

Endocrine disrupting alkylphenols and bisphenol-A in coastal waters and supermarket seafood from Singapore

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Octylphenols, nonylphenols and pentachlorophenols are some of the more commonly encountered groups of surfactants collectively known as alkylphenol ethoxylates. They are used extensively as detergents in industrial cleaners, wetting agents, emulsifiers and in domestic soaps (Talmage, 1994) and evidence have indicated a linkage between these compounds and adverse changes in the reproductive health of humans and animals (Déchaud et al., 1999; Sonnenschein and Soto, 1998). Octylphenols are used in a variety of products such as plastic packing material (Markey et al., 2001). Vom Saal et al. (1998) found that mice exposed to octylphenols experienced significantly lower daily sperm production. Nonylphenols are used as spermicides in condoms, as well as performing the role of antioxidants, stabilizers and emulsifiers in polyvinyl chloride, polystyrene and lubricant oil industries (Sonnenschein and Soto, 1998). It has been shown that exposure to low-level doses of nonylphenol inhibits ATP synthesis in mitochondria (Bragadin et al., 1999). Evans et al. (2000) recently found that marine gastropods exposed to nonylphenols can induce male sexual characteristics in females, as is the well-known case for tributyltin. Pentachlorophenol (PCP) is used as a preservative in the manufacture of insecticides and in the paper industry (Bols et al., 2001). Women chronically exposed to wood preservatives containing PCP may suffer from infertility and adrenal insufficiency (Gerhard et al., 1999).

Bisphenol-A is extensively employed in the production of epoxy resin and polycarbonate plastics for use in food and drink packaging industries (Nakazawa et al., 2002; Staples et al., 1998), although the estrogenic activity of this compound was noted nearly seventy years ago (Dodds and Lawson, 1938). Resins containing bisphenol-A are commonly used to coat metal products such as food cans, bottle caps and water supply pipes. Like diethyl stilbestrol (DES), bisphenol-A is capable of binding to DNA after metabolic activation and has estrogenic properties at low concentrations (Hormonally active agents, 2000). Howdeshell et al. (1999) showed that trans-placental exposure of low doses of bisphenol-

A in mice could bring on early puberty in females. Much of the concern focuses on unborn babies, because sex hormones play an important role during foetal development (Bolten et al., 1989).

Alkylphenols and bisphenol-A primarily enter the marine environment as components of industrial and domestic waste (Fendinger et al., 1995). Singapore, with a population of four million, is arguably one of the most densely populated countries in the world. The small size of Singapore, coupled with limited natural resources, necessitates multiple use of the sea surrounding the island-state. While the port waters of Singapore support an important and significant maritime economy based on container handling and petrochemicals, it is also a sink for treated sewage effluents. The same body of water is now increasingly used for seafood cultivation, recreation and desalination of water for drinking purposes. These somewhat diverging interests require prudent and stringent management of the marine environment. Despite strictly enforced laws governing the discharge of heavy metals and organic contaminants into waterways leading into the sea, there is currently no legislation on controlling the use or discharge of alkylphenols and bisphenol-A. The occurrence, quantity and distribution of these pollutants in the seas around Singapore remain undocumented. In this study we analyzed surface seawater samples from 28 coastal locations around Singapore to determine the presence of alkylphenols and bisphenol-A. Seafood samples purchased from the local supermarket were also examined for these compounds.

Sample analysis was carried out using a Shimadzu (Tokyo, Japan) QP5050 GC-MS equipped with a Shimadzu AOC-20i auto sampler and a DB-5 fused silica capillary column (30 m×0.32 mm I.D., film thickness 0.25 µm, J&W Scientific, Folsom, CA). All standards and samples were analysed in selective ion monitoring mode (SIM) with a detector voltage of 1.5 kV and a scan range of m/z 50 to m/z 500.

Surface seawater samples were collected from 28 locations (see Fig. 1) along the Singapore coastline from May to December 2000. These locations encompassed both inshore (i.e., on Singapore island) and offshore sampling points in the vicinity of industrial areas, jetties, shipyards, marinas and recreational beaches, as well as

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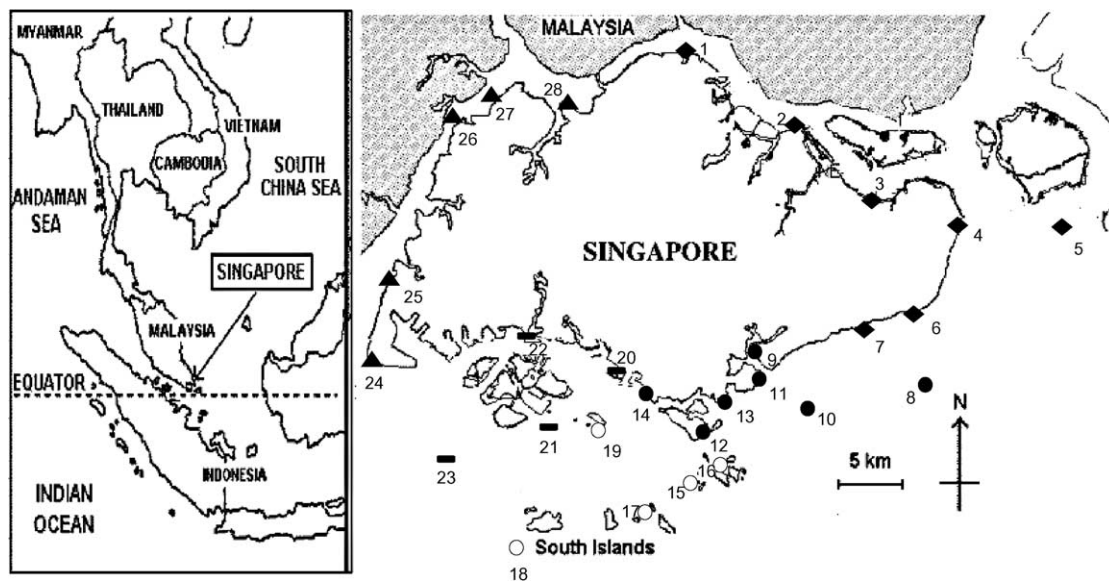


