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The occurrence of selected antibiotics in Hong Kong coastal waters

A. Gulkowska [a], Yuhe He [a], M.K. So [a], Leo W.Y. Yeung [a], H.W. Leung [a], J.P. Giesy [a,b], Paul K.S. Lam [a], Michael Martin [a], Bruce J. Richardson [a,*]

[a] *Centre for Coastal Pollution and Conservation, Department of Biology and Chemistry, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong SAR, People's Republic of China*
[b] *Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Canada*

Pharmaceuticals and personal care products (PPCPs) are a group of emerging chemicals of environmental concern that have remained largely unrecognized until recent advances in low level analytical measurements (Cha et al., 2006; Erickson, 2002; Gros et al., 2006; Lindsey et al., 2001). The growing concern over the occurrence and fate of PPCPs has been attributed not only to the induction of adverse effects in terrestrial or aquatic organisms, but also the potential of some antibiotics to induce resistant bacterial strains in the environment (Hirsch et al., 1998).

Over 3000 chemical substances are used in human and veterinary medicine, including aquaculture and farming practices (Ternes et al., 2004). Such pharmaceuticals include antiphlogistics/anti-inflammatory drugs, β-blockers, lipid regulators, antiepileptics and antibiotics, the latter being amongst the most important environmentally (Petrović et al., 2005). Unlike other micro-pollutants and pesticides, antibiotics are not rigorously tested for environmental fate and effects (Jones et al., 2001), resulting in their ubiquitous distribution in the environment.

The occurrence of pharmaceuticals in the environment has been reviewed in detail by Heberer (2002) and Halling-Sørensen et al. (1998) and references therein. Antibiotics and their metabolites primarily enter the aquatic environment *via* wastewater treatment systems following human consumption and excretion (Daughton and Ternes, 1999). Many researchers have demonstrated the incomplete removal of pharmaceuticals during wastewater treatment processes (Daughton and Ternes, 1999; Hirsch et al., 1999; Öllers et al., 2001; Stumpf et al., 1999; Ternes, 1998); in addition, the high water solubility and poor degradability of antibiotics enhances their passage through filtration, and thus their entry into groundwater and drinking water (Reddersen et al., 2002; Ternes et al., 2002). Other potential sources include unintentional discharges during manufacturing processes; agricultural application of veterinary drugs (including poultry production and fish farming applications); and discharges from landfill leachate (Daughton and Ternes, 1999; Holm et al., 1995; Ternes et al., 2004).

In order to maintain the medicinal effects of pharmaceuticals for prolonged periods, most compounds are lipophilic, able to pass through membranes, and manufactured to resist inactivation before providing their effect (Halling-Sørensen et al., 1998). Although the environmental concentrations of pharmaceuticals are generally low, their properties, together with their continuous usage, eventually result in concentrations that may be sufficient to provoke toxic effects in aquatic or terrestrial ecosystems. Importantly, the synergistic effects of different chemicals sharing common mechanisms of action could be substantial (Daughton and Ternes, 1999).

* Corresponding author. Tel.: +852 2788 7042; fax: +852 2788 7406.
E-mail address: bhbrucer@cityu.edu.hk (B.J. Richardson).

Limited monitoring of pharmaceuticals in the aquatic environment has been conducted in Sweden (Bendz et al., 2005); northern Colorado (Cha et al., 2006; Kim and Carlson, 2007); Vietnam (Le and Munekage, 2004), Idaho (Batt et al., 2006); Germany (Sacher et al., 2001); the United Kingdom (Ashton et al., 2004; Thomas and Hilton, 2004) and Italy (Calamari et al., 2003). However, data from Asian countries, including Hong Kong and China, are especially scarce. To date, there has been only one report demonstrating the detection of ofloxacin, nonfloxacin, roxithromycin and erythromycin–H_2O at very low concentrations in Victoria Harbour, Hong Kong; sulfadiazine, sulfadimidine, sulfamethoxazole and chloramphenicol were also quantified in the Pearl River, south China, at comparatively higher concentrations (Xu et al., 2007).

