Table 5. Histopathological findings in the combined repeated dose toxicity study with reproduction/developmental toxicity screening test for PFHxDA in rats.

Dose (mg/kg/day)		At the end of the administration period (Main group)				At the end of the recovery period (Recovery group)	
		0	4	20	100	0	100
MALES							
Number of examined animals		7	12	12	7	5	5
Liver							
- Centrilobular hypertrophy of hepatocytes	+	0	0	5	0 ┐**	0	5**
	++	0	0	0	7 ┘	0	0
- Centrilobular fatty change	+	0	0	2	7**	0	1
FEMALES							
Number of examined animals		12	12	12	12	5	5
Liver							
- Centrilobular hypertrophy of hepatocytes	+	0	0	0	8**	0	1

Values represent the number of animals with findings.
+: Slight change, ++: moderate change
**: Significantly different from the control group at $P \leq 0.01$.
Brackets in the data columns mean that statistical analysis was performed for a total number of animals with findings in consideration of grades.

Table 6. Reproductive/developmental findings in the combined repeated dose toxicity study with the reproduction/developmental screening test for PFHxDA in rats.

Dose (mg/kg/day)		0	4	20	100
Incidence of females with normal estrous cycle[a] (%)		100	100	100	100
Estrous cycle length[a, b] (days)		4.11 ± 0.22	4.18 ± 0.32	4.03 ± 0.09	4.00 ± 0.00
Number of cohabited pairs		12	12	12	12
Coupulation index (%)	Males	100	100	100	100
	Females	100	100	100	100
Fertility index (%)		91.7	100	100	100
Gestation index (%)		100	100	100	100
Gestation length[b] (days)		22.3 ± 0.5	22.3 ± 0.5	22.3 ± 0.5	22.2 ± 0.4
Number of pregnant females		11 12	12 12		
Number of corpora lutea[b]		16.5 ± 1.1	17.0 ± 1.2	15.8 ± 1.9	16.1 ± 1.6
Number of implantation sites[b]		16.1 ± 1.4	16.6 ± 1.2	15.3 ± 2.1	15.8 ± 1.6
Number of pups delivered[b]		15.2 ± 1.7	16.0 ± 1.7	14.2 ± 2.2	14.6 ± 2.0
Sex ratio of pups (male pups / all pups)[b]		0.505 ± 0.165	0.413 ± 0.158	0.429 ± 0.140	0.492 ± 0.183
Number of live pups[b]	on PND 0	15.1 ± 1.7	15.8 ± 1.6	14.2 ± 2.2	14.5 ± 2.2
	on PND 4	15.0 ± 1.7	13.1 ± 6.3	14.1 ± 2.3	13.7 ± 2.7
Body weight of male pups[b] (g)					
	on PND 0	6.63 ± 0.58	6.67 ± 0.67	6.75 ± 0.69	6.45 ± 0.46
	on PND 1	7.25 ± 0.56	7.12 ± 1.08	7.33 ± 0.75	6.97 ± 0.80
	on PND 4	10.53 ± 0.85	10.63 ± 1.54	10.67 ± 1.14	9.93 ± 1.24
Body weight of female pups[b] (g)					
	on PND 0	6.27 ± 0.51	6.22 ± 0.60	6.40 ± 0.66	6.08 ± 0.50
	on PND 1	6.91 ± 0.51	6.58 ± 1.07	6.98 ± 0.82	6.59 ± 0.79
	on PND 4	10.05 ± 0.79	9.97 ± 1.36	10.15 ± 1.25	9.43 ± 1.31

a: Data of the main group are shown. No significant changes in estrous cycle normality were found in the recovery group, either.
b: Data are shown as the mean ± S.D.

Table 7. Comparison of the NOAELs for the repeated dose and reproductive/developmental toxicity for long-chain PFCAs.

Chemical name	Carbon number	NOAEL (mg/kg/day)		Reference
		Repeated dose toxicity	Reproductive /developmental toxicity	
PFUnA (perfluoroundacanoic acid)	11	0.1	0.3	Takahashi *et al.*, 2014
PFDoA (perfluorododecanoic acid)	12	0.1	0.5	Kato *et al.*, in press
PFTeDA (perfluorotetradecanoic acid)	14	1	3	Current study
PFHxDA (perfluorohexadecanoic acid)	16	4	100	Current study
PFOcDA (perfluorooctadecanoic acid)	18	40	200	Hirata-Koizumi *et al.*, 2012

The NOAELs were established based on the results of in the combined repeated dose toxicity study with reproduction/developmental toxicity screening tests in rats

the thyroids of males. Although the serum levels of thyroid-related hormones were not analyzed in the present study for PFTeDA, it may be a compensatory response of the thyroid to a decrease in thyroid hormone levels because the structural analogue, perfluorodecanoic acid (PFDeA, C10), was previously reported to reduce serum T_3 and/or T_4 levels in rats (Gutshall *et al.*, 1988; Van Rafelghem *et al.*, 1987; Langley and Pilcher, 1985; Gutshall *et al.*, 1989). In the present study, PFHxDA (C16) did not affect the histopathology of thyroids, but increased the thyroid weight in males and decreased serum T_3 level in females. Although these effects of PFHxDA were not consistent between sexes and lacked clear dose-dependency, our results indicate that PFHxDA may slightly affect the thyroid system through a similar mechanism to PFTeDA (C14) and PFDeA (C10). The findings of mechanistic studies on PFDeA (C10) suggested that reduced serum thyroid hormone levels may result from (1) a displacement in the hormones from plasma protein binding sites, leading to an increase in tissue uptake and turnover (Gutshall *et al.*, 1989), and (2) the enhanced metabolism of thyroid hormones in the liver (Shelby and Klaassen, 2006). In our previous studies, we did not detect any effects of PFUnA (C11), PFDoA (C12), and PFOcDA (C18) on the histopathology or weight of the thyroids (Hirata-Koizumi *et al.*, 2012; Kato *et al.*, in press; Takahashi *et al.*, 2014). Serum hormone levels were not measured in these studies.

We previously reported that PFOcDA (C18) reduced forelimb grip strength in females (Hirata-Koizumi *et al.*, 2012). This effect was not observed at the end of the administration period, but appeared at the end of recovery period in both sexes in studies on PFUnA (C11) and PFDoA (C12) (Kato *et al.*, in press; Takahashi *et al.*, 2014). We considered that the reduction observed in grip strength may reflect the muscle weakness associated with a decrease in food consumption and/or body weight. In the present study, PFTeDA (C14) and PFHxDA (C16) reduced hindlimb grip strength, but not that of the forelimb. As with PFUnA (C11) and PFDoA (C12), the effects of PFHxDA (C16) on grip strength only appeared at the end of the recovery period. Hindlimb grip weakness was not necessarily accompanied by a low body weight. Further studies are required in order to clarify the mechanism responsible.

As for reproductive/developmental toxicity, the only effect observed was an inhibited postnatal body weight gain in pups at a maternal toxic dose of PFTeDA (C14). Similar results were observed in the study on PFHxDA (C16), but these changes were not significant. In our previous studies on long-chain PFCAs, postnatal body weight gain in pups was also inhibited at the highest dose (Hirata-Koizumi *et al.*, 2012; Kato *et al.*, in press; Takahashi *et al.*, 2014). In studies performed on PFDoA (C12) and PFOcDA (C18), such effects were accompanied by more severe reproductive/developmental effects, such as the deaths of dams at the end of pregnancy and stillbirths, and with more severe maternal toxic effects than those observed in the present study. The effect of long-chain PFCAs on postnatal development could be attributed to secondary effects due to maternal toxicity such as a low body weight during the lactation period. If PFTeDA (C14) reduced thyroid hormone levels as speculated above, it may be one cause of impaired postnatal development because Hapon *et al.* (2003) reported that hypothyroidism induced by a propylthiouracyl treatment impaired the growth of pups during the lactation period in rats. When the lipophilic properties of long-chain PFCAs (Inoue *et al.*, 2012) are considered, there is also the possibility that they were transferred via breast milk and affected the pups directly.