Fig. 1. Sampling locations of alkylphenols and bisphenol-A in the Singapore Straits and Straits of Johor in February and June 2000. The locations were classified into five overlapping geographical sectors A–E, represented by symbols: Eastern Straits of Johor and NE Singapore (locations 1–7; diamond); Eastern and Central Singapore Straits (8–14, closed circle); Southern Islands (15–19, open circle); Western Singapore Straits (20–23, rectangle) and Northwestern Johor Straits and Western Singapore Straits (24–28, triangle). The proximities to potential alkylphenol and bisphenol sources are indicated as follows: [1] Sembawang Park (shipyard & port), [2] Punggol (near port), [3] Pasir Ris (near sailing club), [4] Changi (municipal treatment plant/Naval base), [5] off Pulau Tekong (shipping lane), [6] Bedok Jetty (shipping lane), [7] East Coast Park (shipping lane/recreational), [8] off East Coast Park (shipping lane), [9] Marina East (near port), [10] off Marina South (near port), [11] Clifford Pier (near port), [12] Sentosa (recreational/near port), [13] Harbourfront, (near Port), [14] Labrador Park (shipping lane/near port), [15] Sister's Islands (Refineries/Shipping lane), [16] St. John's Island (shipping lane), [17] Pulau Hantu (refineries/shipping lane), [18] Raffles Lighthouse (lighthouse), [19] Cyrene Reef (shipping lane/near port), [20] West Coast Park (near sailing club and marina), [21] Jurong Island (industrial area/shipping lane), [22] Jurong Pier (shipyard/ferry terminal/industrial area) [23] Sultan Shoal (shipping lane), [24] Tuas Jetty (industrial area/shipyard), [25] Raffles Marina (sailing club), [26] Sarimbun (floating fish farms), [27] Lim Chu Kang (floating fish farms), [28] Kranji (wood industries).

in shipping lanes, anchorages and near petroleum refineries. Sampling locations (1–28) were geographically grouped into five regions (A–E): Region A—Eastern Johor Straits (locations 1–7); B—Eastern Singapore Straits (locations 8–14); C—Southern Islands (locations 15–18); D—Western Singapore Straits (locations 19–23); E—Western Johor Straits (locations 24–28). A total of six samples were obtained at each location during the day in pre-washed glass bottles. Extraction was performed on unfiltered seawater samples. To prevent possible degradation of analytes, samples were collected and processed on the same day. Salinities of the samples varied between 28 and 35; seawater temperatures ranged between 28 and 32 °C, and pH between 7.9 and 8.6. Seven varieties of fresh seafood samples (blood cockle, *Anadara granosa*; white clam, *Meretrix meretrix*; squid, *Loligo* sp.; tiger prawn, *Penaeus monodon*; swimming crab, *Portunus pelagicus* and carangid fish, *Decapterus russelli*) were purchased from a local supermarket chain between June 2000 and February 2001. Whole (shucked cockle, clam) or partial (muscle tissue of squid, prawn, crab and fish) samples were cut into small pieces immediately after returning to the laboratory and stored at –80 °C until analysis.

The extraction procedure was initially evaluated with artificial seawater (Coral Red Sea Salt® dissolved in deionized water to obtain a final salinity of 33 parts per thousand). This was spiked with a stock solution containing alkylphenols and bisphenol-A at a concentration of 50 µg l⁻¹ (per analyte) in 200 ml of sample, which was adjusted to pH 2 using 1 N HCl. Liquid–liquid extraction (LLE) was performed twice with 50 ml of dichloromethane. To remove trace amounts of water, anhydrous sodium sulphate was added to the organic layer. This layer was pre-concentrated in a rotary evaporator to a total solvent volume of approximately 1 ml, and subsequently cleaned-up using an Oasis-HLB (SPE) cartridge. The eluted extract was reduced in volume to less than 1 ml with nitrogen gas bubbling. Finally, 100 µl of bis(trimethylsilyl) trifluoroacetamide (BSTFA) was added, made up to 2 ml with acetone and kept in a 60°C water bath for 30 min to complete the derivatization. From this, 1 µl was injected to a GC-MS for analysis. A six-point calibration with correlation coefficient of >0.997 was made with naphthalene-d₈, pyrene-d₁₀ and phenanthrene-d₁₀ as internal standards and bisphenol-A-d₁₄ as the surrogate standard. Extraction recoveries and detection limits of each analyte are listed in Table 1. A strict quality control procedure was followed that

included the analyses of spiked and seawater samples with each set of field samples.