The global annual usage of antibiotics has been estimated to be between 100,000 and 200,000 tons, with over 25,000 tons used in China (Kümmerer, 2003). In addition, statistics indicate that more than 70% of drug prescriptions in China are for antibiotics, as compared to 30% in western countries. It has also been estimated that, in China, 15,770 tons per annum of antibiotics were utilized for human usage, with equal or possibly larger amounts used in the agricultural sector (Richardson et al., 2005). The extensive use of antibiotics in China and Hong Kong (including the Pearl River Delta) may imply environmental occurrence at higher concentrations than other parts of the world. In order to monitor baseline concentrations of antibiotics in the marine environment of Hong Kong, this study was conducted to (i) determine the concentrations of five important classes of antibiotics (including penicillins, cephalosporins, quinolones, macrolides, tetracyclines and trimethoprim) in surface water samples from five selected locations; and (ii) assess the potential risk of these antibiotics to the marine ecosystem in Hong Kong.

Antibiotics selected for this survey were chosen *via* anecdotal evidence of the most frequently used pharmaceutical compounds in China. Erythromycin (ERY) and trimethoprim (TMP) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Tetracycline hydrochloride (TET), norfloxacin (NOR), potassium salts of penicillin G (PEN G) and penicillin V (PEN V) and cefalexin (CLX) were obtained from Riedel-de Haën (Seelze, Germany). The sodium salts of cefotaxim (CTX) and cefazolin (CFZ) were purchased from Fluka (Buchs, Switzerland). Oasis® Hydrophilic-Lipophilic Balanced (HLB; 6 cc, 200 mg) solid phase extraction (SPE) cartridges were purchased from Waters (Milford, MA). Milli-Q water was used throughout the study. HPLC-grade methanol and acetonitrile, formic acid (99%) and disodium ethylenediamine tetra-acetate (Na_2EDTA) were purchased from Wako Pure Chemical Industries Ltd. (Japan). Ammonium hydroxide (NH_4OH) was obtained from Riedel-de Haën (Seelze, Germany).

Individual stock antibiotic solutions were prepared by dissolving the compounds in the corresponding solvent solutions at a concentration of 100 μg/mL. Group 1 chemicals included ERY, TET, NOR and TMP, and were dissolved in methanol; Group 2 chemicals included PEN V, PEN G, CLX, CTX and CFZ, and were dissolved in Milli-Q water. The stock solutions were covered by alumina foil to prevent photodegradation and were kept at 4 °C in the refrigerator. New stock solutions were made every three months. Mixed working solutions (1, 5, 10, 20, 50 and 100 ng/mL) were freshly prepared by diluting the individual stock solutions with the same solvent in polypropylene tubes which were stored at 4 °C in the dark.

Surface seawater samples were collected from five locations (Ma Wan, MW; Tsing Yi, TY; Victoria Harbor, VH; Kwun Tong, KT; Tung Lung Chau, TLC) in Hong Kong on 27th December 2006 (Fig. 1). Locations TY, VH and KT were further divided into two sub-areas and were chosen near points of effluent disposal from sewage treatment plants (STPs); two locations (MW and TLC) were selected as reference sites. Detailed information for sampling locations is summarized in Table 1.

Surface water samples (2 L) were collected from each location using a stainless steel bucket which was precleaned by rinsing (in sequence) with methanol, Milli-Q water, and water from the specific location. Water samples were stored in 1 L polypropylene bottles, kept in ice boxes and transported to the laboratory. The samples were then vacuum filtered through glass fiber filters (4.5 μm, Advantec, Toyo Roshi Kaisha Ltd., Japan) to remove particulate matter prior to extraction. The filtered samples were stored in the dark at 4 °C and were extracted within 48 h following collection.

