p]	0.35	0.071[s]	0.48	0.32	5.44[v]	5.61[f]	470	8.5×10^{-4}	270	570	1.0×10^{-3}	330
C3-Phenanthrene	220.31	116[r]	56.5[p]	0.35	0.021[s]	0.16	0.11	5.79[v]	5.97[f]	400	6.8×10^{-4}	74	490	8.3×10^{-4}	90
C4-Phenanthrene	234.34	128[r]	56.5[p]	0.35	0.0059[s]	0.062	0.041	6.07[v]	6.25[f]	220	3.6×10^{-4}	15	270	4.4×10^{-4}	18
1-Methyldibenzothiophene	198.28	112[r]	56.5[p]	0.35	0.33[s]	2.4	1.6	4.70[v]	4.87[f]	150	2.8×10^{-4}	440	190	3.5×10^{-4}	560
2-/3-Methyldibenzothiophene	198.28	112[r]	56.5[p]	0.35	0.33[s]	2.4	1.6	4.70[v]	4.87[f]	250	4.7×10^{-4}	750	320	6.0×10^{-4}	960
4-Methyldibenzothiophene	198.28	112[r]	56.5[p]	0.35	0.33[s]	2.4	1.6	4.69[v]	4.86[f]	320	6.0×10^{-4}	960	400	7.7×10^{-4}	1200
C2-Dibenzothiophene	212.31	117[r]	56.5[p]	0.35	0.095[s]	0.77	0.52	4.88[v]	5.05[f]	1300	2.3×10^{-3}	1200	1700	3.1×10^{-3}	1600
C3-Dibenzothiophene	226.34	128[r]	56.5[p]	0.35	0.027[s]	0.29	0.19	5.55[v]	5.73[f]	1100	1.9×10^{-3}	360	1400	2.4×10^{-3}	460
C1-Chrysene	242.32	173	56.5[p]	0.35	0.013[s]	0.39	0.26	6.11[v]	6.29[f]	39	6.1×10^{-5}	16	50	7.7×10^{-5}	20
C2-Chrysene	256.34	154[r]	56.5[p]	0.35	0.0037[s]	0.070	0.047	6.36[v]	6.54[f]	58	8.4×10^{-5}	4.0	65	9.4×10^{-5}	4.4
C3-Chrysene	270.37	165[r]	56.5[p]	0.35	0.0011[s]	0.026	0.017	6.56[v]	6.75[f]	51	7.0×10^{-5}	1.2	62	8.6×10^{-5}	1.5

[a]Lide, 2010. [b]Chickos et al. 1999. [c]Jonker and Muijs 2010. [d]Walters and Luthy 1984. [e]Paasivirta et al. 1999 [f]Endo et al. 2011. [g]Xie et al. 1997. [h]Suggested values by Sangster Research Laboratory. [i]Kwon and Kwon 2012. [j]van der Heijden and Jonker 2009. [k]Wise et al. 1981 [l]Means et al. 1980 [m]Coon et al. 1988. [n]Wasik et al. 1983. [o]Vadas et al. 1991. [p]Estimated value using Walden's rule, Schüürmann et al. 2008. [q]Mackay and Shiu 1977. [r]Estimated value using MPBPVP v1.43. [s]Estimated value using WSKOW v1.42. [t]Dimitriou-Christidis et al. 2003. [u]Miller and Wasik 1985. [v]Estimated value using ALOGPS 2.1 Program. [w]May and Wasik 1978. [x]Isnard and Lambert 1989. [y]Yalkowsky and Dannenfelser 1992.

described in the literature (Wang et al. 1995; Yim et al. 2011; Kang et al. 2014).