Biological sample extraction was performed using microwave-assisted solvent extraction (MASE) (Kington and Haswell, 1997). A small amount (0.2 g) of thawed tissue was placed in a microwave extraction vessel with 10 ml (20% water content) of tetramethylammonium hydroxide (TMOH) solution and 1 ml of *n*-nonane. The extraction vessel was exposed to microwave radiation at 80% power, and the contents were maintained at 115 °C for 15 min. In MASE, the tissue was completely digested in TMOH. The organic layer was washed and separated with diethyl ether and the extract cleaned-up using an Oasis-HLB (SPE) cartridge. The resulting mixture was derivatized using 100 µl of BSTFA. Finally, 1 µl of extract was injected into the GC-MS for analysis.

Recovery efficiencies and reproducibility of the extraction methods used are shown in Table 1. The relative standard deviations (RSDs) varied between 4.8% and 18.4% for seawater samples and 3.0% and 8.6% for tissue samples. Recoveries ranged between 73.9% and 96.5% for seawater and between 92.0% and 111.1% for tissue samples respectively (Table 1).

The following ten alkylphenols, in addition to bisphenol-A, were detected in seawater samples from 28 locations around Singapore: 4-*n*-butylphenol, 4-*t*-butylphenol, 4-*n*-pentylphenol, 4-*n*-hexylphenol, 4-*n*-octylphenol, 4-*t*-octylphenol, 4-*n*-heptylphenol, 4-nonylphenol, 2,4-dichlorophenol and PCP. The mean alkylphenol and bisphenol-A concentrations at 28 locations are shown in Table 2. Quantities ranged from non-detectable (<2 ng l⁻¹) to a maximum mean value of 3.3 µg l⁻¹ (2,4-dichlorophenol). The most widespread alkylphenol was 4-nonylphenol, which was detected at all 28 locations,

with mean concentrations between 0.02 and 2.76 µg l⁻¹. Mean total concentration of alkylphenols and bisphenol-A in Singapore's seawater samples are shown in Fig. 2. Samples from about half the number of locations contained more than 1 µg l⁻¹ of 4-nonylphenol. In contrast, 4-*n*-pentylphenol was present at low mean concentrations (<0.4 µg l⁻¹) in samples from only a few locations. The majority of other alkylphenols analyzed were found in small quantities (<0.5 µg l⁻¹) with a few exceptions, e.g., mean concentrations of 4-*t*-butylphenol, 4-*n*-heptylphenol and PCP exceeded 2 µg l⁻¹ in a several samples. Bisphenol-A was detected in seawater from most parts of Singapore. The maximum mean concentration of bisphenol-A obtained from a single location was 2.47 µg l⁻¹, although more than 70% of the samples from other locations contained less than 0.4 µg l⁻¹.

Seawater from several sampling locations were characterized by their relatively high concentrations of a variety of alkylphenols as well as bisphenol A (Table 2). In region A, samples from Sembawang Park (location 1) and Changi (location 4) contained all of the 11 targeted compounds, some of which were in excess of 1 µg l⁻¹. In region B and region E, seawater samples from Sentosa (location 12), Harbourfront (location 13) and Tuas Jetty (location 24) were similarly disposed, with several alkylphenol compounds exceeding 1 µg l⁻¹ (e.g., 4-*t*-butylphenol; 4-*n*-pentylphenol; 4-nonylphenol; 2,4-dichlorophenol; PCP). All of these locations experience heavy boat and ship traffic, which may be a major contributing factor to broadband alkylphenol pollution. Region A is also influenced by sewage effluent discharge from two sewage treatment plants in Singapore.

In general, the total concentrations of alkylphenols and bisphenol-A in samples obtained from offshore locations were lower than inshore (Singapore Island)

Table 1

GC/MS analytical conditions, recovery, reproducibility (as percentage relative standard deviation) and limit of detection (LOD) of alkylphenols and bisphenol-A from seawater and biological samples using liquid-liquid extraction (LLE) and microwave-assisted solvent extraction (MASE)