Prior to extraction, 10 mL 5% (w/v) Na_2EDTA were added to 1 L samples as a chelating agent. Extraction was divided into two categories as follows. Group 1: pretreatment of water samples to pH 3 using 5 M formic acid, for the extraction of ERY, TET, NOR and TMP; Group 2: pretreatment of water samples to pH 7.5–8.0 using 0.01% NH_4OH solution for the extraction of PEN V, PEN G, CLX, CTX and CFZ. Samples were extracted using Oasis HLB extraction cartridges. Cartridges were preconditioned prior to loading water samples. For Group 1 chemicals, the cartridges were conditioned by elution with 4 mL acetonitrile followed by 4 mL water; for Group 2 chemicals by elution with 4 mL methanol followed by 4 mL water. An aliquot of water sample (500 mL) was loaded onto the cartridge, eluted at a rate of 1 drop/s, and the eluate discarded. The cartridge was then washed with 4 mL water to remove excess Na_2EDTA. Finally, the target fraction was eluted with 4 mL acetonitrile or methanol for Group 1 and 2 chemicals, respectively. The eluate was reduced to 0.5 mL under a gentle stream of nitrogen. Water was added to the eluate to a final volume of 2 mL, thoroughly mixed, and then transferred into an amber autosampler vial for chemical analysis.

Concentrations of antibiotics in seawater samples were analyzed using high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS). Separation of analytes was performed using an Agilent HP1100 liquid

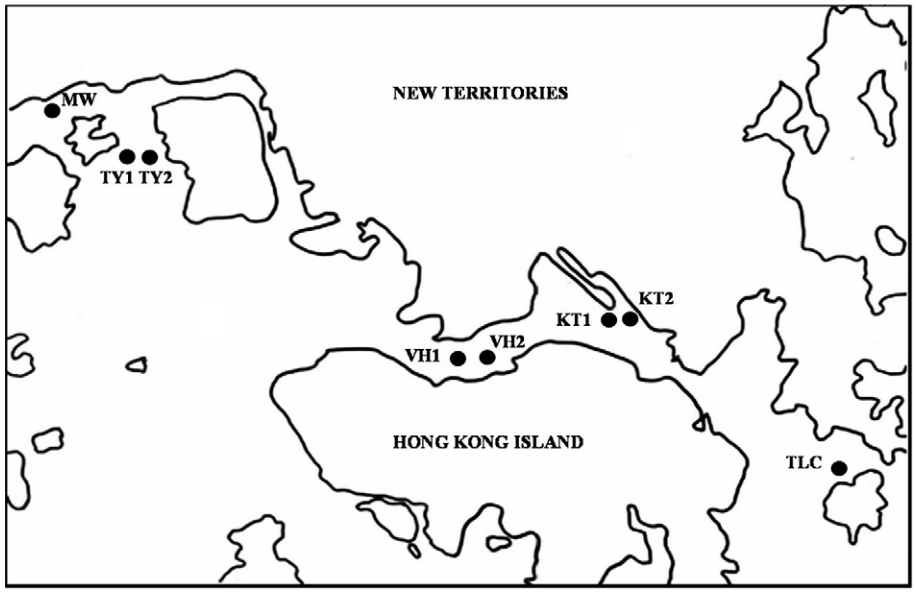

Fig. 1. Map showing sampling locations of surface seawater samples.

Table 1
Information concerning sampling locations for seawater samples in Hong Kong coastal waters (see Fig. 1 for map locations)

Sample ID	GPS	Description
MW	22″21′390 114″03′697	Located near Park Island in Ma Wan; selected as a reference location
TY1	22″20′366 114″04′020	Located near Tsing Yi; waters influenced by outfall from Stonecutters Island STP
TY2	22″20′489 114″04′132	
VH1	22″17′179 114″10′420	Located within Victoria Harbour; waters influenced by outfall from Wan Chai STP
VH2	22″17′014 114″10′775	Located farther away from the Wan Chai STP outfall
KT1	22″18′135 114″13′020	Located near Kwun Tong; waters influenced by outfall from Shatin and Tai Po STPs
KT2	22″18′327 114″13′362	Located farther away from Shatin and Tai Po STP outfall
TLC	22″15′713 114″16′876	Located in Tung Lung Chau; selected as a reference location

