Occurrence, ecological risk assessment and source apportionment of pharmaceuticals, steroid hormones and xenoestrogens in the Ghanaian aquatic environments

Joseph K. Adjei a,1, Alberta D. Dayie a, Justice K. Addo a, Anita Asamoah b, Ernest O. Amoako a, Benedicta Y. Ego b, Ebenezer Bekoe a, Nathaniel O. Ofori a, George A. Adjei a, David K. Essumang a

a Environmental Research Group, Department of Chemistry, University of Cape Coast, Ghana
b Environmental Resources Centre, Ghana Atomic Energy Commission, Ghana

A R T I C L E   I N F O

Handling Editor: Lawrence Lash

Keywords:
Xenoestrogens
APCS-MLR receptor model
Bisphenol A
Ecological risk quotients
Endocrine disruptors
Aquatic environment

A B S T R A C T

Elevated levels of pharmaceuticals, steroid hormones and xenoestrogens (PSHXEs) in the aquatic environment pose a serious threat to the ecological balance. The endocrine disrupting PSHXEs in aquatic systems are linked to several adverse effects like reproductive health impairment, feminization, high mortality rate, decreased biodiversity etc. This study, therefore, sought to investigate the occurrence and the ecological risks posed by some selected PSHXEs and also conduct source apportionment of the PSHXEs in the Ghanaian aquatic environments. A total of 48 samples comprising 24 sediments and water each were taken from six waterbodies in Ghana. The samples were extracted using SPE cartridges for water and QuEChERS-dSPE for sediments. The analyses were done using Shimadzu Prominance UFLC 20A series. Ecological risk assessments were also conducted with the aid of USEPA T.E.S.T., whereas source apportionments were conducted using the APCS-MLR receptor model. Elevated mean total levels of PSHXEs ranging between 12,187 and 52,117 ng/L and 2,022-6,047 ng/g for water and sediment samples respectively were found. The risk quotients (RQ < 1) suggested a high risk posed by PSHXEs in water to organisms at the three trophic levels and also to benthic organisms in sediments of the Ghanaian aquatic environments for a short-term period. The APCS-MLR receptor model suggested three statistically significant sources (p < 0.05) designated by signature PSHXEs as domestic (major), mix hospital and industrial and agricultural waste sources. The source apportionment suggested increased use of steroid estrogens and anabolic drugs among the Ghana populace.

1. Introduction

Pharmaceuticals, steroid hormones and xenoestrogens (PSHXEs) belong to a class of ubiquitous chemicals considered contaminants of emerging concern [1] and 21st-century global environmental issues because of their potency to impact negatively on the health of humans and other organisms [2,3]. Most compounds in this class are endocrine disrupting chemicals (EDC), and they include bisphenol A (BPA), estrone (E1), 17α-estradiol (αE2), 17β-estradiol (βE2), estriol (E3), 17α-ethinylestradiol (EE2), 4-para-nonylphenol (4-NP) and 4-tert-octylphenol (4-t-OP), chlorinated phenols, some phthalates and several others [4-6].

The ubiquitous xenoestrogens such as 4-NP, 4-t-OP and BPA are respectively listed among the EU “priority hazardous” and “priority substances” in the aquatic environment [7]. The three compounds βE2, EE2, and diclofenac, were also included in the EU list of substances of possible concern in the aquatic environment [7,8].

According to literature, all the 177 EDC identified internationally by WHO also have an impact on the nervous system, thus may be collectively termed endocrine and nervous disruptors [9-14].

Literature reports that early-life exposure to even low doses of BPA adversely affects brain function and behaviour in children and future liver function and also induces hepatocarcinogenesis in the liver [15-17]. Jia et al. [18] also associated BPA with carcinogenesis by the notice of direct activation of integrin β1 molecule. González-Rojro et al. [19] implicated BPA in male reproductive impairment, causing

* Corresponding author.
E-mail addresses: joseph.adjei@ucc.edu.gh, extrajoseph2007@yahoo.co.uk (J.K. Adjei).

https://doi.org/10.1016/j.toxrep.2022.06.011
Received 10 May 2022; Received in revised form 10 June 2022; Accepted 18 June 2022
Available online 22 June 2022
© 2022 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
transcription, apoptosis and epigenetic changes during spermatogenesis.

Petrick et al. [20] concluded that long-term oral use of exogenous hormones such as testosterone and related hormones, may be associated with an increased risk of intrahepatic cholangiocarcinoma, a common type of liver cancer.