The concentrations of these chemicals in the seawater and the embryos were measured at 24 and 48 h after the exposure. The effluent water (2 L) collected at the outlet of the embryo tank was serially extracted three times using 50 mL of dichloromethane in a separator funnel. The fish embryos were pooled to approximately 0.2–1.0 g dry weight and the PAHs were extracted in a Soxhlet extractor for 16 h with 200 mL of dichloromethane. Both the water and embryo samples were spiked with surrogate standards (naphthalene-d_8, acenaphthene-d_{10}, dibenzothiophene-d_8, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12}) before extraction. The concentrated extracts were purified using an alumina/silica gel (Al/Si, 10g/20g) chromatographic column (300 × 13 mm i.d.). Alumina oxide (~150 mesh) was activated by heating at 400°C for 4 hours and then deactivated with HPLC grade water (1%, w/w). Silica gel (70 ~ 230 mesh) was activated by heating at 170°C for 12 hours and deactivated with HPLC grade water (5%, w/w). The aromatic hydrocarbon fraction was eluted from the column with 100 mL of dichloromethane. The fraction was further purified by a gel permeation chromatography (GPC) using a high performance liquid chromatography (HPLC) equipped with two size exclusion columns (22.5 × 250 mm, Phenomenex Phenogel 100Å). Dichloromethane was used as the mobile phase at a flow rate of 7 mL min^{-1}. The eluted samples were concentrated and re-dissolved in *n*-hexane, followed by the addition of terphenyl-d_{14} as an internal standard. Individual PAHs were quantified using a gas chromatograph (Hewlett–Packard HP6890) coupled with a quadrupole mass spectrometer (Hewlett–Packard HP5972) using the selected ion monitoring described in the earlier literature (Yim et al. 2011).

For quality control and quality assurance, each set of samples was accompanied by a procedural blank, a matrix spike, and a duplicate sample. The concentrations in the procedural blank were lower than the method detection limit and ranged from 0.13 to 3.36 ng/g for biota and 0.09 to 5.67 ng/L for seawater. Recoveries in matrix spikes were 80 to 110%. Relative percent differences of duplicate samples ranged from 0.19 to 19% for biota and from 0.13 to 21% for seawater. A CRM (Certified Reference Material) (NIST 1947, Lake Michigan fish tissue, National Institute of Standards and Technology, Gaithersburg, USA) was analyzed as an additional quality assurance check. The concentrations of parent PAHs were within 25% of the CRM certified concentrations. Recoveries of surrogate standards in samples ranged from 47% to 82%. All reported concentrations were adjusted for the recoveries.

3. Results and Discussion

Concentrations of the individual PAHs in the seawater and in the fish embryos

Table 2 summarizes all the concentrations of the PAHs measured in seawater and in fish embryos at 24 and 48 h after the experiment started for FIHCO and EIHCO, respectively. It should be noted that the filtered seawater (control) samples contain background level of PAHs. The concentrations of low molecular weight PAHs significantly decreased after 48 h due to their relatively rapid dissolution into the seawater from the oil coating on the gravel. For example, the effluent concentration of fluorene at 48 h after the exposure was 34 ng L^{-1} whereas it was 220 ng L^{-1} at 24 h after the exposure when FIHCO was used. On the other hand, the decrease in the concentration was not as noticeable with regard to high molecular weight PAHs. For example, the effluent concentration of chrysene was 3.6 and 2.6 ng L^{-1} at 24 and 48 h, respectively, when using FIHCO. As shown in Table 1, the contents of fluorene and chrysene in FIHCO were 49 and 17 mg g^{-1}. On the other hand, the water solubility of fluorene (1.6 mg L^{-1}) is much greater than that of chrysene (0.0007 mg L^{-1}), by a factor of more than a thousand (Kwon and Kwon 2012). The observed differences in the effluent concentrations during the test could be explained by the rapid depletion of the low molecular weight PAHs with relatively high water solubilities and/or the rapid uptake by fish embryos.

The changes in the concentration of PAHs in the fish embryos fluctuated less than that in the effluent. In contrast to the rapid equilibrium between the oil coating and the water in the generator column, the concentration in the fish embryos is determined by the uptake and elimination kinetic processes represented in equation 2. While the effluent concentration decreased, the embryos were likely to take up chemicals from the water until the concentration ratio (C_{embryo}/C_w) reached the bioconcentration factor. Although there are limited data, the values in Table 2 support this explanation.