Analytes	Targetions (<i>m/z</i>)	Retention time (min)	LLE			MASE		
			Recovery ^a (%)	RSD (%)	LOD (ng l ⁻¹)	Recovery ^b (%)	RSD (%)	LOD (ng g ⁻¹)
4- <i>n</i> -Butylphenol	179.00	11.98	77.23	11.30	5.80	92.99	4.97	0.942
4- <i>t</i> -Butylphenol	207.00	11.09	83.10	6.80	4.30	93.57	4.55	0.255
4- <i>n</i> -Pentylphenol	179.00	13.20	96.51	16.70	8.60	94.94	3.61	0.228
4- <i>n</i> -Hexylphenol	179.00	14.39	92.80	15.70	3.72	94.78	3.70	0.172
4- <i>n</i> -Octylphenol	179.00	16.35	90.25	7.70	4.22	95.90	3.11	0.205
4- <i>t</i> -Octylphenol	207.00	14.68	95.87	18.40	5.60	nc	nc	0.150
4- <i>n</i> -Heptylphenol	179.00	15.48	86.88	16.90	4.40	95.38	3.27	2.319
4-Nonylphenol	207.00	15.66	95.35	16.50	14.60	109.19	8.62	0.164
2,4-Dichlorophenol	219.00	11.29	87.25	15.50	7.80	92.04	5.71	0.968
Pentachlorophenol	323.00	16.90	79.67	15.30	15.86	104.05	4.93	1.857
Bisphenol-A-d14 (surr)	368.00	18.92	105.00	13.00	nc	111.23	7.65	nc
Bisphenol-A	357.00	18.99	73.89	4.80	6.30	97.64	3.00	1.357

nc = not calculated.

^a *n* = 5.

^b *n* = 3.

Table 2
Mean alkylphenol and bisphenol-A concentrations in Singapore coastal waters

Location no.	Sampling locations	Mean concentration in $\mu\text{g l}^{-1}$ ($n = 6$) (nd = $<0.002 \mu\text{g l}^{-1}$)										
		4- <i>n</i> -butyl-phenol	4- <i>t</i> -butyl-phenol	4- <i>n</i> -pentyl-phenol	4- <i>n</i> -pentyl-phenol	4- <i>n</i> -octyl-phenol	4- <i>t</i> -octyl-phenol	4- <i>n</i> -heptyl-phenol	4-nonyl-phenol	2,4-dichloro-phenol	Pentachloro-phenol	Bisphenol-A
<i>Eastern Straits of Johor and Singapore</i>												
1	Sembawang Park	1.57	2.30	0.05	0.10	0.36	0.14	2.14	1.08	1.44	0.40	1.70
2	Punggol	0.06	0.01	0.02	0.13	0.06	0.04	0.28	0.64	0.03	0.10	2.47
3	Pasir Ris	0.01	0.06	nd	0.05	0.01	0.02	0.02	0.34	1.97	0.01	0.03
4	Changi	0.89	0.27	0.32	0.19	0.53	0.46	2.92	2.08	0.59	0.53	0.75
5	Off-Pulau Tekong (off shore)	nd	0.01	0.01	nd	0.07	0.02	0.02	1.00	nd	0.03	1.02
6	Bedok Jetty	0.01	0.01	nd	0.03	0.02	0.01	0.01	1.26	0.87	nd	0.01
7	East Coast Park	0.00	0.07	nd	0.01	nd	0.01	nd	0.43	0.02	nd	0.02
<i>Eastern and Central Singapore Straits</i>												
8	Off-East Coast Park (off shore)	0.01	0.01	nd	nd	0.05	0.03	0.05	1.00	nd	0.33	0.02
9	Marina East	nd	0.07	nd	0.02	nd	0.01	0.01	0.93	0.01	0.00	0.03
10	Off-Marina (off shore)	nd	0.10	0.00	nd	0.11	0.15	0.09	0.33	nd	0.36	0.33
11	Clifford Pier	nd	0.12	nd	0.02	0.01	0.02	nd	0.67	0.02	0.01	0.04
12	Sentosa	0.59	0.95	0.06	1.86	0.13	0.32	0.07	2.76	1.55	0.09	0.04
13	Harbourfront	0.44	1.06	0.20	0.55	0.19	0.54	1.59	2.40	0.53	1.65	0.17
14	Labrador Park	0.01	0.01	nd	0.03	nd	nd	0.89	0.02	0.17	nd	nd
<i>Southern Islands</i>												
15	Sisters Island (off shore)	0.01	0.03	nd	0.01	0.05	0.04	0.03	1.03	0.05	0.03	0.04
16	St. John Island (off shore)	0.02	0.03	Nd	nd	0.10	0.01	0.01	0.32	nd	0.01	0.05
17	Pulau Hantu (off shore)	0.11	0.01	0.01	0.03	0.02	0.04	0.01	1.63	0.24	0.14	0.19
18	Pulau Satumu (off shore)	nd	0.31	nd	0.03	nd	0.01	nd	0.61	nd	0.05	nd
19	Cyrene Reef (off shore)	nd	0.01	0.01	0.01	0.03	0.02	0.03	0.44	nd	0.10	1.71
<i>Western Singapore Straits</i>												
20	West Coast Park	nd	0.11	nd	0.01	0.01	0.01	0.01	0.77	0.01	0.00	0.03
21	Jurong Island (off shore)	0.02	0.40	0.03	0.18	0.14	0.13	0.06	1.36	3.33	0.13	0.02
22	Jurong Pier	nd	0.01	0.04	0.00	0.11	0.06	0.02	0.28	0.08	1.06	0.97
23	Sultan Shoal (off shore)	0.05	0.03	nd	0.04	0.02	0.02	0.01	0.37	0.08	0.10	0.04
<i>NW Johor, W Singapore Straits</i>												
24	Tuas Jetty	0.68	2.25	0.07	1.03	0.65	0.80	0.07	1.26	1.67	2.15	0.22
25	Raffles Marina	0.01	0.01	nd	0.03	0.01	0.01	nd	0.20	0.06	nd	nd
26	Sarimbun (off shore)	nd	0.05	nd	0.01	nd	0.01	0.01	0.93	0.01	nd	0.01
27	Off Lim Chu Kang (off shore)	0.02	0.05	nd	0.02	0.02	0.02	0.01	1.51	2.93	0.09	0.03
28	Off Kranji (off shore)	0.02	0.05	0.01	0.01	0.01	0.04	0.01	1.01	2.84	0.05	0.04