Steroidal estrogens at pollutant levels have been linked with breast cancer in women and prostate cancer in men [21-23], for which scientific evidence predicted similar occurrences in fish and aquatic mammals. Ethinyl estradiol, a synthetic estrogen, is reported to be more persistent in the environment than natural estrogens and has been attributed to be the major cause of environmental pollution concern [21]. Studies have demonstrated that elevated concentrations of estrogens disrupted fish physiology and caused feminization of male fish by reducing testes size, lowering sperm count and quality, and inducing the production of vitellogenin as well as altering other sex characteristics [21,24-28]. Additionally, in fish, estrogen regulates behaviour patterns such as territorial aggression [29] and changes in immune responses [30,31]. Wojnarowski et al. [32] reported similar effects of estrogen on mammals in addition to disturbances in the regulation of both proapoptotic and anti-apoptotic processes, as well as the occurrence of neoplastic processes causing a drastic decrease in animal welfare. In mammals, elevated levels of estrogens are linked to reproductive disorders [33,34] and increased risk of carcinogenesis [21,35-37]. Additionally, in humans, estrogens have been associated with cancer of the prostate, lung, breast and liver endometriosis [35,36,38,39,20,37] as well as increased risk of cardiovascular diseases [40]. Elevated levels of endogenous estrogen and progesterone affect the central nervous system of women [41], thereby affecting different mood and cognitive processes [42,43]. Zucchi et al. [44] also reported that elevated levels of progesterone affect the gene expression in zebrafish during the early development.

Testosterone as EDC in the aquatic environment, is reported to cause a high proportion of males in some Aquatic organisms [45], the development of male secondary sexual characteristics in female fish, the inhibition of vitellogenin production, and the decrease in reproductive capacity [46,47]. Testosterone can also cause masculinization [48] and neuronal degeneration [49] to arise in mammals.

Primidone, a synthetic anticonvulsant drug [50], is linked with an increased risk of depression and thus suicidal thoughts and/or violent death actions [51]. According to the [52] monograph, primidone is possibly carcinogenic to humans (Group 2B).

Primidone, has been found in groundwater, spring water and well-water [53].

Over a decade, studies have reported the presence of these ubiquitous PSHXEs in several environmental sample matrices from most parts of the world. Most of the earlier literature reported, concentrated on point source samples from domestic and industrial wastewater treatment plants (WWTP) effluent, influents and sludge [54-64], agricultural environment and waste runoff water [65-70]. Quite recently, the presence of PSHXEs in samples from the aquatic environment; streams, sea, lakes, lagoons, rivers and estuaries ([71-81]), and groundwater [82,83], have also been reported.

The significant sources of PSHXEs in the aquatic environments, have been attributed to wastewater effluent from treatment plants [73,84,62], septic fields, [85], agricultural runoff [75], stormwater runoff [86], landfill leachate and biosolids [87,88] as well as effluent from PSHXEs manufacturing facilities, health-care facilities [89] and veterinary facilities [90-91].

Evidence in literature points out that PSHXEs, are ubiquitously present in the environment, being found in almost all matrices, and have detrimental implications on the health of flora and fauna, including humans, when exposed to elevated levels or even trace levels for an extended period. In most Sub-Sahara African countries, especially Ghana, the use of PSHXEs have surged over the decades, and due to the lack of effective drug use control measures, especially those acquired over the counter, coupled with improper disposal methods and lack of proper waste treatment facilities, levels of PSHXEs in the environment and for that matter the aquatic environment may have surged beyond pollution limits. Retrospectively, the elevated levels of these PSHXEs in the Ghanaian aquatic environment may have contributed to the decrease in biodiversity and increase in health-related incidences such as cancer, drug resistance, hormonal imbalances etc., in aquatic organisms, as well as in humans.

Most of the studies reported in literature worldwide aimed at determining the levels of PSHXEs, were done concentrating on point sources like wastewater facilities, storm runoffs, septic tanks, farmlands etc., and only a few studies have reported on the distribution of PSHXEs in the natural environment such as the aquatic environment. Additionally, scientific data on PSHXEs occurrences, the risk posed and sources in the aquatic environment are woefully sparse in sub-Saharan Africa, including Ghana. This study therefore, sought to investigate the occurrence and the ecological risk posed by the levels of some selected PSHXEs in the Ghanaian aquatic environment and further apportion sources of the PSHXEs in the Ghanaian aquatic environment. The study, possibly the first of its kind in Ghana, and Sub-Saharan Africa, also sought to provide scientific data that could inform policy.

2. Materials and methods

2.1. Solvents and reagents

LC grade acetonitrile and methanol, GC grade acetone, analytical grade ammonium formate, and formic acid were obtained from Merck international. The ten components steroids and mixed pharmaceuticals mix standards (200 μg/mL in Acetonitrile, Lot #: A0163575) from Restek Ltd. The roQ QuEChERS kits were from Phenomenex Inc. The chloramphenicol (lot #: L16101350) was from USBiological Life Sciences.

2.2. Sampling

Samples including water and sediments were taken from six (6) major and well-known water bodies in Ghana. Among the waterbodies were three (3) lagoons namely Fosu (FL), Chemu (CL), and Korle (KL), one lake: Ashaiman Dam (AL), and two (2) rivers: Densu (DR), and Kakum (KR) (Fig. 1). Upon careful demarcation of the water bodies for representative samples, four (4) samples each for sediments and water were taken from each waterbody, totalling 48 samples. Sediment samples were wrapped in pre-cleaned aluminium foils whereas water samples were collected in duplicates into pre-cleaned and dry 2.5 L amber glass reagent bottles and or in high-density polyethylene gallons and protected from sunlight. The water samples were preserved by adjusting the pH to about 5 with sulphuric acid.