chromatograph (Agilent, Palo Alto, CA) interfaced with an Applied Biosystems API 2000 triple quadrupole tandem mass spectrometer equipped with a Turbo IonSpray source operated in both negative and positive mode. A 10 μL aliquot of extract was injected onto an XBridge™ C_{18} column (Waters Corp., 2.1 mm i.d. × 50 mm length, 5 μm) equipped with a guard column (Waters Corp., XBridge™ C_{18}, 5 μm, 2.1 mm i.d. × 10 mm length). 0.01% formic acid/water and methanol were used as mobile phases starting at 10% methanol. At a flow rate of 300 μL/min, the gradient was increased to 90% methanol at 10 min before reverting to the original conditions at 13 min. The declustering potential, collision energy, ionization mode and MS/MS parameters for the instrument were optimized individually for each analyte and are summarized in Table 2.

Each ion of interest in the chromatogram was selected and integrated. Concentrations of target analytes were quantified utilising external calibration curves, constructed using external standards of six different concentrations (1, 5, 10, 20, 50 and 100 ng/mL). Standard calibration curves exhibited excellent linearity (correlation coefficient >0.99). Final extracts where concentrations fell outside the range of calibration curves were diluted with methanol to an appropriate factor and re-injected.

It has been demonstrated that ERY, readily dehydrated by the loss of one water molecule, is immediately converted into ERY–H_2O at a pH value below 7.0 (Batt and Aga, 2005). The acid degradation of ERY in aqueous solution has been characterized and the mechanisms have been described (Volmer and Hui, 1998). The degraded form of ERY is most frequently monitored and detected in the environment (Hirsch et al., 1999), and the concentrations of ERY–H_2O are reported in the present study. ERY–H_2O was measured following the method described by McArdell et al. (2003), with modification. The pH of aqueous solutions was adjusted by 5 M formic acid, instead of using 3 M sulphuric acid. Phosphoric acid could not be used as it has been shown to cause degradation of antibiotics during the evaporation process (Lindsey et al., 2001).

Procedural and field blanks, as well as procedural and matrix spike recoveries were conducted to ensure the accuracy of the sampling, extraction and analytical procedures. Field blanks were used to check possible sources of contamination during sampling and were performed using

Table 2
MS/MS parameters for individual antibiotics

Analyte	Acronym	Mode of ionization	Transition monitored	Declustering potential (V)	Collision energy (eV)
Erythromycin	ERY	+	*716 → 158*	42	38
			716 → 558	33	26
Norfloxacin	NOR	+	320 → 302	39	34
			320 → 276	38	31
Trimethoprim	TMP	+	*291 → 123*	57	34
			291 → 261	46	31
Tetracycline	TET	+	*445 → 410*	33	36
			445 → 428	54	34
Penicillin G	PEN G	–	*333 → 192*	−31	−13
			333 → 171	−40	−21
Penicillin V	PEN V	–	*349 → 208*	−40	−21
			349 → 114	−36	−25
Cefazolin	CFZ	–	*453 → 321*	−25	−17
			453 → 251	−22	−17
Cefotaxime	CTX	–	*454 → 239*	−60	−26
			454 → 394	−30	−17
Cephalexin	CLX	–	346 → 268	−45	−17
			346 → 189	−30	−17

pre-filled individual 500 mL water bottles, transported to the field and exposed to the same conditions as the real water samples, then returned for analysis. Procedural blanks were used to check possible sources of contamination during extraction procedures. Both procedural and field blanks were extracted following the same procedures as detailed above, except that Milli-Q water was used. The concentrations of all target analytes in the blank samples were below the corresponding method detection limits (MDLs). MDLs were determined following US EPA guidelines (Berthouex and Brown, 2002) and were defined as the variability of multiple analyses of seven Milli-Q and surface seawater samples spiked at 5 and 20 μg/L, respectively. MDLs were calculated by multiplying the standard deviation of each corresponding analyte in all seven samples by the Student's *t*-variate for a one-sided *t*-test at the 99% confidence level with a degree of freedom of $n - 1$. The MDLs ranged from 2 to 22 ng/L for Milli-Q water and 2 to 13 ng/L for seawater samples.