Comparison with the model results

Figure 2 shows a comparison between the maximum solubility of each chemical species in seawater using Raoult's law (the values are also shown in Table 1) and the measured concentration in the seawater effluent for (a) FIHCO and (b) EIHCO, respectively. As shown, the measured concentrations were generally within

Table 2. Concentration of test chemicals measured in the effluent water and fish embryos using FIHCO and EIHCO

Compound	Concentration in the effluent (ng L^{-1})						Concentration in embryo (ng g^{-1} lipid weight)											
							Lateolabrax maculates						*Paralichthys olivaceus*					
	24 h			48 h			24 h			48 h			24 h			48 h		
	Control	FIHCO	EIHCO	Control	FIHCO	EIHCO	Control	FIHCO	EIHCO	Control	FIHCO	EIHCO	Control	FIHCO	EIHCO	Control	FIHCO	EIHCO
Acenaphthylene	0.48	0.45	0.25	0.40	0.31	0.33	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Acenaphthene	0.82	23	22	1.3	2.7	4.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fluorene	8.5	220	230	7.3	34	60	120	330	420	67	450	680	ND	1700	5100	380	970	1800
Phenanthrene	9.3	300	300	5.2	92	150	980	2500	2600	520	2700	3900	2000	12000	31000	5800	8000	13000
Anthracene	1.7	14	16	0.58	4.7	6.1	44	83	72	24	73	77	120	300	780	ND	260	400
Fluoranthene	1.4	1.8	1.3	0.51	0.91	1.1	59	73	50	41	60	74	180	230	430	170	140	210
Pyrene	0.93	4.9	5.0	0.35	2.9	4.3	80	140	130	65	130	190	210	490	1200	320	380	480
Benz[*a*]anthracene	0.52	1.2	1.1	0.33	0.52	0.63	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Chrysene	1.3	3.6	4.2	0.18	2.6	3.3	50	97	130	33	94	140	150	300	580	130	280	350
Benzo[*b*]fluoranthene	ND	ND	ND	ND	0.23	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Benzo[*k*]fluoranthene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Benzo[*a*]pyrene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Indeno[*1,2,3-cd*]pyrene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dibenz[*a,h*]anthracene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Benzo[*ghi*]perylene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dibenzothiophene	2.9	760	720	2.0	270	390	330	3600	4000	400	4800	6700	1000	18000	54000	1300	13000	24000
1-Methylnaphthalene	13	1500	830	4.1	180	84	250	380	350	34	380	250	720	1100	1200	760	630	510
2-Methylnaphthalene	14	1400	690	5.5	180	82	450	550	520	95	320	290	990	1400	1800	1000	720	770
C2-Naphthalene	22	2200	1700	19	330	450	2300	6100	7400	450	9800	12000	5400	33000	51000	6500	22000	28000
C3-Naphthalene	24	2600	2100	25	840	1400	2400	7500	7200	940	16000	16000	4600	56000	120000	6000	46000	67000
C4-Naphthalene	18	790	670	8.8	400	660	4500	3500	5200	970	6900	7300	6100	24000	70000	5100	25000	33000
C1-Fluorene	21	1400	1400	22	440	850	ND	2400	2800	440	2800	3700	ND	10000	36000	2700	6800	12000
C2-Fluorene	2.9	230	260	6.4	120	210	ND	1700	1800	570	2600	2500	ND	9600	34000	2600	7800	10000
C3-Fluorene	5.1	220	370	11	100	150	ND	ND	ND	760	1200	1100	ND	6900	27000	ND	3900	5600
1-Methylphenanthrene	0.53	360	310	0.45	180	260	250	1100	1100	330	1400	1900	850	7700	20000	1300	4800	7700
2-Methylphenanthrene	1.2	300	270	0.70	170	260	300	1000	1100	390	1400	1700	960	6900	18000	1600	4400	7100
3-Methylphenanthrene	0.83	290	280	0.51	140	220	310	820	1000	280	1000	1400	720	5400	14000	1000	3200	5000
4/9-Methylphenanthrene	0.78	530	480	0.39	310	440	510	2100	2100	610	3000	3400	1400	12000	30000	2400	9700	14000
C2-Phenanthrene	4.7	240	280	4.7	140	170	960	2400	2700	940	2700	3700	3200	13000	30000	5100	9300	11000
C3-Phenanthrene	3.5	160	190	ND	54	56	590	1100	1200	980	1900	1900	2800	4300	7800	4800	4600	5700
C4-Phenanthrene	1.9	58	84	ND	13	22	ND	ND	580	300	590	460	1100	1400	1600	960	830	1600
1-Methyldibenzothiophene	0.87	710	660	0.55	560	690	590	2700	2700	670	4000	4900	1600	18000	44000	2600	16000	23000
2-/3-Methyldibenzothiophene	1.2	1000	940	0.77	750	980	710	3600	3900	880	5000	6500	2200	26000	67000	3200	19000	30000
4-Methyldibenzothiophene	1.5	1200	1000	1.2	1000	1200	830	4400	4600	1100	6900	8000	2500	30000	76000	3900	27000	39000
C2-Dibenzothiophene	5.6	450	470	ND	390	460	1500	5300	6000	1500	6900	8300	4500	41000	94000	6600	29000	37000
C3-Dibenzothiophene	2.2	230	270	ND	99	120	500	1400	1700	470	1500	2100	1600	7900	16000	2200	6800	7200
C1-Chrysene	0.32	3.3	4.2	ND	1.7	2.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C2-Chrysene	ND	1.4	2.6	ND	0.68	0.76	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C3-Chrysene	ND	ND	0.92	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND: not determined