2.3. Sample pre-treatments and extractions

2.3.1. Water

Samples with obvious particles were filtered and the filter paper was extracted with methanol thrice to remove adhered compounds to prevent losses. Before solid phase extraction (SPE), the pH of each 1.0 L water sample was adjusted to about pH = 6.0-6.5 using 25.0 mM ammonium formate buffered solution.

The water samples were extracted following USEPA method 539 with slight modification. The Strata-X-AW SPE cartridge (6 mL) was first conditioned with a 5.0 mL methanol and then equilibrated with 5.0 mL milliQ deionized water (18 MW·cm). The 1.0 L samples were loaded onto the SPE cartridges with the aid of a vacuum manifold at a flow rate between 8 and 10 mL/min. The loaded SPE cartridges were then washed with 5.0 mL 5.0 % methanol/water mixture. The cartridge was dried under vacuum for 5 min. Three successive elution of the analytes from the cartridge were done using 3.0 mL methanol each. The extracts were concentrated to near dryness using a pure stream of
nitrogen gas. It was then reconstituted with acetonitrile to 1.0 mL and filtered with a 0.22 µm disk filter into 1.5 mL vials prior to HPLC-UV instrumental analysis.

2.3.2. Sediment

The sediments samples were freeze-dried and pulverized to fine particles before extractions. The samples were extracted using a slightly modified QuEChERS method (AOAC 2007.01) to improve recovery. Here, 2.0 g of homogenized sediments sample was added to 1.0 mL MilliQ DI water (18 MΩ.cm) and 9.0 mL acetonitrile mixture in a roQ centrifuge tube. Two grams (2 g) of the RoQ QuEChERS salts (MgSO4 and sodium acetate mix) were added to the content, followed by the addition of 3.0 mL of acetone and the final content was vigorously shaken by hand for 1.0 min. The contents were then centrifuged at 4000 rpm for 5 min, after which the supernatant was poured into a 15 mL roQ dispersive SPE (dSPE) tube containing MgSO4, C18E and PSA for clean-up. The contents were shaken vigorously by hand for 30 s and centrifuged at 4000 rpm for 5 min. The cleansed supernatant was transferred into a test tube and concentrated to near dryness with a stream of pure nitrogen gas over a water bath set at 40 °C. The extract was then reconstituted in acetonitrile to 1.0 mL and further transferred into a 1.5 mL vial with the aid 0.22 µm syringe disk filter prior to HPLC-UV analysis.

2.4. HPLC-UV analysis of the PSHXEs

2.4.1. Quality control

Six (6) level external standard calibration curves were created for each of the analytes at concentrations of 0.1, 0.5, 1.0, 2.0, 5.0, 10.0 mg/L in acetonitrile. An initial calibration verification (ICV) standard (2.5 mg/L) was analysed to validate the calibration curve. Continuous calibration verification standards (10.0 mg/L) were also analysed each day prior to each batch analysis to verify the robustness of the instrumental method. System suitability test (SST) using US Pharmacopoeia (USP) criteria were conducted for each batch analysis to ascertain the propriety of the instrumental analysis. A Reagent blank i.e. extracted MilliQ DI water spiked at 1.0 mg/L level with native standards was also extracted and analysed in replicates (n = 7) to ensure ongoing precisions and recovery (OPR) of the method in accordance with EPA methods 539 and 542. The detection limit was measured and computed at the 0.1 mg/L of the spiked reagent blank.

2.4.2. Instrumental analysis

The extracts were analysed using Shimadzu prominence UFLC 20A series coupled with UV–vis SPD 20 AX detector. The compounds analysed were Bisphenol A, chloramphenicol, 17-alpha-ethynylestradiol, 17-beta-estradiol, estrone, diclofenac sodium salt, primidone, testosterone, progesterone, 4-tert-octylphenol, 4-para-nonylphenol. The dimensions of the Phenomenex Luna 3 µm C18 column used were 150 mm × 4.6 mm (i.d.) and it was maintained at a temperature of 40 °C in an oven throughout the analysis.

The HPLC gradient solvent elution method was performed using the tabulated solvent systems and gradient time elution (Table 1).

Solvent A: 0.14 % H3PO4 in water (pH = 3.0) and Solvent B: 100 % Acetonitrile.

The sample injection volume was 5.0 µL and the elution was done at a flow rate of 0.8 mL/min. The UV–vis detector was operated in the dual wavelengths mode at 222 nm (channel 1) and 256 nm (channel 2). The flow cell was also kept at a temperature of 15 °C.

2.4.3. Ecological risk assessment

The ecological risks associated with the levels of PSHXEs in the aquatic environments were assessed using the risk quotients (RQ). The RQ were calculated by first estimating the toxicity of the PSHXEs with the aid of USEPA’s “toxicity estimation software tool (T.E.S.T) which employs a quantitative structure activity relationship (QSAR) model of the chemicals. The “Consensus QSAR toxicity estimation method” was

![Fig. 1. A map showing the locations of the various sampling sites.](image-url)
employed with the following toxicity endpoints for three species that are a representation of the aquatic food chain.