Recovery tests were performed by spiking a mixture of external standards (100 ng/mL, 200 μL) to the same volume of Milli-Q or seawater sample, followed by extraction in duplicate simultaneously with duplicate unspiked samples using the same extraction procedures as environmental samples. The sample eluates of the unspiked samples were then spiked with an equivalent volume of standard as the spiked samples to simulate a theoretical 100% recovery. The relative recovery was then calculated as the ratio of the average concentration of analyte in pre-spiked samples to those in post-spiked samples. The results for the recovery tests are summarized in Table 3. In general, acceptable recoveries were achieved for all target chemicals ranging from 71% to 100% for procedural recovery and 93% to 116% for matrix-spike recovery. All reported data were not corrected for recoveries.

The concentrations of PEN V, PEN G, CTX and CFZ were below MDLs in all samples. It has been suggested that penicillins can be easily hydrolyzed in the aquatic environment (Daughton and Ternes, 1999), which may explain these results. The concentrations of the remaining five antibiotics (ERY–H_2O, TET, NOR, TMP and CLX) are summarised in Table 4.

ERY–H_2O was detected in all the samples and the concentrations ranged from 9.50 to 486 ng/L. CLX, TMP, TET and NOR were detected at lower frequencies and concentrations, which ranged from <MDL to 182 ng/L, <MDL to 21.8 ng/L, <MDL to 122 ng/L and <MDL to 8.00 ng/L, respectively. The greatest concentrations of ERY–H_2O (486 ng/L), TET (122 ng/L), NOR (8.00 ng/L), TMP (21.8 ng/L) and CLX (182 ng/L) were observed in surface waters collected from location VH2, situated within Victoria Harbour. The next highest concentrations of ERY–H_2O (87.0 ng/L) and TMP (9.70 ng/L) were measured in samples collected at location KT2. The high concentrations at these locations may be due to proximity to the Wan Chai East outfall, and Shatin and Tai Po STP outfalls. Victoria Harbour receives domestic sewage from the nearby urbanized areas of Hong Kong Island and

Table 3
Recoveries (%), field blanks and procedural blanks (ng/L) for individual antibiotics (acronyms as in Table 2)

Target analyte	Procedural blank (ng/L)	Field blank (ng/L)	Procedural recovery (%) ($n = 12$)	Mean matrix-spike recovery (%) ($n = 2$)
ERY–H_2O	<3	<3	100	100
TET	<5	<5	95	99
NOR	<18	<18	94	116
TMP	<2	<2	97	101
PEN V	<22	<22	100	96
PEN G	<8	<8	99	95
CLX	<12	<12	80	94
CTX	<22	<22	89	93
CFZ	<5	<5	71	103

Table 4
Concentrations of individual antibiotics in surface waters from Hong Kong (acronyms as in Table 2)

Sampling locations	Concentration (ng/L)				
	ERY–H_2O	TET	NOR	TMP	CLX
MW	16.0	3.00	<MDL	<MDL	15.1
TY1	19.1	<MDL	<MDL	2.30	10.8
TY2	14.9	<MDL	<MDL	4.30	10.0
VH1	56.6	12.0	2.30	4.20	47.0
VH2	486	122	8.00	21.8	182
KT1	40.3	2.30	<MDL	5.50	34.5
KT2	87.0	6.40	<MDL	9.70	41.2
TLC	9.50	<MDL	<MDL	<MDL	<MDL

MDL, method detection limit.

Kowloon. As most sewage treatment facilities have not been designed for antibiotic removal in effluents (Göbel et al., 2004; Lindberg et al., 2005), it is not surprising that the comparatively higher antibiotic concentrations occurred within Victoria Harbor.