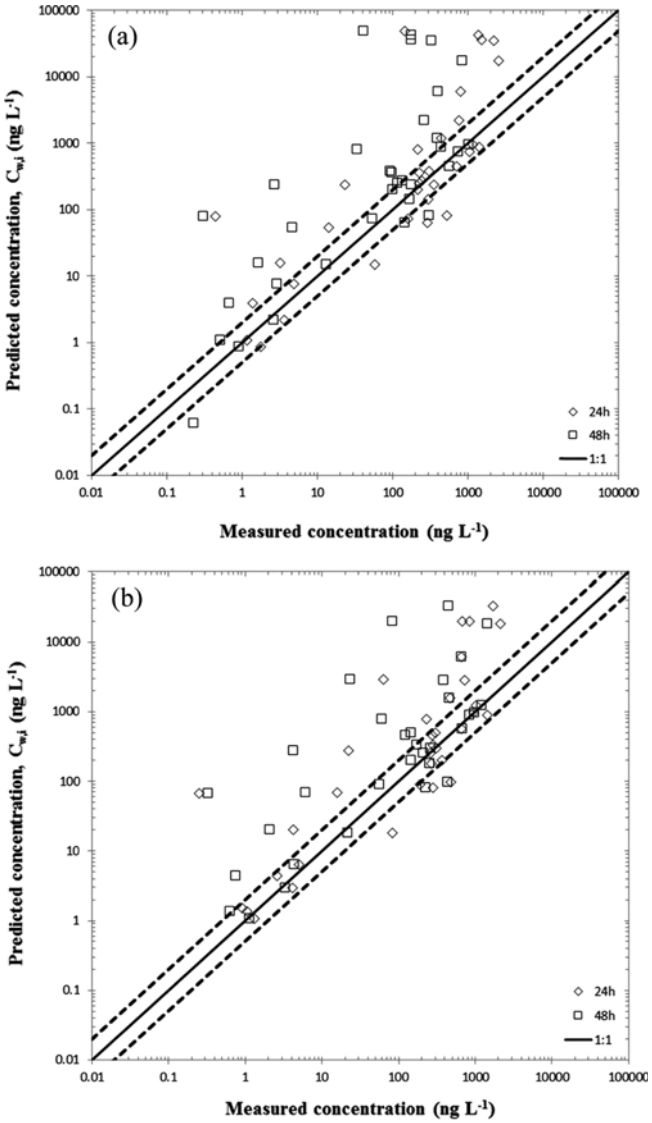

Fig. 2. Comparison of the concentrations monitored at 24 h (diamonds) and 48 h (squares) after the exposure with theoretical solubilities estimated using Raoult's law for (a) FIHCO and (b) EIHCO. Solid lines represent 1:1 relationships and dashed lines represent 10:1 and 1:10 lines