Based on the present results, the NOAELs for the repeated dose and reproductive/developmental toxicity were concluded to be 1 and 3 mg/kg/day for PFTe-

DA (C14) and 4 and 100 mg/kg/day for PFHxDA (C16), respectively. When the NOAELs were compared with those of PFUnA (C11), PFDoA (C12), and PFOcDA (C18) from our previous studies, the toxic potency of PFCAs was found to become weaker as the carbon chain length increased from C12 to C18 (Table 7). Since the previous comparative studies on the hepatic effects of PFCAs demonstrated increases in toxic potency due to an increase in the length of carbon chains up to C8 in rodents (Kudo *et al.*, 2006; Permadi *et al.*, 1993), the toxic potency of PFCAs was considered to be the strongest when the carbon length was C8 to C12. A clear chain length-dependent downward trend was observed in the renal elimination of PFCAs with a carbon chain length from C6 to C10 in rats (Ohmori *et al.*, 2003; Kudo *et al.*, 2001), and active renal tubular reabsorption via organic anion transport proteins was considered to be responsible for this (Han *et al.*, 2012). On the other hand, Wolf *et al.* (2008, 2012) reported that PFCAs of longer chain lengths induced more activity from mouse and human PPARα than those of shorter chain lengths up to C9 in transiently transfected COS-1 cells; therefore, not only toxicokinetic, but also toxicodynamic factors may contribute to the chain length-dependent toxicity of PFCAs with carbon chain lengths up to C8. Regarding PFCAs with carbon chain lengths of C11 and above, although no data is currently available to explain the cause of the chain length-dependent differences in toxic potencies, medium chain fatty acids (typically C6-C12) are known to be absorbed better from the gastrointestinal tract than long-chain fatty acids (typically longer than C12) (Ramirez *et al.*, 2001). Considering structural similarities, the gastrointestinal absorption of longer chain PFCAs may be poorer than that of PFCAs with shorter carbon chains. In order to clarify the cause of the differences in the toxic potencies of long-chain PFCAs, we are planning to first analyze serum PFCA levels in rats given different long-chain PFCAs.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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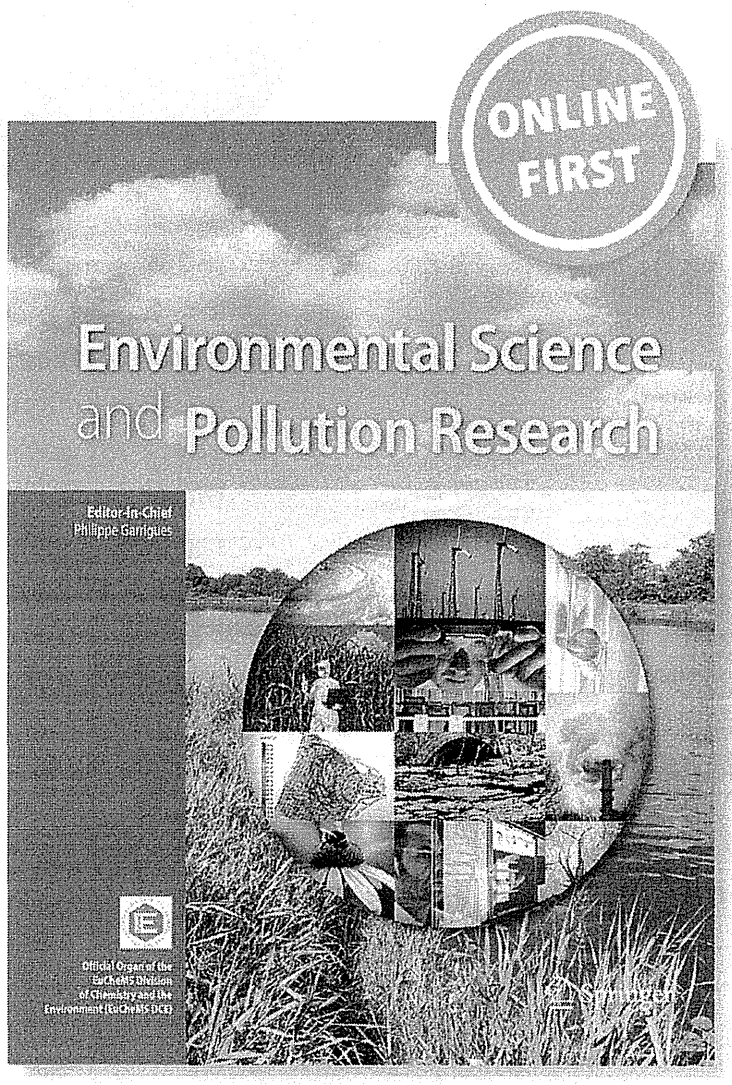

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Occurrence of 1153 organic micropollutants in the aquatic environment of Vietnam

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Abstract The rapid increase in the number and volume of chemical substances being used in modern society has been accompanied by a large number of potentially hazardous chemicals being found in environmental samples. In Vietnam, the monitoring of chemical substances is mainly limited to a small number of known pollutants in spite of rapid economic growth and urbanization, and there is an urgent need to examine a large number of chemicals to prevent impacts from expanding environmental pollution. However, it is difficult to analyze a large number of chemicals using existing methods, because they are time consuming and expensive. In the present study, we determined 1153 substances to grasp a pollution picture of microcontaminants in the aquatic environment. To achieve this objective, we have used two comprehensive analytical methods: (1) solid-phase extraction (SPE) and LC-TOF-MS analysis, and (2) SPE and GC-MS analysis. We collected 42 samples from northern (the Red River and Hanoi), central (Hue and Danang), and southern (Ho Chi Minh City and Saigon-Dongnai River) Vietnam. One hundred and sixty-five compounds were detected at least once. The compounds detected most frequently (>40 % samples) at μg/L concentrations were sterols (cholesterol, beta-sitosterol, stigmasterol, coprostanol), phthalates (bis(2-ethylhexyl) phthalate and di-*n*-butyl phthalate), and pharmaceutical and personal care products (caffeine, metformin). These contaminants were detected at almost the same detection frequency as in developed countries. The results reveal that surface waters in Vietnam, particularly in the center of large cities, are polluted by a large number of organic micropollutants, with households and business activities as the major sources. In addition, risk quotients (MEC/PNEC values) for nonylphenol, sulfamethoxazole, ampicillin, acetaminophen, erythromycin and clarithromycin were higher than 1, which indicates a possibility of adverse effects on aquatic ecosystems.

Keywords Screening analysis · Micropollutants · GC/MS · LC/TOF-MS · Pesticides · PPCPs

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Introduction

Urbanization, industrialization, and intensive farming are having a negative impact on Vietnam's environment. As a result, surface water of rivers running through residential and industrial areas has been increasingly polluted by organic contaminants (Ministry of Natural Resources and Environment (MONRE) 2010). Untreated medical, industrial, and municipal wastewater are combined in municipal sewage systems and then discharged to canals and rivers. In particular, water pollution problems originating in domestic wastewater were clearly evidenced in large cities (MONRE 2010). For example, Ho Chi Minh City [HCMC, the most densely populated city in Vietnam (GSO 2013)], discharges 413,000 m^3 of wastewater per day, Hanoi discharges 155,000 m^3/day, and Hue-Danang discharge 58,800 m^3/day.

Water pollution was also found in rural or suburban areas of these cities. The main cause of water pollution in rural areas is pesticide and fertilizer residuals (Dang and Thiemann 2002;

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Anyusheva et al. 2012). Statistical data show that pesticide consumption rapidly increased from 66,000 t in 2005 to 124 000 t in 2012 (GSO 2013). Because of poor cropping practices, pesticides and fertilizers are often overused and enter waterways. High-density industrial development and agricultural activities in certain major river basins may also pollute rivers. For example, surface water of the Red River and Saigon-Dongnai River (SDR) is extensively used for irrigation, drinking, and cooking. Therefore, water pollution may affect large numbers of the population. The Red River is one of the main sources of water in northern Vietnam and has the second largest basin, covering 26 % of the area of Vietnam (MONRE 2006). Another important basin is that of the SDR; this basin encompasses the southeast principal economic zone including HCMC, Binhduong, Dongnai, and Baria-Vungtau provinces. These provinces comprise the most important industrial area in the country, with a high rate of economic growth.

In Vietnam, there have been few studies focusing on a small number of organochlorine pesticides, PCBs, PAHs, and others in surface or in sediments (e.g., Nhan et al. 2001; Dang and Thiemann 2002; Nguyen et al. 2007; Duong et al. 2008; Pham et al. 2010; Lamers et al. 2011). Owing to rapid economic growth and urbanization, monitoring of a large number of chemicals is needed to prevent expansion of environmental pollution. However, it is difficult to analyze such large numbers using existing methods because of the substantial time and expense involved with operating multiple definitive tests. We have developed novel screening methods that can measure hundreds of chemicals simultaneously (Jinya et al. 2013). In the present study, we applied the methods to river water in Vietnam and analyzed 1153 substances composed of 843 semi-volatile organic compounds (SVOCs) and 310 polar organic compounds (POCs), to elucidate the pollution status of the aquatic environment in Vietnam. From the results, a complete pollution picture of the aquatic environment in the country is portrayed.