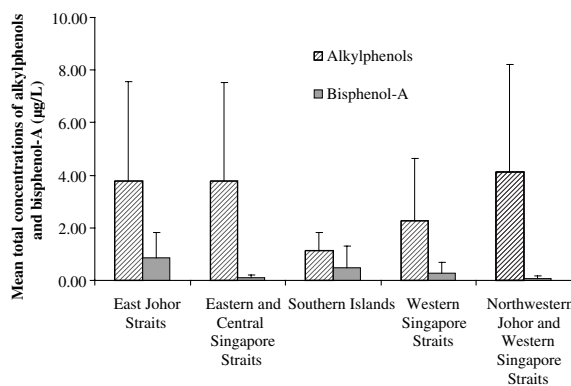


Fig. 2. Mean concentration \pm standard error of total alkylphenols and bisphenol-A in seawater from Singapore waters. A total of 28 sampling locations were grouped into five geographical sectors (for details refer to Table 2).

samples (see Table 2). Locations in region C were generally characterized by low concentrations ($<0.5 \mu\text{g l}^{-1}$) of these compounds, although 4-nonylphenol was detected in excess of $1 \mu\text{g l}^{-1}$ at two offshore islands.

Localized effects, however, were also apparent. Seawater samples from several locations, unlike those highlighted previously, were characterized by containing high concentrations of only a few types of alkylphenols, with little or no presence of other compounds. Samples from Punggol (location 2 in region A) contained a high concentration of bisphenol A but other alkylphenol compounds (except for 4-nonylphenol) were only present in low mean concentrations not exceeding $0.3 \mu\text{g l}^{-1}$. In region E, samples from Lim Chu Kang (location 27) and Kranji (location 28) contained little or no butyl-, pentyl- and octyl-phenols, but 2,4-dichlorophenol was present in large quantities in excess of $2.5 \mu\text{g l}^{-1}$ (Table 2). While it is difficult to determine the sources of these specific pollutants, the resultant localized concentrations of alkylphenols are clear. This cannot be entirely attributed to poor circulation in the Johor Straits, which is well known (e.g., Lim, 1984a,b; Koh et al., 1991; Wood et al., 1997). As demonstrated in the case of two sampling locations in region B, the relatively diverse and high concentrations of alkylphenols found in samples from the Harbourfront (location 13) are in contrast to the seemingly less polluted waters sampled from Labrador Park (location 14) 500 m away.

In general, alkylphenol concentrations obtained in this study are comparable to those reported elsewhere in the world, although only a few studies have been done in the marine environment. For example, concentrations of up to $0.92 \mu\text{g l}^{-1}$ dissolved 4-nonylphenol were reported from Hamilton Harbour in Lake Ontario, Canada (Bennie et al., 1997) and $53 \mu\text{g l}^{-1}$ (total extractable concentration up to $330 \mu\text{g l}^{-1}$) from the vicinity of sewage treatment works at River Aire in England (Blackburn and Waldock, 1995). Nonylphenol up to 45

$\mu\text{g l}^{-1}$ was reported in the Glatt River, Switzerland (Ahel et al., 1994) and is widely distributed in concentrations up to $5 \mu\text{g l}^{-1}$ (Ahel et al., 2000). Concentrations of 4-nonylphenol in the Weisse Elster, a tributary of the Elbe River in Germany, ranged from 0.08 to $0.22 \mu\text{g l}^{-1}$ (Heemken et al., 2001). Lower concentrations below $0.1 \mu\text{g l}^{-1}$ were reported downstream in the Elbe estuary and in the North Sea (Heemken et al., 2001). Total extractable nonylphenols from water samples in the Tees estuary, England was $5.2 \mu\text{g l}^{-1}$ (Blackburn and Waldock, 1995). These values are within the range of concentrations of total extractable nonylphenols obtained in seawater samples around Singapore, which are between 0.02 and $2.76 \mu\text{g l}^{-1}$ (Table 2).

Compared to nonylphenol, octylphenol is distributed in lower concentrations, e.g., the total extractable octylphenol level in the Tees estuary in England was $13 \mu\text{g l}^{-1}$ (Blackburn and Waldock, 1995), and in the Elbe estuary, less than $0.02 \mu\text{g l}^{-1}$ (Heemken et al., 2001). At most sites in the Great Lakes and St Lawrence River in Canada, octylphenol was not detectable except at Humber and Hamilton Harbour, Lake Ontario, where the levels exceeded $0.08 \mu\text{g l}^{-1}$ (Bennie et al., 1997). The highest detected mean concentration of 4-*t*-octylphenol in this study ($0.804 \pm 0.003 \mu\text{g l}^{-1}$ at Tuas Jetty) was lower than the laboratory threshold limits of male rainbow trout and the amount required to induce imposex in marine gastropods (Routledge et al., 1998; Oehlmann et al., 2000). However, relatively high octylphenol concentrations exceeding $0.1 \mu\text{g l}^{-1}$ at several locations around Singapore is a cause for concern.