1. Fathead minnow (Pimephales promelas) LC50 (96 hr)
2. Daphnia magna LC50 (48 hr)
3. Tetrahymena Pyriformis IGC50 (48 hr)

The predicted toxicity values from the T.E.S.T models were used in calculating the predicted no-effect concentration (PNEC) values (ng/L) using an assessment factor (AF) of 1,000 for acute toxicity associated with both water and sediments [92; Laurenson et al., 2014] since experimental toxicity data for the PSHXEs is sparse. The measured environmental concentrations (MEC, ng/L) were used in the RQ calculations.

\[
RQ = \frac{MEC_X}{PNEC_X}
\]

(1)

where \( RQ \) is the risk quotient, \( MEC_X \) is the measured environmental concentration of X in sediments and water, \( PNEC_X \) is the predicted no-effect concentration (PNEC) of X in water (ng/L) using an assessment factor (AF) of 1,000 for acute toxicity associated with both water and sediments, \( X \) is the chemical of interest.

\[
PNEC_X = \frac{\text{predicted toxicity value of } X \text{ in water}}{\text{AF}}
\]

(2)

Where X is the chemical of interest.

For sediments, the PNECs were calculated by the equilibrium partitioning method using the following equations:

\[
PNEC_{\text{sediment}} = \frac{K_{\text{oc-water}} \times PNEC_{\text{water}} \times 1000}{\text{RHO}_{\text{solid}}} \times F_{\text{oc-solid}} \times K_{\text{ooc}}
\]

(3)

where \( K_{\text{oc-water}} = \text{partition coefficient of sediment-water (m}^3\text{m}^{-3}) \), \( \text{RHO}_{\text{solid}} = \text{bulk density of wet sediment (1,300 kg/m}^3) \), \( F_{\text{water-sed}} = \text{volume fraction of water in sediment (0.8 m}^3\text{m}^{-3}) \), \( F_{\text{ooc-solid}} = \text{volume fraction of solids (0.2 m}^3\text{m}^{-3}) \) in sediment respectively. \( F_{\text{oc-solid}} = \text{the weight fraction of organic carbon in sediment solids (0.05 kg/kg)} \), \( RHO_{\text{solid}} = \text{density of solid phase (2500 kg/m}^3) \), \( C_{\text{oc}} \) and \( C_W \) are respective concentrations of X in sediments and water. The values used are the recommended default value from the European chemicals agency [93] for environmental exposure assessments.

\[
RQ < 0.1 \text{ suggests minimum risk, } 0.1 \leq RQ < 1 \text{ suggests medium risk and } RQ \geq 1 \text{ suggests high risk. It is worth noting that RQ for levels } < \text{LOQ were calculated using half the values of respective LOQ.}
\]

### 2.4.4. Data collection and statistical data analysis

The Shimadzu labsolution software was used to collect sample data and statistics from the instrumental analysis. Further multivariate statistical analyses of data were also done with Microsoft excel toolpak and IBM SPSS version 22.0 software. Source apportionment using the APCS-MLR receptor model [5,94-96] on data was also conducted using the IBM SPSS version 22.0. Details on the mathematical explanation of the APCS-MLR receptor model for source apportionment are provided in our earlier study [5]. The ecological risk assessments were conducted with the aid of the USEPA’s ‘toxicity estimation software tool (T.E.S.T)” version 5.1.1 [97].

### 3. Results and discussions

#### 3.1. Quality control results

Pass results were recorded for all the SST done using USP performance criteria. The levels of standards used for the calibration curves showed linear responses with correlation coefficients, \( R^2 > 0.995 \) and response factors (%) ranging between 3.7 % and 13.9 % (Table 2). Figs. 2 and 3 respectively show calibration levels chromatogram for BPA and a well-resolved chromatogram for the PSHXEs. Also, the ICV and CCV standards respectively recorded mean recoveries ranging between 97.4 % and 104.2 % and 81.2 – 106.9 % (Table 2), indicating a robust instrumental method used. The mean percent recoveries of the fortified (spiked) blank used for OPRs ranged between 78.1 % and 125 % whereas that of spiked samples ranged between 74.9 % and 117.4 % for water and 64.3–108.9 % for sediment samples respectively (Table 2).

Fig. 2. A calibration peaks chromatogram of Bisphenol A for the six calibration levels at λ = 222 nm.