Materials and methods

Materials

All solvents, *n*-hexane, acetone, and dichloromethane (DCM) for pesticide residue analysis, methanol of LC-MS grade, Na_2HPO_4, and NaH_2PO_4 were supplied by the Kanto Chemical Company (Tokyo, Japan). Reagents of target compounds and internal standards were purchased from Wako Pure Chemical Industries (Osaka, Japan), Kanto Chemical Company, and Sigma-Aldrich (Tokyo, Japan). Purified water was obtained using a Millipore Milli-Q Advantage system (Nihon Millipore K.K., Tokyo, Japan).

Water sample collection

All 42 samples were collected in March 2013. Fourteen samples were collected from the Red River (Fig. 1a, upstream to downstream). In Hanoi, three samples were taken in urban zones including the Kimnguu River (HN1), Lu River (HN2), and Tolich River (HN3). Other two samples (HN4, HN5) were collected from the Nhue River in a suburban zone of Hanoi (Fig. 1b). Figure 1c is for Hue (five samples, in an urban area HU4, and in a rural area HU1, HU2, HU3, HU5). Figure 1d is for Danang (seven samples, in an urban area DN3, DN7, and in a suburban area DN1, DN2, DN4, DN5, DN6). Four out of six HCMC samples were taken in the Thamluong (HCM7), Nhieuloc-Thinghe (HCM8), Logom (HCM9), and Tauhu (HCM10) canals (Fig. 1e), which appeared to be wastewater canals within urban areas. Since water from these canals has been collected and treated at wastewater treatment plants, water quality has improved (HCMC PC 2014). However, their surface water quality still does not meet national standards. Other two samples were taken from Anha (HCM6) and Kenhdoi (HCM11) canals in a suburban zone. For the SDR, three of five samples were collected from the Saigon River (HCM2, HCM3, HCM4) and one from the Dongnai River (HCM1); there was one other sample from the downstream of these two rivers (HCM5) (Fig. 1e). Detailed information and figures of sampling sites are given in Duong et al. (2015).

Surface water at the center of a stream was sampled from a bridge with a stainless steel bucket, which was pre-cleaned with solvents, purified water, and sample water. Each water sample was stored in a 1-L glass bottle previously washed with solvents and purified water. Bottles containing water samples were kept in an icebox and transported to our laboratory.

Sample extraction and analysis

The GC-MS and GC-MS-MS analytical method for 950 SVOCs was undertaken according to the method of Jinya et al. (2013). A water sample (1 L), spiked with 1 mL of phosphate buffer (1 M, pH 7.0) to adjust the pH of each sample to 7, was fitted inside a vacuum manifold (3M Company, St. Paul, MN, USA) with flow rate less than 100 mL/min in a sequence with a glass microfiber disk (GMF 150, 47 mm, Whatman, Maidstone, UK), a styrene-divinylbenzene disk (Empore™ SDB-XD, 47 mm, 3M Co.), and an active carbon disk (Empore™ AC, 47 mm, 3M Co.). These disks were preconditioned by passing 10 mL of DCM, 10 mL of acetone, 10 mL of methanol, and 20 mL of purified water through them before use. After passing water sample through the disks, water remaining in the disks was completely removed using a vacuum for 30 min. The GMF and XD disks were eluted together with 5 mL of acetone (twice), followed by 5 mL of DCM. The AC disk was eluted with 5 mL of acetone (twice).

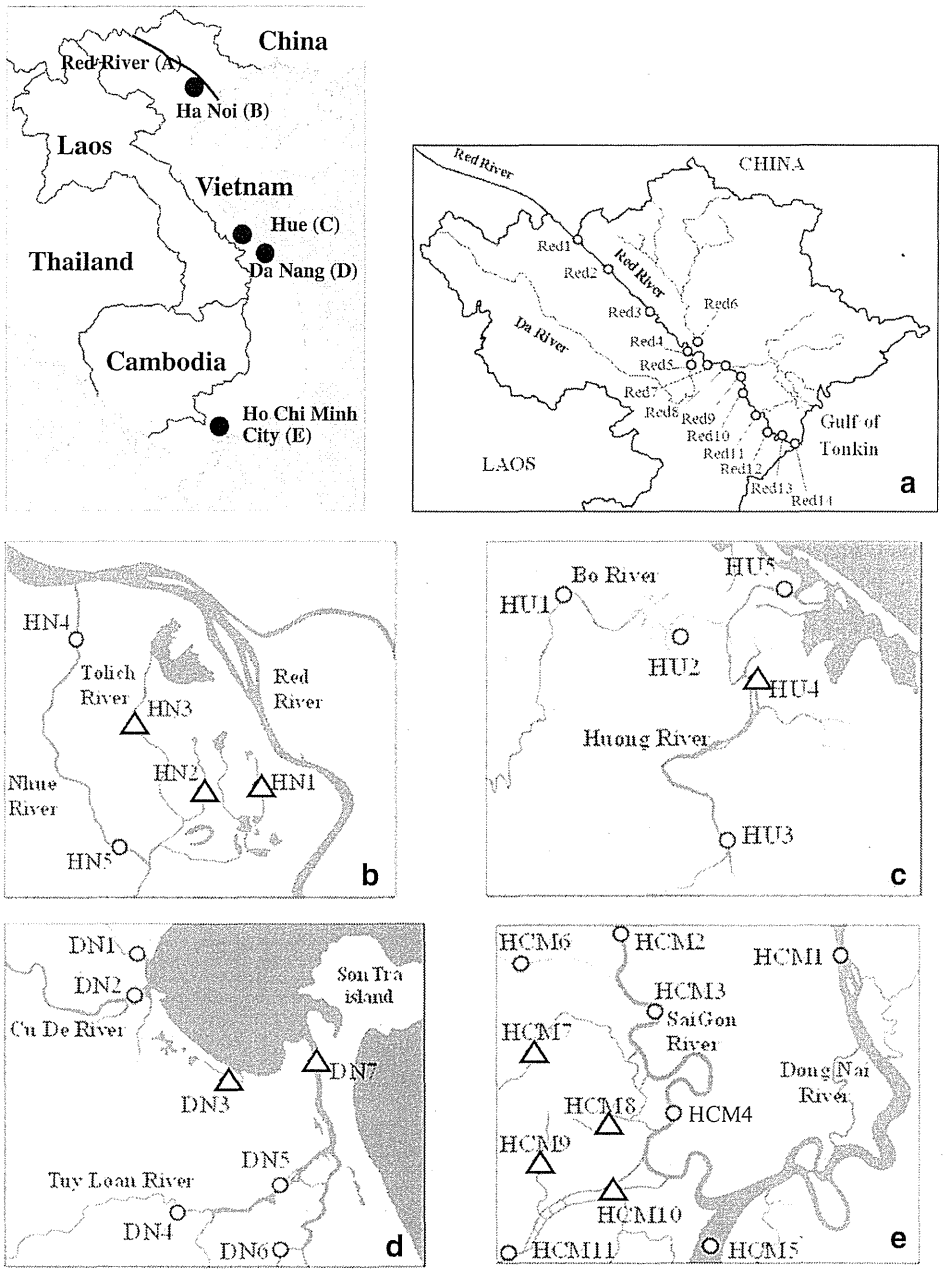

Fig. 1 Location of 42 sampling sites. **a** Red River, **b** Hanoi, **c** Hue, **d** Danang, **e** Ho Chi Minh City and Saigon-Dongnai River. *Empty triangles* represent urban area; *empty circles* represent suburban area

The eluates were combined and concentrated into 1 mL with a nitrogen stream. The concentrate was diluted with 10 mL of hexane and dehydrated by adding Na_2SO_4 (preheated at 700 °C for 6 h). The dehydrated solution was concentrated to 1 mL, and then mixed internal standards (IS; 4-chlorotoluene-d_4, 1,4-dichlorobenzene-d_4, naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, fluoranthene-d_{10}, chrysene-d_{12}, perylene-d_{12}) were added prior to instrumental analysis [GC-MS-SIM/Scan (QP-2100 Plus, Shimadzu, Tokyo, Japan) and GC-MS-MS-SRM (TSQ Quantum XLS, Thermo Fisher Scientific, Yokohama, Japan)].