Bisphenol-A detected in this study range between 1 ng l^{-1} to values exceeding $2 \mu\text{g l}^{-1}$ (Table 2). The mean maximum concentration is higher than those reported from the Elbe River in Germany ($0.776 \mu\text{g l}^{-1}$) (Heemken et al., 2001) and is comparable to maximum concentrations reported in Tokyo Bay, Japan (Matsumoto et al., 1977). Concentrations of pentachlorophenol and 2,4-dichlorophenol detected in this study were considerably lower than the World Health Organization (WHO) drinking water guidelines of 9 and $40 \mu\text{g l}^{-1}$, respectively. The total alkylphenols and bisphenol-A concentration is lower than the Environmental Protection Agency (US-EPA) maximum permissible concentration range of phenols 60 – $400 \mu\text{g l}^{-1}$ (EPA, 1984).

Alkylphenols and bisphenol-A were also detected in all seafood samples purchased from a local supermarket. We found up to 530.4 ng g^{-1} wet weight (w.w.) of total alkylphenols (see Fig. 3) and between 13.3 and 213.1 ng g^{-1} w.w. of bisphenol-A in prawn, crab, blood cockle, white clam, squid, and fish (Table 3). The highest nonylphenol concentration (197.0 ng g^{-1} w.w.) amongst the seafood samples analyzed was detected in prawns. However, the highest concentrations of 2,4-dichlorophenol (191.0 ng g^{-1} w.w.), PCP (146.0 ng g^{-1} w.w.) and bisphenol-A (213.1 ng g^{-1} w.w.) were found in crab

Table 3

Alkylphenols and bisphenol-A in seafood samples purchased from a local supermarket in Singapore

Analytes	Concentration (ng g ⁻¹ wet weight \pm SE) (n = 5)					
	Prawn (<i>Penaeus monodon</i>)	Crab (<i>Portunus pelagicus</i>)	Blood cockle (<i>Anadara granosa</i>)	White clam (<i>Meretrix meretrix</i>)	Squid (<i>Loligo</i> sp.)	Fish (<i>Decapterus russelli</i>)
4- <i>n</i> -butylphenol	4.5 \pm 1.1	20.0 \pm 0.8	25.3 \pm 6.1	23.7 \pm 5.4	11.5 \pm 3.7	19.3 \pm 5.1
4- <i>t</i> -butylphenol	21.3 \pm 17.0	24.0 \pm 2.1	8.8 \pm 5.6	6.5 \pm 3.7	13.9 \pm 13.1	19.0 \pm 9.0
4- <i>n</i> -pentylphenol	3.7 \pm 8.3	8.0 \pm 6.0	6.3 \pm 1.4	5.5 \pm 1.2	5.7 \pm 2.6	5.4 \pm 2.1
4- <i>n</i> -hexylphenol	7.0 \pm 12.2	9.0 \pm 5.0	5.9 \pm 0.8	4.3 \pm 2.4	6.0 \pm 3.3	6.0 \pm 3.1
4- <i>n</i> -octylphenol	23.0 \pm 18.9	4.1 \pm 2.3	5.4 \pm 3.3	4.5 \pm 2.0	5.5 \pm 1.9	3.3 \pm 1.0
4- <i>t</i> -octylphenol	20.4 \pm 15.8	20.2 \pm 1.2	6.7 \pm 2.7	44.9 \pm 21.2	10.2 \pm 5.4	31.4 \pm 15.0
4- <i>n</i> -heptylphenol	10.2 \pm 6.7	5.1 \pm 2.1	6.0 \pm 4.5	5.7 \pm 3.3	4.1 \pm 1.7	4.4 \pm 1.5
4-nonylphenol	197.0 \pm 13.1	103.1 \pm 36.0	54.0 \pm 6.1	46.6 \pm 11.4	64.8 \pm 13.7	60.5 \pm 10.4
2,4-dichlorophenol	10.7 \pm 7.5	191.0 \pm 22.0	152.4 \pm 37.0	139.7 \pm 43.5	84.1 \pm 20.2	141.2 \pm 22.8
Pentachlorophenol	47.8 \pm 24.5	146.0 \pm 10.8	107 \pm 56.1	71.4 \pm 82.8	41.8 \pm 47.4	37.7 \pm 3.9
Bisphenol-A	13.3 \pm 8.4	213.1 \pm 20.2	56.5 \pm 40.0	27.4 \pm 29.1	118.9 \pm 107.1	65.6 \pm 47.4