### Table 2

| Name                | Ret. Time | R² | RF % | RSD | LOQ, ng/L | Mean ICV Recovery (%) | Mean CCV Recovery (%) | CCV % RSD | Mean spiked Recovery (%) | Maximum Spikes, %RSD | Mean Recovery OPR (%RSD | Mean Recovery OPR % RSD |
|---------------------|-----------|----|------|-----|-----------|------------------------|-----------------------|------------|--------------------------|-----------------------|------------------------|------------------------|
| Bisphenol A         | 14.8      | 0.999 | 10.9 | 9   | 99.8      | 102.1                  | 1.2                   | 107.5      | 108.9                    | 13.5                  | 124.4                  | 6.0                    |
| Chloramphenicol     | 18.1      | 0.995 | 7.7  | 6   | 97.4      | 93.6                   | 8.6                   | 74.9       | 101.0                    | 12.0                  | 118.6                  | 4.4                    |
| 17-alpha-Ethinylestradiol | 22.5 | 0.998 | 8.5  | 19  | 99.1      | 100.7                  | 3.4                   | 94.2       | 92.0                     | 16.1                  | 125.0                  | 6.3                    |
| 17-beta-Estradiol   | 23.1      | 0.998 | 11.8 | 54  | 99.7      | 96.1                   | 7.5                   | 112.4      | 100.6                    | 6.9                   | 110.8                  | 5.6                    |
| Estrone             | 23.7      | 0.998 | 13.9 | 19  | 104.2     | 100.8                  | 2.9                   | 85.0       | 99.3                     | 14.5                  | 115.1                  | 18.4                   |
| Diclofenac Sodium Salt | 24.1   | 0.998 | 13.0 | 86  | 99.0      | 81.2                   | 6.5                   | 101.5      | 64.3                     | 8.4                   | 85.3                   | 8.4                    |
| Primidone           | 24.5      | 0.999 | 3.7  | 10  | 98.6      | 101.9                  | 2.1                   | 112.9      | 107.5                    | 3.5                   | 122.0                  | 8.7                    |
| Testosterone        | 26.6      | 0.999 | 7.5  | 22  | 103.6     | 103.5                  | 3.5                   | 88.7       | 99.5                     | 14.4                  | 117.3                  | 17.3                   |
| Progestosterone     | 28.4      | 0.998 | 4.9  | 65  | 100.1     | 105.2                  | 3.0                   | 107.7      | 102.6                    | 18.8                  | 91.3                   | 3.8                    |
| 4-tert-Octylphenol  | 30.9      | 0.996 | 8.5  | 5   | 99.4      | 106.9                  | 2.7                   | 89.0       | 74.0                     | 8.0                   | 84.1                   | 4.8                    |
| 4-Para-Nonylphenol  | 32.9      | 0.999 | 8.9  | 18  | 101.5     | 104.6                  | 1.3                   | 117.4      | 98.8                     | 10.8                  | 78.1                   | 4.0                    |
These recoveries were within the limits specified by EPA methods 539, 542 and 1694. The limits of quantitative (LOQ) computed ranged between, 5.0 – 86.0 ng/L (Table 2). The method employed was robust with good levels of accuracy and precision for the analysis of environmental samples. The QC results are comparable to that obtained by Patrolecco et al. [98] in similar work, where the proposed developed method had recoveries of 65 – 104% and LOQ between 10 and 1,100 ng/L. Similar recoveries and quantitative limits between 70.7% and 118.7% and 25–50 ng/L for the estrogens were also reported by Cais et al. [99] for water samples. Whereas the recoveries for sediments samples were comparable to values reported by Peng et al. [100]. The recoveries obtained for chloramphenicol (CP) in this study are comparable to the 77.9 – 102.5% obtained by Qin et al. [101].

### 3.2. Levels of the PSHXEs analytes in water samples

The mean total levels of the PSHXEs in the water samples ranged from 12,187 ng/L for River Densu to a maximum of 52,177 ng/L for the Chemu lagoon (Table 3). In hierarchical order, samples from Kakum River (34,775 ng/L), Korle lagoon (34,031 ng/L) etc also recorded elevated levels only next to Chemu lagoon. These elevated levels recorded especially for CL may be attributed to the high domestic and industrial activities along and around the lagoon. It is worth noting that CL is situated along the Tema industrial area and the harbour expanse, the industrial hub of Ghana.

Among the analytes, primidone recorded the highest concentrations in most of the samples with mean concentrations ranging between 2,209 and 17,284 ng/L, of which CL recorded the highest (Table 3). Next in the ranking order were levels of 4-t-OP, which ranged between 752 and 16,879 ng/L, of which CL again recorded the highest level, followed closely by Kakum River (15,814 ng/L). The dominant presence of primidone may suggest an increased use and improper disposal of the anticonvulsant among the Ghanaian populace, which is a cause for concern.

BPA, a well-known EDC and IARC classified carcinogen, also recorded mean levels between 448 and 6363 ng/L (Table 3). Except for CL, all the water samples from the waterbodies studied were below the Canadian Federal environmental quality guidelines of 3,500 ng/L BPA for aquatic life protection [102]. The BPA mean levels recorded in this study were comparable to the levels of up to 1,340 ng/L recorded in China’s aquatic environment by various studies [71,103-106,91,107] and that between <LOQ – 763 ng/L recorded in Skaneateles Lake [108], although relatively higher levels for Chemu lagoon and Densu River, were found. The mean 4-NP levels in this study (Table 3) were also comparable with levels up to 1,758.4 ng/L and 262.39–1442.72 ng/L reported for Luoma Lake [71] and Taihu Lake [109] in China respectively. Peng et al. [103] reported levels up to 5,050 ng/L for urban rivers in Guangzhou, significantly higher than the maximum recorded in this current study. About 50% of the samples

### Table 3

Mean concentrations (n = 4) in ng/L of PSHXEs in water samples from the studied sites.