For the analysis of 300 POCs, 1 mL of phosphate buffer (1 M, pH 7.0) was added to a water sample (500 mL) and filtered with a 1.2-μm glass fiber filter (Whatman, GF/C). Suspended solids (SS) were subjected to ultrasonic extraction with methanol twice. The filtrate was passed through a PS-2 Sep-Pak short cartridge (Waters Corporation) and an AC 2 Sep-Pak (Waters) using a Chratec Sep-Pak Concentrator (SPC 10-C; Chratec, Kyoto, Japan) with a flow rate of 10 mL/min, and then rinsed with 10 mL of purified water. The cartridges were then dried with nitrogen to remove water for 40 min. The cartridges were eluted with methanol (5 mL)

Table 1 Concentrations (μg/L) of the chemicals found and the numbers of chemicals found (in parentheses)

Group	Type of compound	Compound	*N*	Mean-max value of measured concentration (number of detected compound)					
				Red River (14 samples)	Hanoi (5 samples)	Hue (5 samples)	Danang (7 samples)	Saigon–Dongnai River (5 samples)	HCMC (6 samples)
Household chemicals	Leaching from tire	2(3H)-benzothiazolone, 2-(methylthio)-benzothiazol, acetophenone, benzyl alcohol, phenylethyl alcohol	5	nd	3.9–6.9 (4)	0.043–0.087 (2)	nd	0.0044–0.022 (1)	3.3–12 (5)
	Petroleum		25	2.4–8.8 (22)	23–37 (24)	1.1–4.3 (22)	4.1–8.0 (19)	2.9–4.7 (21)	33–100 (25)
	Plasticizers	Bis(2-ethylhexyl)phthalate, bisphenol A, butyl benzyl phtalate, di(2-ethylhexyl) adipate, diethyl phthalate, di-*n*-butyl phthalate, triphenylphosphate	7	4.3–17 (7)	14–22 (6)	0.11–0.47 (3)	1.3–5.1 (4)	2.3–4.0 (7)	13–38 (6)
	Disinfectants	2-methylphenol, 3- and 4-methylphenol, phenol	3	0.011–0.040 (1)	15–28 (2)	nd	nd	nd	17–63 (3)
	Others	4-methyl-2,6-di-t-butylphenol; 4-*tert*-octylphenol; nonylphenol	3	0.051–0.11 (1)	3.7–7.0 (3)	0.017–0.086 (1)	0.020–0.044 (1)	0.056–0.11 (2)	7.2–28 (3)
Industrial chemicals	Intermediates	2,4-dichloroaniline; 2-ethyl-1-hexanol; 2-phenylphenol; 3,4-dichloroaniline; 3,5-dimethylphenol; biphenyl; dicyclohexylamine; quinoline	8	0.026–0.076 (1)	3.9–5.4 (7)	0.048–0.067 (1)	0.40–1.8 (2)	0.10–0.16 (1)	4.2–21 (6)
	PAHs	1,3-dimethylnaphthalene; 2,6-dimethylnaphthalene; 2-methylnaphthalene; fluoranthene; phenanthrene; pyrene	6	3.2–44[a] (3)	0.090–0.17 (3)	nd	11–74[a] (3)	nd	0.35–1.3 (4)
	PCBs		32	0.057–0.15[a] (2)	1.8–5.3[a] (14)	0.086–0.14[a] (1)	0.18–0.35[a] (3)	0.19–0.27[a] (2)	1.7–7.6[a] (28)
	Paint/solvent	Isophorone	1	0.010–0.14 (1)	0.23–5.2 (1)	0.069–0.26 (1)	nd	nd	0.35–1.4 (1)
Pesticides	Fungicides	Azoxystrobin, carbendazim, cyprodinil, epoxiconazole, ethoxyquin, hexachlorobenzene, isoprothiolane, tricyclazole	8	0.12–0.29 (2)	0.14–0.21 (3)	0.029–0.11 (3)	0.17–0.35 (2)	0.15–0.28 (4)	0.15–0.22 (5)
	Herbicides	Acetochlor, alachlor, ametryn, atrazine, bensulfuron-methyl, butachlor, diuron, flufenacet, naproanilide, prometryn, siduron, tebuthiuron	12	0.11–0.29 (4)	0.16–0.34 (5)	0.025–0.12 (3)	0.13–0.90 (2)	0.075–0.21 (4)	0.54–1.2 (4)
	Insecticides	Acetamiprid; a-HCH; aldrin; carbofuran; *cis*-chlordane; trans-chlordane; dimethoate; fenobucarb; fenoxycarb; imidacloprid; o,p'-DDD; p,p'-DDD+o,p'-DDT; p,p'-DDE; permethrin 1; permethrin 2; piperonyl butoxide; promecarb;	17	0.039–0.14[a] (3)	1.8–2.9 (12)	0.036–0.10 (3)	0.034–0.22 (7)	0.054–0.10 (5)	1.0–3.0 (9)
	Sterols	Cholestanol, cholesterol, coprostanol, beta-sitosterol, stigmasterol	5	5.3–17 (4)	121–194 (5)	3.8–6.5 (4)	9.6–39 (4)	8.6–11 (4)	58–159 (4)
PPCPs	Antibiotics	Ampicillin, clarithromycin, erythromycin, griseofulvin, lincomycin, oleandomycin, roxithromycin, spiramycin, sulfadiazine,	13	0.017–0.24 (3)	3.7–5.5 (10)	nd	0.12–0.86 (2)	0.26–0.63 (2)	2.1–4.4 (10)

Table 1 (continued)

Group	Type of compound	Compound	*N*	Mean-max value of measured concentration (number of detected compound)					
				Red River (14 samples)	Hanoi (5 samples)	Hue (5 samples)	Danang (7 samples)	Saigon–Dongnai River (5 samples)	HCMC (6 samples)
	Other pharmaceuticals	sulfanilamide, sulfamethoxazole, sulfapyridine, trimethoprim Acetaminophen, atenolol, acetohexamide, antipyrine, caffeine, carbamazepin, cimetidine, cotinine, diethyltoluamide, lidocaine, hexamethylenetetramine, L-menthol, losartan, metformin, nicotine, phenacetin, propranolol, sulpiride, testosterone, theophylline	20	0.058–0.25 (4)	22–38 (14)	0.14–0.55 (2)	0.27–1.5 (7)	1.1–3.1 (8)	17–60 (16)
Total number of detected compounds			165	58	113	46	56	61	129

N number of detected compounds detected in all 42 samples at least once, *nd* not detected

[a] Concentrations were calculated in the unit of ng/L

and DCM (3 mL). After combining the eluates and the extract from SS, the mixture was concentrated to 50 μL and then spiked with 40 μL of three IS (5 μg/L, mixture of methomyl-d_3, pirimicarb-d_6, imazalil-d_5). The concentrate was diluted to 1 mL with purified water, filtered through a 0.2-μm syringe filter (Millex-LG) into an analysis vial, and subsequently measured by LC-TOF-MS.

Analytical quality control

Method accuracy and precision were studied by recovery studies using surface water and effluent of sewage treatment plants spiked at different concentrations. The procedure blanks were analyzed every 6 samples to check for cross-contamination and interference.

For SVOC analysis, quality control measures were as described by Jinya et al. (2011, 2013). Two hundred-two SVOCs were selected as model compounds (MCs) having a wide range of physicochemical properties (structure, functional group, boiling points (145–536 °C)). The MCs included polycyclic aromatic hydrocarbons (PAHs), amines, alkyl phenols, halogenated phenols, phthalates, benzenes, alcohols, and some classes of pesticides. Recoveries were determined by analyzing purified and environmental sample spiked standards at two concentrations (0.1 and 0.5 μg/L). Most of the model compounds, which are representative of the target SVOCs, had recoveries of over 50 % (Jinya et al. 2013). Method detection limits (MDL) of chemicals measured by SIM and/or SRM were 0.0004–0.3 μg/L. The MDL of compounds measured by TIM were 0.005 to 0.5 μg/L.

For the polar substance analysis, the recoveries of 264 MCs from spike experiments at 0.05 and 0.2 μg/L were determined using purified water (replication $n=7$ for each level of concentration) and effluent wastewater ($n=5$) to be in the range 50–120 %. The relative standard deviation (RSD) values for recovery tests using purified water were in the range 3–25 % and the RSD of effluent samples between 5 and 30 %. Quantitation was performed by IS method using a peak area obtained at 100 V of fragmentor voltage. MDLs of POCs ranged from 0.008 to 0.4 μg/L. The correlation coefficients of calibration curves are higher than 0.99 for all the compounds analyzed.

Results and discussion

Detection of micropollutants in surface water samples

One hundred and sixty-five out of 1153 target compounds were detected at least once in surface water samples (Table S1). The total number of compounds found in Hanoi and HCMC samples were similar (113 and 129 compounds, respectively; Table 1), and two to three times higher than at

other sampling sites (Red River 58, Hue 46, Danang 56, SDR 61). Overall, the concentrations of substances detected in Hanoi and HCMC were much higher than in Hue, Danang, the Red River, and SDR (except for fungicides and herbicides; Fig. 2), because of differences in population density and economic activity. When comparing data from large cities and other sites, household chemicals, PAHs, and sterols had nearly identical numbers of detected compounds but vastly different total concentrations. The numbers and concentrations of fungicides and herbicides did not vary greatly between sites. PCBs, insecticides, and pharmaceutical and personal care products (PPCPs) were found in much higher numbers and concentrations in the large cities than at other sites (Table 1). When comparing the number and concentrations of detected organic compounds between urban and suburban area of cities, sampling sites in Hanoi urban area (HN1, HN2, and HN3) had high concentrations of household chemicals and PPCPs compared to those in suburban areas (HN4 and HN5; Fig. 2). This pattern was also observed among samples collected in urban area and suburban area of HCMC, Danang, and Hue (Fig. 2).