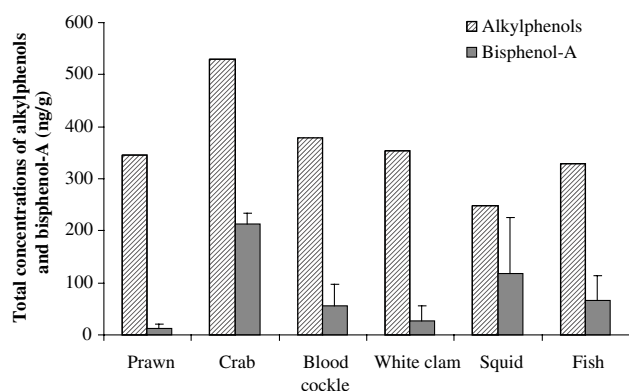


Fig. 3. Total alkylphenol and bisphenol-A in seafood samples from a local supermarket.

samples. The sand-dwelling, filter-feeding white clam appeared to be the least contaminated seafood item with the lowest amounts of total alkylphenols, although the tissue concentration of 2,4-dichlorophenol exceeded 100 ng g⁻¹ w.w. (see Table 3). Alkylphenols in squid muscle ranged between 4.1 and 84.1 ng g⁻¹ w.w. Interestingly, the lowest concentration of bisphenol-A was recorded in prawns (13.3 ng g⁻¹ w.w.). Our preliminary survey indicates that alkylphenols and bisphenol-A are present in seafood products commercially available to the public in Singapore. In comparison, mean octophenol and nonylphenol values obtained from clams and squid from the Adriatic coast of Italy were similar or higher (octylphenol: 2.7–18.6 ng g⁻¹ w.w.; nonylphenol: 243–696 ng g⁻¹ w.w.; Ferrara et al., 2001). Few other studies have examined alkylphenols in marine organisms, but freshwater fish analyzed for nonylphenol contained up to 112 ng g⁻¹ w.w. in the Saar River in Germany (Wenzel et al., 2004), 110 ng g⁻¹ w.w. in Lake Biwa in Japan (Tsuda et al., 2000) and 300 ng g⁻¹ w.w. in the Sakarya River in Turkey (Uguz et al., 2003). These values are generally higher than the values obtained for the marine pelagic fish *Decapterus russelli* examined in this study.

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References

- Ahel, M., Giger, W., Schaffner, C., 1994. Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment—II. Occurrence and transformation in rivers. *Water Research* 28, 1143–1152.
- Ahel, M., Molnar, E., Ibric, S., Giger, W., 2000. Estrogenic metabolites of alkylphenol polyethoxylates in secondary sewage effluents and rivers. *Water Science and Technology* 42, 15–22.
- Bennie, D.T., Sullivan, C.A., Lee, H.-B., Peart, T.E., Maguire, R.J., 1997. Occurrence of alkylphenols and alkylphenol mono- and diethoxylates in natural waters of the Laurentian Great Lakes Basin and the upper St Lawrence River. *Science of the Total Environment* 193, 263–275.
- Blackburn, M.A., Waldock, M.J., 1995. Concentrations of alkylphenols in rivers and estuaries in England and Wales. *Water Research* 29, 1623–1629.
- Bols, N.C., Brubacher, J.L., Ganassin, R.C., Lee, L.E.J., 2001. Ecotoxicology and innate immunity in fish. *Developmental and Comparative Immunology* 25, 853–873.
- Bolten, N.J., Tapanainen, J., Kovisto, M., Vihko, R., 1989. Circulating sex hormone binding globulin and testosterone in newborn and infants. *Clinical Endocrinology* 37, 201–207.
- Bragadin, M., Perin, G., Iero, A., Manente, S., Rizzoli, V., Scutari, G., 1999. An in vitro study on the toxic effects of nonylphenols (NP) in mitochondria. *Chemosphere* 38, 1997–2001.
- Déchaud, H., Ravard, C., Claustrat, F., de la Perrière, A.B., Pugeat, M., 1999. Xenoestrogen interaction with human sex hormone-binding globulin (hSHBG). *Steroids* 64, 328–334.
- Dodds, E., Lawson, W., 1938. Molecular structure in relation to estrogenic activity. Compounds without a phenanthrene nucleus. *Proceedings of the Royal Society of London B* 125, 222–232.
- Environmental Protection Agency 1984. Methods 604. Phenols in Federal Register. October 26, Part VIII, 40, cfr, Part 136, 58.