an order of magnitude of those predicted by Raoult's law, especially for values measured 24 h after the exposure. The concentrations in the effluent were in general lower than the predicted maximum concentrations for the effluent samples 48 h after exposure. Although complete mass balance for individual PAHs was not assessed, this decrease might be caused by (1) the depletion of the PAHs from the oil coating, (2) the decreased desorption rate from the oil coating, (3) evaporation of dissolved PAHs to air, and (4) the decreased concentration of dissolved PAHs by absorption by fish embryos.

Figure 3 shows the logarithm of the ratios of the concentrations in the fish embryos to those in the seawater (C_{embryo}/C_w) in two experiments using FIHCO ((a) and (c)) and EIHCO ((b) and (d)) with respect to the lipid-water partition coefficients (K_{lipw}) of PAHs. The ratio was greater after 48 h than after 24 h for most of the chemicals. As mentioned previously, this is probably due to the decrease in the concentration in the effluent and the increase in the concentration in the fish embryos. Interestingly, for the flounder embryos the values of log (C_{embryo}/C_w) for all PAHs with log $K_{lipw} < 5$ were close to the values of log K_{lipw}, suggesting that equilibrium was attained between the embryos and the seawater within the duration of the toxicity test. However, the ratios for the sea bass embryos were approximately an order-of-magnitude lower than those for the flounder embryos, although a similar tendency for the ratios to increase with increasing log K_{lipw} values was observed. Because the embryo sizes for the two species were not significantly different, this difference is not likely to be due to the difference in the uptake rate constant (k_u) that depends on organism size. It would be due to the higher transformation rate of PAHs in the sea bass embryos. The higher biotransformation activities (CYP1A expression levels) in the embryonic sea bass implies that a greater rate of elimination takes place, which leads to low residual concentrations of the PAHs in the embryos (Jung et al. 2015). On the other hand, 48 h was insufficient to attain phase equilibrium for the more hydrophobic PAHs with log $K_{lipw} > 5$. This suggests that the concentration in fish embryos may be lower than expected from the equilibrium assumption that is often used for the evaluation of the ecological impacts of oil spills. This would be important when sensitive effects need to be taken into account, such as during the hatching period after a spill.

Figure 4 describes the theoretical uptake kinetics of two model PAHs with different hydrophobicities (phenanthrene and benzo[*a*]pyrene) by fish embryos to illustrate kinetics. The uptake and elimination rate constants were estimated, based on the assumption that the overall mass transfer is limited by diffusion in the water boundary layer. The estimated aqueous diffusivities were 6.81×10^{-10} and 5.32×10^{-10} m^2 s^{-1} for phenanthrene and benzo[*a*]pyrene, respectively (Lee et al. 2012). The typical size of a fish embryo was in the order of 10^{-6} m. The thickness of the water boundary layer around the embryo was estimated to be 10^{-3} m under static conditions (Kwon et al. 2006). The uptake and elimination rate constants were estimated using the film diffusion model (Gobas et al. 1986; Kwon et al. 2006). The uptake rate constants were estimated to be 5.9 ×