| Name               | Ashaiman Lake | Chemu Lagoon | Fosu Lagoon | Kakum River | Densu River | Korle Lagoon |
|--------------------|---------------|--------------|-------------|-------------|-------------|--------------|
| Bisphenol A        | 1,186         | 6,363        | 1,007       | 509         | 1,993       | 448          |
| Chloramphenicol    | 2,624         | 336          | 248         | 7,270       | 390         | 905          |
| 17-Alpha-Ethynylestradiol | 1,308    | 1,181        | 635         | 167         | 785         | 1,739        |
| 17-beta-Estradiol  | 5,338         | 4,734        | 907         | <LOQ        | <LOQ        | <LOQ         |
| Estrone            | 1,399         | 1,150        | 1,478       | 1126        | 677         | 4,152        |
| Dichlofenac Sodium Salt | <LOQ        | 1,368        | <LOQ        | <LOQ        | 1168        | 3,590        |
| Primidone          | 10,076        | 17,284       | 8,652       | 7,630       | 2,209       | 2,511        |
| Testosterone       | 214           | 228          | 117         | 225         | 662         | 5,292        |
| Progestosterone    | 970           | 785          | 666         | 950         | 1,372       | 12,288       |
| 4-tert-Octylphenol | 6,129         | 16,879       | 11,986      | 15,814      | 1,624       | 752          |
| 4-Para-Nonylphenol | 564           | 1,869        | 781         | 1,085       | 521         | 1,597        |
| Mean total         | 29,807        | 52,177       | 26,476      | 34,775      | 12,189      | 34,031       |
studied had 4-NP levels below the Canadian water quality guideline of 1,000 ng/L for 4-NP [110], and about 33 % had levels of NP significantly greater than the Canadian criteria.

The mean levels of diclofenac (<LOQ – 3590 ng/L) recorded in the waterbodies studied were lower than the mean levels between 1,010 and 10,200 ng/L reported by Gumbi et al. [111] for Umgeni River in South Africa. On average, 50 % of the samples analysed for diclofenac recorded values <LOQ (Table 3) that were less than the 100 ng/L allowable level set by the EU water framework directive [112].

The elevated levels of the xenoestrogens, 4-t-OP and BPA in samples from Chemu and Fosu lagoons may be, attributed to the industrial activities along the stretch of these waterbodies that may have introduced these chemicals into the natural environment since these are industrial chemicals used in the production of plasticizers and antioxidants in plastic and resins. Also, the presence of 4-t-OP and BPA in most water samples, may be due to the increased domestic usage and improper disposal of materials containing these plasticizers.

The steroid hormones recorded mean levels between <LOQ for 17-beta-estradiol at Kakum River to the highest mean level of 12288 ng/L for progesterone in Korle lagoon (Table 3). Estrone recorded mean levels between 677 and 4152 ng/L (Table 3), which were significantly higher than levels up to 89 ng/L (Zhou et al., 2019) and <LOQ – 472 ng/L [6] respectively reported in European and Chinese aquatic environment. The levels of steroid hormones, especially EE2, recorded in the current study are comparable to the levels up to 450 ng/L in the Potomac River in Virginia reported by Arya et al. [113]. Testosterone levels recorded in this study were comparable to the levels up to 214 ng/L reported in surface waters [114-117] except for the Korle lagoon, which recorded significantly higher mean levels (Table 3).

The elevated levels of steroid hormones, especially synthetic estrogens, may be, attributed to the increased use of birth control medications, steroid anabolic and veterinary drugs among the populace, compounded by the improper disposal of waste containing these drugs. Which, per the results, seemed more pronounced in samples from AL, CL and KL, and may be attributed to runoffs from residential and farming activities such as livestock production [118-120] around these waterbodies.

The elevated levels of PSHXEs, especially the xenoestrogens; 4-t-OP, BPA and primidone in water samples, may have dire implications on the health of fauna and flora in the waterbody, which may also affect human health. For instance, the elevated level of primidone in the water may have contributed significantly to the risk of violent death and cancers [51,52] fish, mammals and thus humans that derive life from the waterbodies. Also, the elevated levels of estrogenic 4-t-OP may have contributed significantly to the upsurge in incidences such as altered sex hormone levels and hypothalamic-pituitary-suppression, reproductive disorders such as testicular atrophy and impaired spermatogenesis [121] in organisms and thus humans that derive their food and drinking water from the water bodies. The levels of BPA may have also contributed to human health problems such as liver and brain malfunction, especially in children [15,17], hepatocarcinogenesis and other forms of cancers [16,18] as well as reproductive impairment in adolescents and adults [19] as well as organisms that depend on these waterbodies for survival. These BPA levels may have also contributed to the decrease in biodiversity in the Ghanaian aquatic environment.

The elevated levels of steroid hormones, especially the estrogens, may have contributed to feminization, reproductive and developmental impairment in fish and aquatic mammals [21,24,25,33,36,34,27,28], increased incidences of breast, prostate and other forms of cancers as well as cancer-related effects in humans [21,35,36,20,37] deriving their livelihood from these waterbodies.