- Evans, S.M., Kerrigan, E., Palme, N., 2000. Causes of imposex in the dogwhelk *Nuccella lapillus* (L.) and its use as a biological indicator of tributyltin contamination. *Marine Pollution Bulletin* 40, 212–219.
- Fendinger, N.J., Begley, W.M., McAvoy, D.C., Eckoff, W.S., 1995. Measurement of alkylphenol ethoxylate surfactants in natural waters. *Environmental Science and Technology* 29, 856–863.
- Ferrara, F., Fabietti, F., Delise, M., Bocca, A.P., Funari, E., 2001. Alkylphenolic compounds in edible molluscs of the Adriatic Sea (Italy). *Environmental Science and Technology* 35, 3109–3112.
- Gerhard, I., Frick, A., Monga, B., Runnebaum, B., 1999. Pentachlorophenol exposure in women with gynecological and endocrine dysfunction. *Environmental Research* 80, 383–388.
- Heemken, O.P., Reincke, H., Stachel, B., Theobald, N., 2001. The occurrence of xenoestrogens in the Elbe River and the North Sea. *Chemosphere* 45, 254–259.
- Hormonally Active Agents in the Environment, 2000. National Academy Press, Washington, DC.
- Howdeshell, K.L., Hotchkiss, A.K., Thayer, K.A., Vandenbergh, J.G., Vom Saal, F., 1999. Exposure to BPA advance puberty. *Nature* 401, 763–764.
- Kingston, H.M., Haswell, S.J., 1997. Microwave-Enhanced Chemistry. American Chemical Society, Washington, DC.
- Koh, H.L., Lim, P.E., Sieh, K.C., Zamali bin Midun, 1991. Simulation of hydrodynamic regimes in the Johor Strait, Malaysia. In: Chou, L.M., Chua, T.E., Khoo, H.W., Lim, P.E., Paw, J.N., Silvestre, G.T., Valencia, M.J., White, A.T., Wong, P.K. (Eds.), Towards an integrated management of tropical coastal resources. ICLARM Conference Proceedings 22, National University of Singapore, Singapore National Science and Technology Board and International Center for Living Aquatic Resources Management, Philippines, pp. 103–107.
- Lim, L.C., 1984a. Coastal fisheries oceanographic studies in Johor Strait, Singapore II. Hydrological condition in the East Johor Strait. *Singapore Journal of Primary Industries* 12, 17–39.
- Lim, L.C., 1984b. Coastal fisheries oceanographic studies in Johor Strait, Singapore III. Hydrological condition in the West Johor Strait. *Singapore Journal of Primary Industries* 12, 128–146.
- Markey, C.M., Michaelson, C.L., Sonnenschein, C., Soto, A.M., 2001. Alkylphenols and bisphenol A as environmental estrogens. In: Metzler, M. (Ed.), *Endocrine Disruptors Part I*. Springer Verlag, Berlin, Heidelberg, pp. 127–153.
- Matsumoto, G., Ishiwatari, R., Hayna, T., 1977. Gas chromatographic-mass spectrometric identification of phenols and aromatic acids in river waters. *Water Research* 11, 693–698.
- Nakazawa, H., Yamaguchi, A., Inoue, K., Yamazaki, T., Kato, K., Yoshimura, Y., Makino, T., 2002. In vitro assay of hydrolysis and chlorohydroxy derivatives of bisphenol A diglycidyl ether for estrogenic activity. *Food and Chemical Toxicology* 40, 1827–1832.
- Oehlmann, J., Schulte-Oehlmann, U., Tillmann, M., Markert, B., 2000. Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the laboratory part I. Bisphenol-A and octylphenols as xeno-estrogens. *Ecotoxicology* 9, 383–397.
- Routledge, E.J., Sheahan, D., Desbrow, C., Brighty, G.C., Waldock, M., Sumpter, J.P., 1998. Identification of estrogenic chemicals in STW effluent.2. In vivo responses in trout and roach. *Environmental Science and Technology* 32, 1559–1565.
- Staples, C.A., Dorn, P.B., Klecka, G.M., O'Block, S.T., Harris, L.R., 1998. A review of the environmental fate, effects, and exposures of bisphenol A. *Chemosphere* 36, 2149–2173.
- Sonnenschein, C., Soto, A.M., 1998. An updated review of environmental estrogen and androgen mimics and antagonists. *Journal of Steroid Biochemistry and Molecular Biology* 65, 143–150.
- Talmage, S.S., 1994. Environmental and Human safety of major surfactants. In: *Alcohol Epoxylates and Alkylphenol Epoxylates*. The Soap and Detergent Association. Lewis Publication, Boca Raton, Florida, p. 374.
- Tsuda, T., Takino, A., Kojima, M., Harada, H., Muraki, K., Tsuji, M., 2000. 4-nonylphenols and 4-tert-octylphenol in water and fish from rivers flowing into Lake Biwa. *Chemosphere* 41, 757–762.
- Uguz, C., Togan, I., Eroglu, Y., Tabak, I., Zengin, M., Iscan, M., 2003. Alkylphenol concentrations in two rivers of Turkey. *Environmental Toxicology and Pharmacology* 14, 87–88.
- Vom Saal, F.S., Cooke, P.S., Buchanan, D.L., Palanza, P., Thayer, K.A., Negel, S.C., Parmigiani, S., Welshons, W.V., 1998. A physiologically based approach to the study of bisphenol-A and other estrogenic chemicals on the size of reproductive organs, daily sperm production, and behavior. *Toxicology and Industrial Health* 14, 239–260.
- Wenzel, A., Böhmer, W., Müller, J., Rüdell, H., 2004. Retrospective monitoring of alkylphenols and alkylphenol monoethoxylates in aquatic biota from 1985 to 2001: Results from the German Environmental Specimen Bank. *Environmental Science and Technology* 38, 1654–1661.
- Wood, A.K.H., Ahmad, Z., Md. Shazili, N.A., Yaacob, R., Carpenter, R., 1997. Geochemistry of sediments in Johor Strait between Malaysia and Singapore. *Continental Shelf Research* 17, 1207–1228.