We screened 13 plasticizers; seven of these [bis(2-ethylhexyl)phthalate (DEHP), bisphenol A, butyl benzyl phthalate, di(2-ethylhexyl)adipate (DEHA), diethyl phthalate (DEP), di-*n*-butyl phthalate (DBP), triphenylphosphate (TPP)] were detected in very high concentrations (μg/L level) at each sampling site. Maximum and average values of total detected concentrations were 38 and 13 μg/L in HCMC, 22 and 14 μg/L in Hanoi, and 17 and 4.3 μg/L in the Red River. DEHP was predominant, with high concentrations accounting for 71 % of the mean concentration of plasticizers detected in the Red River, 75 % in Hanoi, 76 % in SDR, and 65 % in HCMC.

The highest concentration of PAHs was 1334 ng/L (mean 64 ng/L), about three times lower than the value in a previous report from Vietnam (Duong et al. 2014) and about four times lower than in Tianjin, China (Kong et al. 2014). The number of detected PCBs (32) was similar to that reported by Duong et al. (2014), but their total concentrations were <7.6 ng/L

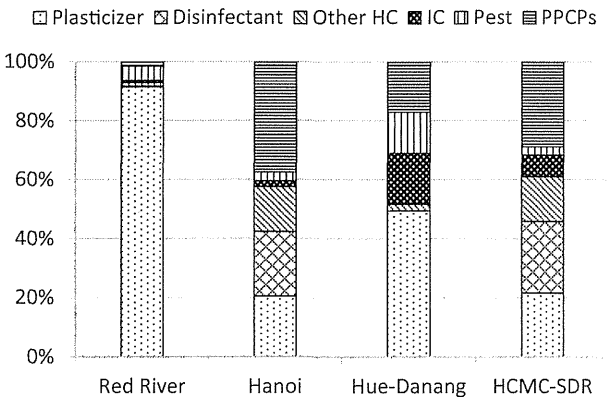

Fig. 3 Percentages of concentrations of compounds detected at each location (*other HC*: other household chemicals; *ICs*: industrial chemicals)

(mean 0.54 ng/L), two times lower than previously reported values.

Only five out of the 12 sterols examined were observed and occurred at the highest concentration compared with the other compounds detected in this survey (Hanoi (194 μg/L), HCMC (159 μg/L)). A ratio of coprostanol/cholesterol ≥0.2 indicates sewage contamination (Grimalt et al. 1990). Generally, values near or greater than 0.2 were found in populous locations such as Hanoi (site HN1 0.96, HN2 0.86, HN3 0.88, HN4 0.37, HN5 0.82), Red10 (0.32, downstream of Hanoi), urban areas of Hue (HU4 0.19) and Danang (DN3 0.46), and HCMC (HCM6 0.30, HCM7 0.67, HCM9 0.79, HCM10 0.3, HCM11 0.20) (Table S1). Glassmeyer et al. (2005) suggested that a ratio exceeding 0.3 indicates fecal contamination. This means that wastewater containing feces from households was directly discharged into rivers or canals in urban areas, and domestic wastewater treatment plants were not operating effectively.

Thirty-three PPCPs were found in the survey, among which 13 compounds were antibiotics (ampicillin, clarithromycin, erythromycin, griseofulvin, lincomycin, oleandomycin, roxithromycin, spiramycin, sulfadiazine, sulfamethoxazole, sulfanilamide, sulfapyridine, and trimethoprim). The total

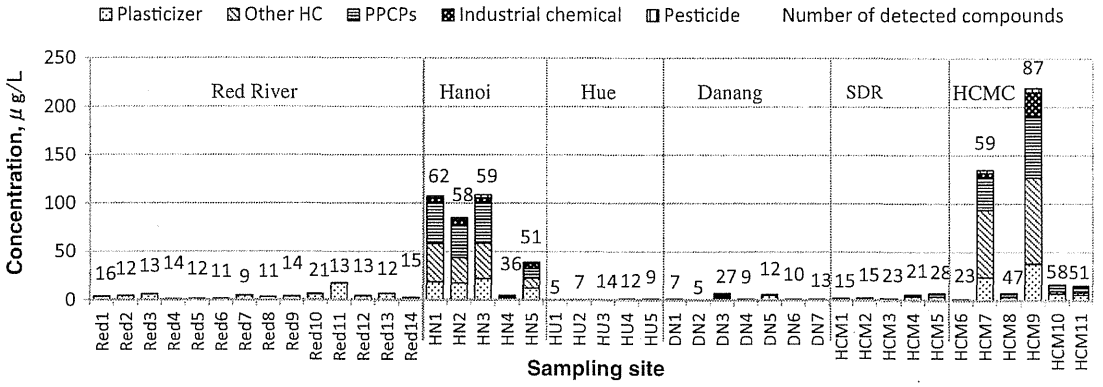

Fig. 2 Concentrations and number of compounds detected at each sampling site

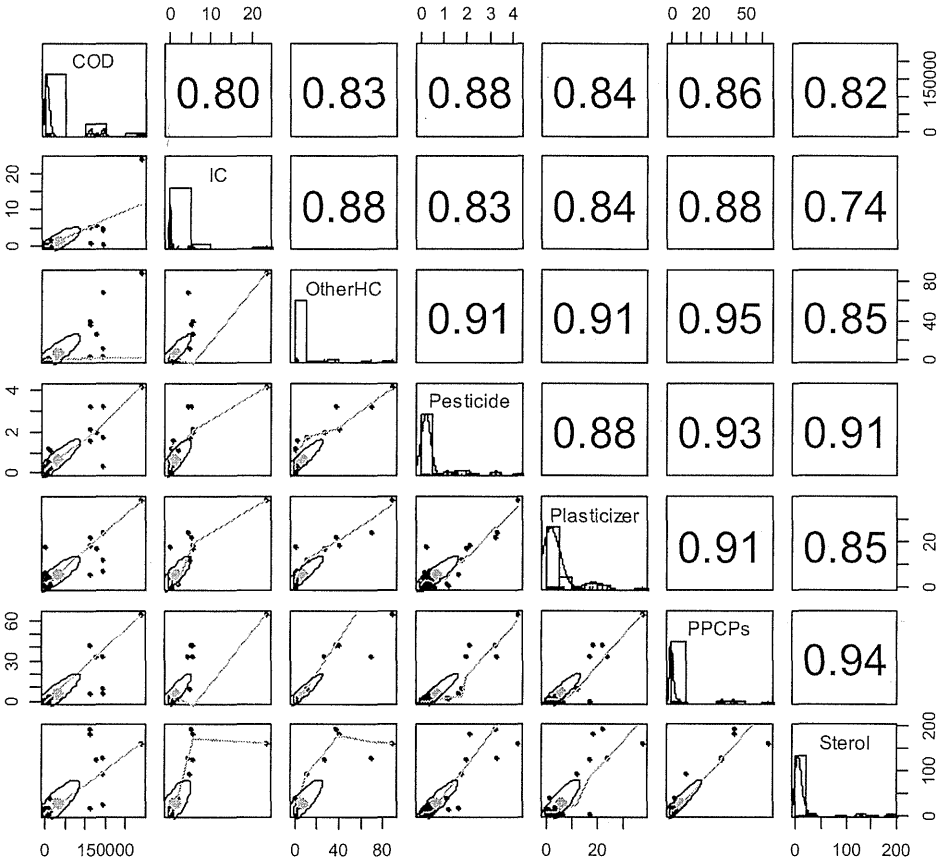

Fig. 4 Correlation between groups of detected organic compounds (*COD* definition of chemical oxygen demand, *IC* industrial chemical, *Other HC* other household chemical)

concentration of all detected antibiotics was highest in Hanoi (5.5 μg/L; mean 3.7 μg/L), followed by 4.4 μg/L in HCMC (mean 2.1 μg/L). In Vietnam, antibiotics are dispensed without a doctor's prescription (Nguyen et al. 2011) and may enter the environment through feces or urine. However, it is possible that important point sources of antibiotics are hospitals because hospital wastewater contains high levels of antibiotics, and removal values through wastewater treatment plants are smaller than those in developed countries (Duong et al. 2008).

Distribution of micropollutants in surface waters

More than 50 % of total micropollutant concentrations detected in both urban and suburban areas were household chemicals (Red River 92 %, Hanoi 58 %, Hue-Danang 52 %, HCMC-SDR 71 %; Fig. 3). The distributions of contaminants in the environment of Hanoi and HCMC-SDR were nearly identical but were very different to those of Hue-Danang and the Red River (Fig. 3).