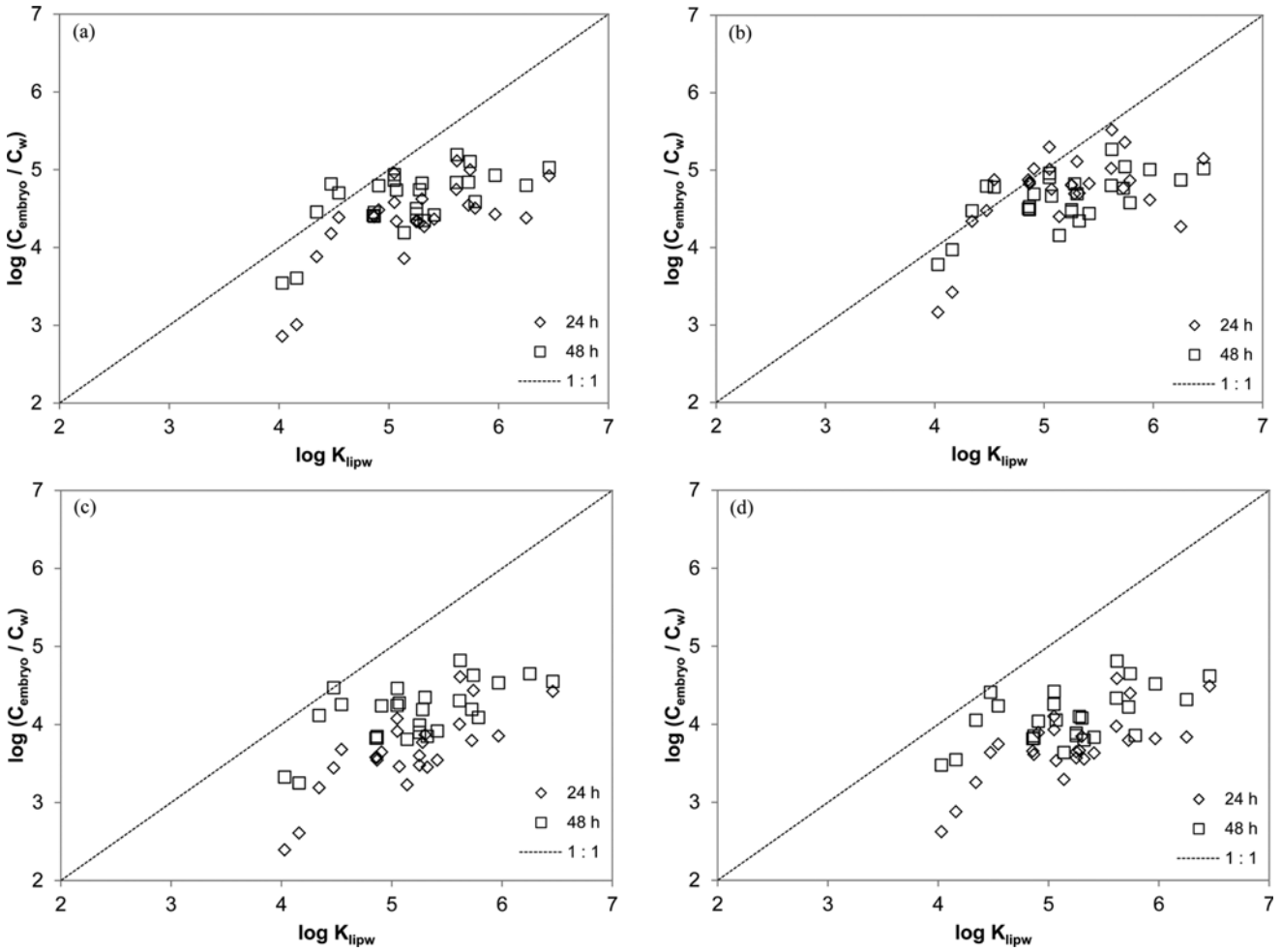

Fig. 3. Ratios of concentration in fish embryos to that in water (in log C_{embryo}/C_w) with respected to chemical's lipid-water partition coefficient (log K_{lipw}) in the literature for (a) FIHCO and (b) EIHCO with olive flounder (*P. olivaceus*), and (c) FIHCO and (d) EIHCO with spotted sea bass (*L. maculates*). The dashed line represents a 1:1 relationship