Dilution effects may be observed in the comparatively lower concentrations of antibiotics at VH1 and KT1, locations that are situated farther away from effluent outfalls. Only a trace amount of ERY–H_2O (9.50 ng/L) was found in samples from TLC, the reference point. Similarly, relatively small amounts of ERY–H_2O (16.0 ng/L), TET (3.00 ng/L) and CLX (15.1 ng/L) were detected in samples from the other reference station, MW. It should be noted that the water quality at MW may be affected, to a certain extent, by discharges from the Pearl River in China.

A comparison of four selected antibiotic concentrations (ERY–H_2O, TET, NOR and TMP) in surface waters from different countries is shown in Table 5. Exceptionally high concentrations of NOR and TMP were detected in surface (NOR, 6,060,000 ng/L; TMP, 1,040,000 ng/L) and bottom (NOR, 4,040,000 ng/L; TMP, 2,030,000 ng/L) layer waters in shrimp ponds in Vietnam, in which antibiotics are often applied for disease prevention (Le and Munekage, 2004). Apart from this extreme situation, concentrations of TET and TMP recorded in Hong Kong coastal waters were, in general, comparable to those measured in other countries, including Snake Creek USA, River Tyne UK, and the Ebro River, Spain (Gros et al., 2006; Lindsey et al., 2001; Roberts and Thomas, 2006). The maximum TMP concentration from VH2 (21.8 ng/L) measured in the present study was relatively lower than levels in River Lutter (120 ng/L) from Germany (Hirsch et al., 1998), even though the sample was taken from the vicinity of a municipal STP. The maximum NOR concentration (8.0 ng/L) was comparable to the levels measured in Victoria Harbour in a previous study (Xu et al., 2007). However, it was lower than the levels downstream of a STP in Brisbane, Australia (n.d. – 80 ng/L; Costanzo et al., 2005) and in the Pearl River in China (13 and 251 ng/L) in areas where the river is the sole water receiver for treated and untreated wastewater from Guangzhou (Xu et al., 2007).

The concentrations of ERY–H_2O in the present study were found to be generally greater than those from the

Table 5
Global comparison of antibiotic concentrations in surface water samples

Sampling location	Sample type	Concentration (ng/L)				Reference
		ERY–H_2O	TET	NOR	TMP	
Hong Kong coastal environment	Surface seawater	9.50–486	<MDL-122	<MDL-8.00	<MDL-21.8	Present study
Victoria Harbour, Hong Kong	Surface seawater	28.1[a] (2004) 12.3[a] (2005)	n.a.	5.2[a] (2004) 4.2[a] (2005)	n.a.	Xu et al. (2007)
Pearl River, China	Surface seawater	423[a] (high water season) 636[a] (low water season)	n.a.	13[a] (high water season) 251[a] (low water season)	n.a.	
River Tyne, United Kingdom	Surface river water	<4–70	n.a.	n.a.	4–19	Roberts and Thomas (2006)
Shrimp ponds in mangrove areas, Vietnam	Surface layer water	n.a.	n.a.	60,000–6,060,000	80,000–1,040,000	Le and Munekage (2004)
	Bottom layer water	n.a.	n.a.	80,000–4,040,000	80,000–2,030,000	
River Lutter in Bielefeld, Germany	River water	620	n.a.	n.a.	120	Hirsch et al. (1998)
Ebro River, Spain	Surface river water	n.a.	n.a.	n.a.	<LOD-20	Gros et al. (2006)
Downstream of a STP in Brisbane, Australia	Surface river water	n.a.	n.a.	n.d. – 80	n.a.	Costanzo et al. (2005)
Po River, North Italy	Surface river water	1.40–15.9	n.a.	n.a.	n.a.	Calamari et al. (2003)
	Surface river water	4.5	n.a.	n.a.	n.a.	
Snake Creek, United States	Surface water	n.a.	110	n.a.	n.a.	Lindsey et al. (2001)

n.a., not analyzed; n.d., not detected; LOD, limit of detection.
[a] The maximum concentration of antibiotic was reported.