Plasticizers are commonly used, and with millions of tons produced worldwide annually (Koch et al. 2003), these chemicals have become widespread in the environment (Fromme et al. 2002; Fauser et al. 2003). In the present study, plasticizers were a large proportion of detected contaminants, accounting for 21–22 % in Hanoi and HCMC-SDR, 50 % in Hue-Danang, and up to 91 % in the Red River. A likely source of plasticizers in the environment of large cities is storm water (Clara et al. 2010; Björklund et al. 2009). However, in the case of Hue-Danang and the Red River, untreated wastewater from craft villages is considered the main plasticizer source. Craft villages are classified into many different groups according to their products, such as textiles, construction materials, recycled metal, paper, or plastics. Most of these villages are in northern and central Vietnam, and the Red River basin has the largest number of craft villages, accounting for 60 % of all such villages in the country (MONRE 2008). All these villages have been facing environmental pollution problems. Pollution in these villages has not decreased and, in fact, has tended to increase. This may explain why industrial chemicals constituted large proportions of the contaminant composition in Hue-Danang (17 %).

Many pharmaceuticals and their metabolites have been detected in aquatic environments (Hereber 2002; Caliman and Gavrilescu 2009). In the present study, PPCPs contributed greatly to the total distribution; 37 % in Hanoi, 29 % in HCMC-SDR, and 17 % in Hue-Danang (Fig. 3). Because PPCPs are one of the major contaminants in surface samples

Table 2 List of most frequently detected compounds in 42 samples

Compound	Type of compound	LOD (ng/L)	Number > LOD	Number > 0.1 μg/L	Number > 1 μg/L	Number > 10 μg/L	Maximum (μg/L)	Median (μg/L)
Beta-sitosterol	Sterol	100	42	42	31	7	25.2	1.98
Cholesterol	Sterol	100	42	42	34	8	70.6	1.66
Stigmasterol	Sterol	100	42	42	30	2	16.4	1.84
PCB #1	PCB	0.03	35	0	0	0	0.32[a]	0.11[a]
Dicyclohexylamine	Intermediate	8	34	14	3	0	3.32	0.07
Coprostanol	Sterol	10	29	22	12	6	57.8	0.12
4-Methyl-2,6-di-t-butylphenol	Antioxidant	25	28	7	0	0	0.41	0.04
Bis(2-ethylhexyl)phthalate	Plasticizer	10	27	26	24	6	19.0	2.25
Cotinine	Nicotine metabolite	8	27	10	5	0	2.84	0.01
Di-*n*-butyl phthalate	Plasticizer	10	26	19	7	0	4.92	0.08
Triphenylphosphate	Plasticizer	20	26	1	0	0	0.14	0.01
p,p'-DDE	Insecticide	0.03	25	0	0	0	4.14[a]	0.04[a]
Di(2-ethylhexyl)adipate	Plasticizer	10	24	14	0	0	0.44	0.03
Atrazine	Herbicide	10	24	0	0	0	0.03	0.01
Lidocaine	Anesthetic/antiarrhythmic	8	23	4	0	0	0.23	0.02
Diethyl phthalate	Plasticizer	10	22	15	6	0	7.49	0.03
Bisphenol A	Plasticizer	10	21	9	2	0	7.82	0.01
Carbendazim	Fungicide	8	19	9	0	0	0.21	nd
Metformin	Antidiabetic	8	19	13	7	0	8.25	nd
Ethoxyquin	Fungicide	8	18	6	0	0	0.29	nd
Tricyclazole	Fungicide	8	18	0	0	0	0.10	nd
4-*tert*-Octylphenol	Nonionic detergent metabolite	10	17	3	0	0	0.85	nd
Fenobucarb	Insecticide	8	17	1	0	0	0.22	nd
Caffeine	Food product	10	17	16	8	1	13.0	nd

LOD limit of detection

[a] Calculated concentrations have units ng/L

of crowded cities, more research is needed on their fates and effects in the environment. Pesticides and industrial chemicals comprised only 3 and 2 % in Hanoi, and 3 and 7 % in HCM-SDR, respectively, or 2–7 times lower than the rates found in Hue-Danang.

Correlations between organic compounds detected in surface waters

The water quality parameters pH, total suspended solids (SS), and chemical oxygen demand (COD) were measured in this survey (Table S1). COD was observed in the range from 0.32 to 240 mg/L. Seven sampling sites had COD values more than 5 times higher than Vietnam's 20 mg/L national surface water quality regulation (QCVN 08: 2008/BTNMT; HN1, HN2, HN3, HCM7, HCM9, HCM10, and HCM11). These sites are located in urban areas of Hanoi and HCMC. There were strong, positive correlations between COD and all groups of detected organic compounds (industrial chemicals, household chemicals, pesticides, plasticizers, and sterol; Fig. 4). Therefore, it can be said that there was no specific sources of contaminants, and surface water has become polluted by wastewater discharges from domestic, hospitals, factories, and agricultural activities.

Most frequently detected compounds in surface waters

Twenty-four substances were found frequently (≥40 % samples, with detected concentrations >LOD; Table 2), including 4 sterols [beta-sitosterol, cholesterol, stigmasterol (100 %), and coprostanol (69 %)], 6 plasticizers [DEHP (64 %), DBP and TPP (62 %), DEHA (57 %), DEP (52 %), bisphenol A (50 %)], 6 pesticides [pp'-DDE (60 %), atrazine (57 %), carbendazim (45 %), ethoxyquin, tricyclazole (43 %), fenobucarb (40 %)], 4 PPCPs [cotinine (64 %), lidocaine (55 %), metformin (45 %), caffeine (40 %)], 2 industrial chemicals [PCB#1 (83 %), dicyclohexylamine (81 %)], and 2 household chemicals [4-methyl-2-6-di-t-butylphenol (67 %), 4-*tert*-octylphenol (40 %)]. The substances showing high concentrations (>1 μg/L) were sterols such as cholesterol (81 %), beta-sitosterol (74 %), stigmasterol (71 %), coprostanol (29 %), phthalate plasticizer of DEHP

Table 3 The MEC/PNEC ratios of detected compounds

Compound	MEC (μg/L)	PNEC (μg/L)	MEC/PNEC
Nonylphenol	26.9	0.21[a]	128
Sulfamethoxazole	2.16	0.027[b]	80
Ampicillin	0.64	0.075[c]	8.6
Acetaminophen	5.64	1[b]	5.6
Erythromycin	0.09	0.02[b]	4.3
Clarithromycin	0.17	0.07[b]	2.4
Sulfadiazine	0.11	0.135[b]	0.8
Bisphenol A	7.82	11[a]	0.7
Propranolol	0.13	0.244[b]	0.5
Trimethoprim	0.18	2.6[b]	0.1
Lincomycin	2.66	82[b]	0.03
Roxithromycin	0.05	4[b]	0.01
Atenolol	0.27	30[b]	0.01
Cimetidine	0.19	35[b]	0.01
Carbamazepin	0.03	13.8[b]	0.002
Sulfapyridine	0.03	21.61[b]	0.002

[a] MOE 2001

[b] Verlicchi et al. 2012

[c] Kümmerer and Henninger 2003

(57 %), DBP (17 %), followed by caffeine and metformin (19 %).

Of the plasticizers, DEHP was detected at the highest concentrations (>1 μg/L at each sampling location) of 19 μg/L (HCMC), 13.5 μg/L (Hanoi), and 13.0 μg/L (Red River), followed by bisphenol A (HCMC 7.82), DEP (HCMC 7.49, Hanoi 6.41), DBP (Danang 4.92, Red River 4.22, HCMC 3.24, Hanoi 1.45). Other studies have also suggested high detection frequencies (>50 %) of these substances in surface waters but at higher concentrations. For example, Clara et al. (2010) reported DEHP with a detection frequency of 100 %, and maximum concentration of 34 μg/L; DEP 100 %, 9.2 μg/L; DBP 53 %, 8.7 μg/L. Higher concentrations have also been reported in France, Germany, and Canada [DEHP: maximum 44 μg/L, DEP 25 μg/L (Dargnat et al. 2009); DEHP 97.8 μg/L, DBP 8.8 μg/L (Fromme et al. 2002); DEHP 70 μg/L (Barnabé et al. 2008), respectively].

In our study, we detected caffeine in surface waters at a maximum concentration of 13.0 μg/L, much lower than the 91.6 μg/L reported by Duong et al. (2014) but higher than in other studies [1.43 μg/L (Kong et al. 2014); 6.9 μg/L (Edwards et al. 2015)]. Another PPCP, metformin (an antidiabetic), was detected at the highest concentration compared with other PPCPs found in this survey (Hanoi 8.23 μg/L at maximum, HCMC 2.25 μg/L), albeit these concentrations were more than two times lower than the maximum concentration found in a study in China (20 μg/L; Kong et al. 2014). Lower concentrations of metformin in surface waters have been observed in many developed countries, such as the maximum 735 ng/L in France (Vulliet and Cren-Olivé 2011) and 1700 ng/L in Germany (Scheurer et al. 2009).