10^4 and 4.6×10^4 L kg^{-1} h^{-1}, and the elimination rate constants were estimated to be 0.52 and 0.0021 h^{-1}, for phenanthrene and benzo[*a*]pyrene, respectively. The degree of equilibration was represented by $(C_{embryo}/C_w) / (k_u/k_e)$ to compare two compounds with different concentration scales. Whereas 24 h was sufficient to attain equilibrium between fish embryos and seawater for phenanthrene, the expected value of C_{embryo}/C_w was less than 10% of the value of K_{lipw} (or k_u/k_e) for benzo[*a*]pyrene. This theoretical model analysis supports lower values of C_{embryo}/C_w for the more hydrophobic PAHs.

Implications for environmentally-relevant exposure

Although oil spills are episodic environmental disasters in which the exposure concentration may significantly change during important biological periods such as the spawning of fish embryos, most studies on the ecotoxicity of oils have been conducted without considering the time-course changes in the oil composition after the spill. As discussed previously, during the spawning time of fish embryos, the equilibrium assumption is only valid for less hydrophobic PAHs, whereas it cannot be used for more hydrophobic PAHs with log $K_{lipw} > 5$. Thus, the uptake and elimination kinetics should be considered for the evaluation of the adverse effects of highly hydrophobic components as an individual chemical or from complex mixtures. Without considering the significant decrease in the aqueous concentrations of low molecular weight PAHs, the potential toxic effects of oil spills may be exaggerated. On the other hand, the time required for equilibrium may be much longer for more hydrophobic components, hence the internal concentration may be much lower than expected from equilibrium partitioning during an important biological stage such as spawning.

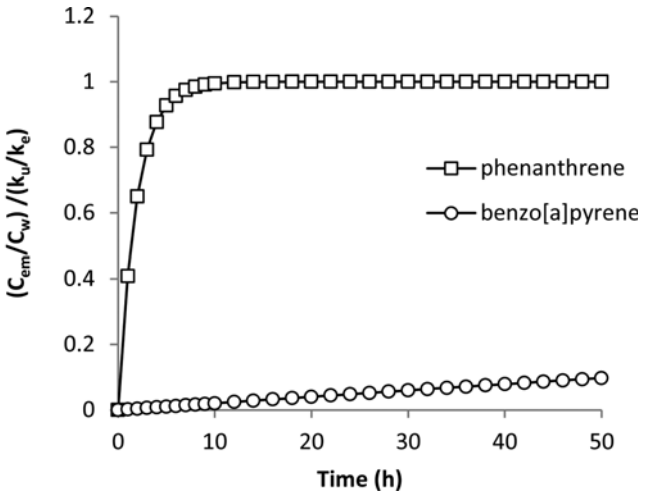

Fig. 4. Modeled changes in the concentration ratio (C_{embryo}/C_w) with exposure time. In order to compare phenanthrene and benzo[*a*]pyrene in one chart, the concentration ratio was normalized by the kinetic bioconcentration factor (k_u/k_e), assuming that no metabolic transformation took place

4. Conclusions

A flow-through exposure system was constructed and its performance was evaluated for determining the developmental toxicity caused by spilled oils in marine fish embryos, with the aim of improving the ability to study oil spills using laboratory scale equipment. The decrease in the effluent concentration was more significant for the less hydrophobic PAHs than for the more hydrophobic PAHs due to depletion of the oil coating, evaporation, and absorption by embryos. The equilibrium assumption was valid for the concentration ratios (C_{embryo}/C_w) of the less hydrophobic PAHs, whereas the kinetic limitation may be important for the more hydrophobic PAHs within the limited duration of the hatching period. Thus, the evaluation of ecotoxicological endpoints using the exposure system introduced in this study would enable more realistic simulations of the conditions that exist after oil spills, reflecting the fluctuating concentrations of the toxic chemicals although it is not possible to standardize field conditions. The dynamic exposure system could be also applied to benthic species after refinement.

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