Life and biodiversity in Korle lagoon have significantly reduced over the years, which may partly be attributed to the elevated levels of some steroid hormones, especially testosterone that may have caused masculinization, reduced eggs in female fish and increased proportion of male fish in the aquatic environment [45,48].

Two-way ANOVA analysis at the 95 % CL suggested no significant differences (p = 0.255) in the sites for the mean levels of PSHXEs in the water samples.

3.3. Levels of the PSHXEs analytes in sediments

The mean total levels of the PSHXEs in sediments samples analysed ranged from a minimum of 2,022 ng/g in Ashaiman Lake to a maximum of 6,047 ng/g in Korle lagoon (Table 3). The next in magnitude just to KL was DR (5,970 ng/g) and CL (5,755 ng/g) in respective ranks (Table 3). The elevated mean total levels recorded in these sediment samples may have a dire health effect on aquatic organisms, especially benthic organisms in the waterbody [3]. These elevated levels may be attributed to the high human, commercial and industrial activities along the expanse of these waterbodies. Whereas the relatively lower mean levels recorded for the rest of the waterbodies could be due to the lower levels of the earlier mentioned activities along their expanse and the periodic dredging which removes contaminated sediments. Thus reducing the levels since most of the PSHXEs, especially 17-β-E2 and 17-α-EE, strongly sorb to Sediments [122]. The mean total levels in this study were relatively lower than the levels reported by Peng et al. [103], with a maximum mean value of 6,350 ng/g and range levels up to 20,500 ng/g.

Among the PSHXEs, 4-NP recorded the highest mean level (2,439 ng/g) in KL, followed closely by estrone (2365 ng/g) in DR. The elevated levels of 4-NP may be attributed to the industrial, commercial and domestic activities along the stretch of the lagoon since 4-NP is known to be a precursor and or part of products such as detergents, paints, pesticides, personal care products, and plastics [123]. For instance, the burning of electronic waste at Agbogbloshie, a known electronic waste recycling hub in Accra and close to the lagoon, may have contributed leachates of 4-NP from plastics to the lagoon. The elevated levels may also be attributed to the fact that 4-NP accumulates in sediments [124]. Contrarily that of estrone may be attributed more to domestic activities in the densely populated communities along the lagoon stretch. Similar trends were reported by Peng et al. [103] where 4-NP dominated among PSHXEs, but mean levels of 4440 ng/g and levels up to 14,400 ng/g reported for 4-NP in Guangzhou Urban rivers were significantly higher than levels recorded for the current study.

The mean levels of BPA in the sites studied (Table 3) were comparable with the mean level of 173 ng/g reported by Pent et al. (2017).

The relatively low levels of primidone to other PSHXEs in the sediments samples compared to its dominance in the water samples of the same sources may be attributed to the low sorption ability of the compound to Sediments [125].

Two-way ANOVA conducted at the 95 % CL suggested a statistically significant difference (p < 0.05) between the sites for the mean levels of PSHXEs in the sediment samples.

3.4. Risk assessment results

3.4.1. Water

From the computed PNEC values for the PSHXEs in water (Supplementary Table S1), the acute ecological risks (RQ) computed ranged between 0.004 and 9.00 for Daphnia magna LC50 and 0.00 – 2.48 for T. Pyriformis IGC50 for 2.0 days period whereas RQ ranging between 0.01 and 15.55 for fathead minnow LC50 for a period of 4 days (Supplementary Table S2). The results (Supplementary Table S2) showed that primidone, although recorded the highest concentrations in water, it generally possesses low risk (RQ < 0.1) to the three trophic levels studied for the short term.

For the LC50, about 55 %, 27 %, 36 %, 55 %, 18 % and 55 % of the PSHXEs in water from AL, DR, KR, CL, FL and KL respectively recorded a high risk level (RQ > 1.0) to fish in the waterbodies for a period of 4 days. The elevated risk in this regard may have affected significantly the population and the biodiversity of fish in these aquatic systems,
especially AL, CL and KL. The elevated risk, $RQ = 15.55$ recorded for progesterone, diclofenac ($RQ = 6.90$), 4-NP ($R = 4.20$) and testosterone ($RQ = 3.21$) in the Korle lagoon (Supplementary Table S2), may have contributed significantly to the decrease in fish population and biodiversity in the waterbody.

For LC50DM, about 36 %, 18 %, 18 %, 45 %, 18 %, and 45 % of the PSHXEs in water from AL, DR, KR, CL, FL, and KL respectively recorded high risk levels (RQ>1.0) (Supplementary Table S2) lethal to half the population of water flea and the likes in two days. The highest risk was recorded for 4-t-OP in CL (RQ = 9.27), followed by that in KR (RQ = 8.70), and FL (RQ = 6.59) in respective order (Supplementary Table S2). The phenols, 4-t-OP and 4-NP recorded the highest elevated risk in almost all the water samples studied (Supplementary Table S2).