Environmental risk assessment of organic compounds

The ratios of the measured environmental concentration (MEC, maximum concentration in surface water) and the predicted no effect concentration (PNEC) were used to assess the environmental risk of detected compounds. The MEC/PNEC values were <1 indicating no toxic potential (Quinn et al. 2008). Of the 16 substances that were evaluated, six substances, nonylphenol (nonionic detergent metabolite), sulfamethoxazole, ampicillin, erythromycin, clarithromycin (antibiotic), and acetaminophen (analgesic), had MEC/PNEC >1 (Table 3). It indicates that these compounds are of concern and may warrant tier three toxicity assessment.

Conclusions

In the present study, 165 out of 1153 micropollutants examined were detected in surface waters, and of which more than 100 contaminants occurred at μg/L level of concentrations in Hanoi and HCMC. Rivers in large cities were heavily polluted by a large number of organic microcontaminants, mainly from domestic sources such as PPCPs, plasticizers, and other household chemicals. Compared with large cities, Hue-Danang, the Red River, and Saigon-Dongnai River were less contaminated, and their pollutant sources were not just domestic but also agricultural and small-scale industries. The most frequently detected contaminants were plasticizers (DEHP, DEP, DBP, bisphenol A), dicyclohexylamine, and PPCPs (caffeine, metformin, cotinine). Their concentrations were high (>1 μg/L) higher than those found in international studies. One of the causes of serious pollution is that construction speed of sewage treatment plants does not catch up economic growth and urbanization. Therefore, accelerated construction of sewage treatment plants and enlightenment about chemicals are necessary to prevent expansion of pollution. Nonylphenol, sulfamethoxazole, ampicillin, acetaminophen, erythromycin, and clarithromycin had risk quotients (MEC/PNEC) >1, suggesting these chemicals may be causing ecological harm, although further detailed field study is required to confirm this hypothesis.

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Combining Passive Sampling with a GC-MS-Database Screening Tool to Assess Trace Organic Contamination of Rivers: a Pilot Study in Melbourne, Australia

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Abstract This study assessed the suitability of passive sampler extracts for use with a GC-MS-database rapid screening technique for around 940 organic chemicals. Chemcatcher™ passive sampler systems containing either Empore™ SDB-XC or C18FF disks were deployed at 21 riverine sites in and near Melbourne, Victoria, Australia, for a period of 28 days during September–October 2008. Methanolic elution of the SDB-XC and C18FF disks produced an extract that, after evaporation and inversion into hexane, was compatible with the GC-MS-database method enabling over 30 chemicals to be observed. The sources of the non-agricultural chemicals are still unclear, but this study was conducted in a relatively dry season where total rainfall was approximately 40 % lower than the long-term mean for the catchment during the study period. Thus, the risks may be greater in wetter seasons, as greater quantities of chemicals are likely to reach waterways as the frequency, extent and intensity of surface run-off events increase. This study provides valuable information for policy and decision-makers, both in Australia and other regions of the world, in that passive sampling can be conveniently used prior to analysis by multi-residue techniques to produce data to assess the likely risks trace organic chemicals pose to aquatic ecosystems.

Keywords GC-MS AIQS-DB multi-residue method · SVOCs · Comprehensive analysis · Passive sampling · Yarra Valley · Victoria · Australia

1 Introduction

Managing the effects of trace organic chemical contaminants in waterways requires information, often far more than we (as scientists and/or managers) can afford to directly measure at all the places at all of the times, and for all the chemicals of interest. The measurement of nutrients and salts in surface and groundwater is now routine and offered cheaply by many commercial laboratories; however, this is not so for many other contaminants of concern, including many pesticides, pharmaceutical and personal care products (PPCPs) and industrial chemicals. Measurement of trace organic chemicals (TrOCs) in waters can be expensive in Australia, which inhibits monitoring by water authorities (as providers of water), catchment management authorities (as

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custodians of the natural environment) and consumers (e.g. irrigators, industry and household and domestic water users), potentially resulting in increased risk to the natural environment. Strategies and/or tools are therefore required to focus monitoring and risk assessment programs in a cost-effective manner; the use of new sampling methods and preliminary screening of samples using rapid assessment tools are thus an increasingly attractive prospect for waterways managers.

When assessing organic substances, many analytical methods may have to be used to cover a large number of known compounds (Gómez et al. 2011), with concomitant financial implications associated with operating multiple, definitive chemical analytical screens using gas and/or liquid chromatography-based methods for volatile, semi-volatile non-polar and polar organic industrial, agricultural and domestic chemicals. In 2005, Kadokami et al. reported a new screening method that combined a mass-structure database with gas chromatography-mass spectrometry (GC-MS) to create a system that can screen samples for more than 940 semi-volatile compounds, including numerous halogenated and non-halogenated hydrocarbons, polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyl compounds (PCBs), pharmaceutical and personal care products (PPCPs) and pesticides. The Kadokami et al. (2005) automated identification and quantification system with database (AIQS-DB) method was originally developed for screening unknown pollutants in environmental samples after incidents, e.g. fish kills, food contamination scares or the unknown number of harmful chemicals discharged into the environment after disasters such as large-scale fires and earthquakes, and has since successfully been applied to both water and sediment samples from rivers and estuaries in Japan (Kadokami et al. 2009; Pan et al. 2014), in Vietnam (Kadokami et al. 2012; Duong et al. 2014) and Victorian wastewater treatment plant effluents (Allinson et al. 2012). Consequently, when applied to river water, as in this study, this GC-MS-database (DB) method can give a good indication of the presence of some TrOCs that would not otherwise be identified if samples were to be subjected to only a very limited number of analytical screens for a limited number of target chemicals.

Collection of grab (or spot) samples is most commonly used to characterise TrOC residues in surface waters, although integrative sampling with passive samplers (or passive sampling) is becoming a more commonly used alternative. A 'passive sampler' can be defined as a device that is able to acquire a sample from discrete location without the active media transport induced by pumping or purge techniques (ITRC 2006). Hence, most passive samplers consist of a receiving phase with high affinity for organic contaminants, separated from the aquatic environment by a diffusion limiting membrane. Some of the most commonly used devices that rely on diffusion and sorption to accumulate analytes in the sampler are semi-permeable membrane devices (SPMDs) and passive in situ samplers (such as the Chemcatcher™ system (CC)). Passive samplers had been little utilised on natural water samples in Australia at the inception of this study. In Victoria, one of the first detailed studies using passive samplers occurred between 2004 and 2006 when Rose and Kibria (2007) deployed SPMDs containing trimethyl pentane in irrigation canals in northern Victoria. The monitoring found three insecticides (endosulfan, chlorpyrifos and parathion methyl) in the passive sampler solvents. Elsewhere in Australia, a number of relatively recent studies have detected residues of atrazine, diuron, hexazinone and simazine in rivers and estuaries on the eastern seaboard of Australia using the CC passive sampling system (Escher et al. 2006; Muller et al. 2008; Shaw et al. 2009a, b, 2010; Stephens et al. 2009; O'Brien et al. 2011), and Mueller et al. (2011) tracked POPs in the Brisbane River, but prior to this study, there had been no significant studies investigating TrOCs in surface waters in Victoria using the CC system.

The sampling rates of chemicals into passive samplers are dependent on a range of factors, both intrinsic to the passive samplers themselves and extrinsic factors (Leonard et al. 2002). Extrinsic environmental factors that may affect uptake include flow rate, water temperature and changes in the sorptive properties of the water (due to dissolved organic matter and suspended particulates; Leonard et al. 2002). Intrinsic factors that may affect uptake include the polarity of the contaminant (as measured by its octanol-water partition coefficient, K_{ow}), the diffusivity of the molecules that have to pass through the aqueous boundary layer, sampler design, exposure time and concentrations of chemicals in the surrounding water. Perhaps of most importance is the type of receiving phase used in the passive sampler relative to target analytes, and whether the receiving phases are used with rate limiting membranes or without them ('naked'). The purpose of rate limiting membranes is literally that suggested by their name—to reduce the rate at which chemicals are adsorbed by the receiving

phase. For instance, the sampling rates (Rs) reported by Shaw et al. (2009a, b) when using naked sulphonated polystyrene divinylbenzene-reverse phase sulphonated disks (Empore™ SDB-RPS disks; Rs: atrazine 0.44 L/day; simazine 0.36 L/day) were about three times higher than when the same experiment was conducted with SDB-RPS disks and a polyether sulphone (PES) membrane (Rs: atrazine and simazine, 0.140 L/day). By reducing the sampling rates, the length of time before all available sites in the receiving phase are saturated is increased. This in turn allows for lengthier exposure periods in the field. In that context, SDB-RPS disks were used naked for 10 days for monitoring a polar herbicide (amitrole) in New South Wales by Sánchez-Bayo et al. (2013), whereas Allinson et al. (2014) were able to use Empore™ SDB-XC disks covered by a PES membrane for 28 days to target similarly polar herbicides in Victorian surface waters. At the inception of this study, receiving phases appeared to be being chosen by expert opinion based on their use to extract target analytes from water samples in the laboratory, although since that time, there has been some systematic study comparing the performance of different receiving phases in the field (e.g. SDB-RPS, SDB-XC and C-18 disks for the monitoring of nonylphenolethoxylates and nonylphenol in aquatic ecosystems; Ahkola et al. 2014).