Po River in North Italy (Calamari et al., 2003), but were comparable to the levels from the Pearl River in China (Xu et al., 2007). It should be noted that the concentrations of ERY–H_2O measured in Victoria Harbor during our study, and that of Xu et al. (2007), vary considerably. This could be the result of differences in sampling times and locations. In addition, in the present study, water samples were collected near the outfall of STPs which may not have been the case in the study of Xu et al. (2007).

An evaluation of the ecological risk to aquatic organisms associated with exposure to ERY and TMP was performed by comparing the measured concentrations of ERY and TMP to predicted no effect concentrations (PNECs). The PNECs for ERY and TMP are 78,000 and 26,264 ng/L, respectively (Ashton et al., 2004). The concentrations of both antibiotics detected in the waters of the Hong Kong coastal environment are all significantly lower than the corresponding PNEC, indicating a minimal risk to aquatic organisms. Nevertheless, uncertainty exists in the risk characterisation process, as PNECs are derived from traditional toxicity end-point data, in which these substances may have different modes of actions and the chronic effects may not have been taken into consideration (Ashton et al., 2004).

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Detection of high concentrations of ^{137}Cs in Walleye pollock collected in the Sea of Japan

Takami Morita *, Ken Fujimoto, Yoko Minamisako, Katsuhiko Yoshida

Marine Productivity Division, National Research Institute of Fisheries Science, Fisheries, Research Agency, Fukuura 2-12-4, Kanazawa-ku, Yokohama, Kanagawa 236-8648, Japan

^{137}Cs is an anthropogenic gamma emitting radionuclide, and has a long physical half-life (about 30.1 years) and a tendency to accumulate in muscle tissues (Young and Folsom, 1979). These characteristics have made ^{137}Cs an important indicator of radionuclide pollution (Yoshitome et al., 2003). In Japan, much attention has been paid to the ^{137}Cs concentrations in marine organisms, which are important food sources (Nagaya et al., 1990; Kasamatsu and Ishikawa, 1997). Most ^{137}Cs detected in marine organisms collected around Japan at present has been attributed to well-known sources, i.e., the global fallout brought from the atmospheric nuclear weapon tests from 1954 to 1980 and the Chernobyl accident on April 26, 1986 (Igarashi et al., 1996).

The National Research Institute of Fisheries Science (NRIFS) has carried out a long term monitoring program for radioactive pollution in marine organisms caught around Japan in order to confirm the safety of these organisms as food sources. In this monitoring program, all samples were commercially collected and prepared to subsamples including the whole-body (all internal organs removed), muscles, livers, internal organs (without the livers the genitals), testes and ovaries. Each small subsample from one individual contained such low concentrations of the artificial radionuclides that they could not be detected, so samples for measurement were prepared by pooling subsamples from individuals. The whole-body, muscle, internal organs, testes and ovary samples consisted of tissues from 4 to 6, 10 to 30, 4 to 6, 15 to 20 and 15 to 20 individuals, respectively. All subsamples were dried in an oven, carbonized in a gas furnace, and ashed in an electric furnace. Gamma rays were measured using a high pure germanium semiconductor detector and all radioactivity measurements were corrected for decay from the sampling date. The sampling points and date for the species used in this study are shown in Table 1.

We detected high concentrations of ^{137}Cs in certain Walleye pollock, *Theragra chalcogramma*, caught in our monitoring program in 2000. Table 2 presents the concentrations of ^{137}Cs in each part of this species in the period 1996–2004. The normality of the data set, except for 2000, of ^{137}Cs concentrations in the muscle and the whole-body samples at each site was first assessed using the Chi square test for goodness of fit. All data sets except for the muscle sample at site D were normally distributed ($P < 0.05$). The Kolmogorov–Smirnov test indicated that ^{137}Cs concentrations in 2000 in the muscle samples (sampling sites A, B and C) and in the whole-body samples (sampling sites A and C) were significantly higher than

* Corresponding author. Tel./fax: +81 45 788 7654.
E-mail address: takam@affrc.go.jp (T. Morita).