In recognition of the potential risks that TrOCs pose to aquatic ecosystems, and the lack of information on the levels of such chemicals in Victorian freshwaters, this study was initiated to compare the utility of two different, but common receiving phases for the CC passive sampler system under field conditions in conjunction with a 'single-shot' multi-residue testing method. To that end, CCs were deployed at 21 sites in urban, peri-urban and rural waterways in and around Melbourne in September–October 2008 and retrieved samples prepared for measurement of approximately 940 semi-volatile organic chemicals by GC-MS-DB.

2 Materials and Methods

2.1 Study Sites

Twenty-one sites were monitored in and around Melbourne for this study (Fig. 1), which was conducted independent of, but in conjunction with a larger, complementary multi-disciplinary study of waterways in and around Melbourne. In that context, this manuscript is a companion piece to the papers by Wightwick et al. (2012, 2013) and Allinson et al. (2014) who reported the levels of fungicides, metals and herbicides, respectively, in the surface waters and sediments in riverine ecosystems in the Middle and Upper Yarra catchment area, some of which were used for this study. The sites were selected for a variety of reasons: either because they were already part of Melbourne Water's Yarra River water quality monitoring program (e.g. site 23), were in a section of very high regional importance (as defined by PPWRRHS 2007; e.g. site 18), were downstream of a tributary with of very high regional importance (e.g. site 1), downstream of a tributary with known industrial pollution or waste water treatment plant (WWTP) impacts (e.g. site 3, 16, 17) or being sampled as part of other concurrent Melbourne Water funded research (e.g. sites 22, 23 and 24 used by Schäfer et al. 2011).

Most of the study sites (75 %) were within the Yarra River catchment, with two study sites on the Mornington Peninsula and two sites in an agricultural location in the Barwon River catchment (sites 22, 23 and 21, 24, respectively). The Yarra River watershed is approximately 4000 km^2 and home to approximately 2 million people in the city of Melbourne (the capital and most populous city in the state of Victoria). The city is located at the northern-most point of a large natural bay (Port Phillip Bay), with the city centre itself positioned on the estuary of the Yarra River (Fig. 1). The metropolitan area extends along the eastern and western shorelines of Port Phillip Bay and more than 25 km inland. The Yarra River flows 240 km from its headwaters to the sea in Port Philip Bay, and, in general, water quality is good in the upper catchment, but deteriorates downstream because of diffuse pollution from changed land use, particularly from agriculture and urban development (PPWRRHS 2007). Specifically, the upper sections of the Yarra River and its main tributaries (above site 1; Fig. 1) flow through forested, mountainous areas that have been reserved for water supply purposes for more than 100 years. Most of the land in the upper-middle section (above site 1) has been cleared for agriculture, although there is significant peri-urban development. The land downstream of site 1 is primarily urban and industrial. The lowest 10-km section of the Yarra River is estuarine. In the urbanised area of the Yarra River catchment, stormwater has a major impact on the river's water quality. Several small (10,000–50,000 population equivalents) wastewater treatment plants (WWTPs) discharge treated effluent into the Yarra River (either

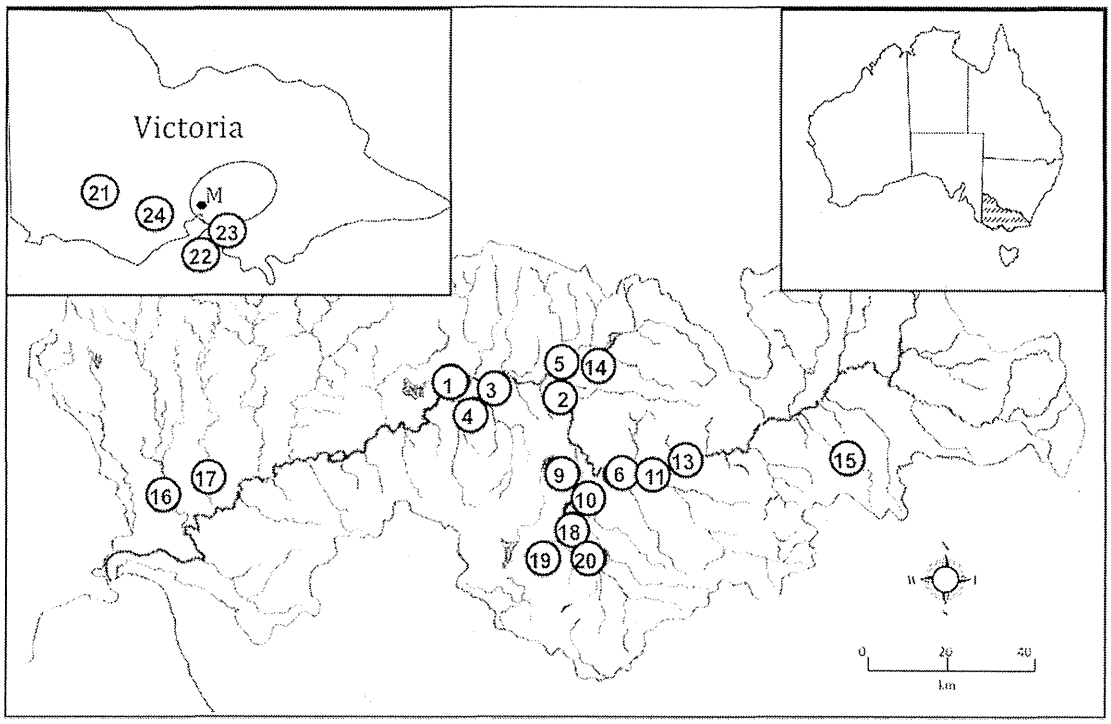

Fig. 1 Approximate location of sampling sites in and around the city of Melbourne (M) in the Yarra River catchment (*oval outline*), Barwon River catchment (sites 21 and 24) and on the Mornington Peninsula (sites 22 and 23), in Victoria, Australia

directly or via a tributary), although most of Melbourne's domestic and industrial sewage is transferred to two large WWTPs in the south and west of the city and after treatment discharged to the ocean.

2.2 Passive Sampling

A single type of passive sampler was used in this study, namely the Chemcatcher™ passive sampler system (CC). The CC system consists of machined polytetrafluoroethylene (PTFE) body that protects the chromatographic receiving phase (see Kingston et al. 2000). In this study, the CC system was fitted with either an Empore™ SDB-XC disk or an Empore™ C18FF disk (47 mm; 3 M, MN, USA) as the receiving phase and a polyethersulfone (PES) membrane (Sterlitech Corp, WA, USA) as the diffusion-limiting membrane. The SDB-XC and C18 disks were conditioned by soaking them in methanol (1 h) after which they were rinsed with deionised water on a disk extraction manifold; the PES membranes were conditioned in 50:50 methanol/water for 1 h then deionised water for 1 h. Samplers containing the two types of receiving phases were deployed in October 2008 for time-integrated monitoring to allow first a qualitative assessment (i.e. presence/absence) and then, where possible, a semi-quantitative assessment (i.e. based on estimated time-weighted average water concentrations) of semi-volatile chemicals in the catchment. For full details of CC preparation and field deployment, readers are directed to the Supplementary Information in Allinson et al. (2014).

Passive samplers were disassembled at DEPI Queenscliff Centre, and the receiving disk and PES membrane dried at 35 °C on a hotplate for approximately 1.5 h. Each disk was wrapped separately in aluminium foil, labelled, placed inside another labelled plastic bag and stored at 4 °C until analyte elution. One disk from each of the site replicates was haphazardly chosen and was eluted with methanol (5 mL) into a glass tube and the resulting solution evaporated to dryness with N_2. The samples were reconstituted in hexane (1 mL), and in this dissolved form, the sample was transported to the University of Kitakyushu for multi-residue screening.

The AIQS-DB method identifies and quantifies chemical substances by using a combination of retention times, mass spectra and internal standard calibration curves registered in the database. In order to obtain accurate results, a GC-MS has to be adjusted to designated conditions that are almost the same as the