In the case of the IGC50 (Supplementary Table S2), about 9 %, 18 %, 36 %, 9 % and 27 % of the PSHXEs in water from AL, KR, CL, FL and KL respectively recorded high risk levels (RQ>1.0) likely to inhibit the growth of 50 % of the T. pyriformis population after 48 h. PSHXEs in the Densu River generally had no significant growth inhibition effects on the population. According to the model results, progesterone and testosterone have no known growth inhibition effects on the T. Pyriformis population. On average, the growth inhibition effects of the PSHXEs on the T. Pyriformis population are no match to the population death toxicity impacts likely to be experienced by the fish population in these waterbodies.

3.4.2. Sediments

For sediments, the chronic toxicity for Daphnia magna LC50 was used for calculating the risk posed to benthic species and their likes in the waterbody. These were conducted in accordance with European REACH and US Toxic Substances Control Act regulations. The RQ computed ranged between 0.01 and 23.4 (Supplementary Table S3). The results (Supplementary Table S3) showed that about 45 %, 18 %, 36 %, 55 %, 45 % and 64 % of PSHXEs in the sediment recorded high risk levels (RQ>1) that may lethally affect or immobilize 50 % of benthic organisms in AL, KR, DR, CL, FL, and KL respectively for a short period of 2 days. These elevated risks recorded, especially for CL and KL, may have dire consequences on the health of benthic organisms and cause for concern. Among the individual PSHXEs, the order of elevated risk posed to the benthic organisms in the Ghanaian aquatic environments according to their occurrence frequencies was 4-t-OP (100 %) > 4-NP (100 %) > EE2 (83 %) > BPA (67 %) > E1 (50 %) > Diclofenac (33 %) > progesterone (17 %) > β-E2 (17 %) > testosterone (17 %) whereas, the pharmaceuticals primidone and chloramphenicol generally posed medium and no risk respectively to the benthic organisms in all sites sampled (supplementary Table S3). The order suggested that the phenolic xenoestrogens are widely distributed at elevated risk levels in the Ghanaian aquatic environment and may have had dire consequences on the ecosystem, especially the health of benthic organisms. Table 4.

3.5. Source apportionments

The factor analysis conducted using varimax with Kaiser Normalization rotation as part of APCS-MLR predicted three (3) significant components (sources) (p = 0.000) designated according to signature PSHXEs as domestic wastes (FAC1), mix hospital/industrial wastes (FAC2) and agricultural wastes (livestock/aquaculture waste, FAC3) sources (Fig. 4). Domestic, mix hospital and industrial, and agricultural waste (including livestock and aquaculture wastes leachates) waste sources contributed about 39 %, 32 % and 14 % respectively to the total variance in the model after rotation. The model summaries of the APCS-MLR conducted at 95 % CL showed adjusted R-squares between 0.94 and 0.999 for the models.

The models statistically suggested domestic waste as the major significant source (p = 0.018) of PSHXEs in the various sites. The model also suggested in ranking order, testosterone ($r = 0.96$), progesterone ($r = 0.95$), diclofenac ($r = 0.79$), estrone ($r = 0.77$) and EE2 ($r = 0.531$) as statistically significant (p < 0.05) and most important predictors of the domestic source in respectively (Fig. 4). The pictorial form of the model conducted, i.e. APCS-MLR with enhanced model accuracy to create an ensemble with boosting for more accurate predictions suggested 99.7 % accuracy in the model for domestic waste with the same predictors confirming the APCS-MLR model pictorially (Figs. 4 and 5a). The signature PSHXEs for the domestic sources were comparable to

Table 4

Mean Levels (n = 4) in ng/g dry weight (dw) of PSHXEs in Sediment samples from the studied sites.

| Name | Ashaiman Lake | Chemu lagoon | Fosu lagoon | Kakum River | Densu River | Korle Lagoon |
|------|---------------|---------------|-------------|-------------|-------------|--------------|
| Bisphenol A | 94 | 133 | 179 | 143 | 118 | 256 |
| Chloramphenicol | 105 | 384 | 157 | 381 | 151 | 52 |
| 17-Alpha-Ethynylestradiol | 141 | 212 | 116 | 123 | 309 | 297 |
| 17-beta-Estradiol | <LOQ | 39 | <LOQ | <LOQ | 103 | 179 |
| Estrone | 804 | 913 | 691 | 400 | 2365 | 256 |
| Diclofenac Sodium Salt | 191 | 929 | 191 | 54 | 387 | 998 |
| Primidone | 81 | 97 | 75 | 77 | 381 | 458 |
| Testosterone | 177 | 319 | 248 | 201 | 408 | 211 |
| Progesterone | 140 | 777 | 260 | 117 | 90 | 556 |
| 4-tert-Octylphenol | 148 | 829 | 491 | 181 | 1192 | 345 |
| 4-Para-Nonylphenol | 244 | 1123 | 372 | 580 | 465 | 2439 |
| Mean Total | 2022 | 5755 | 2719 | 2082 | 5970 | 6047 |
Fig. 5. A pictorial presentation of APCS-MLR/MALR predictor importance for the three PSHXEs sources (a) Domestic waste (b) Mix hospital and industrial waste (c) Agricultural waste.
those reported in the literature [126,77,127,63,64]. Ojogboro et al. [128] suggested humans, thus domestic sources to be a major source of steroid hormones in the aquatic environment which agrees with this study. The results from the model confirmed the earlier assertion that there is increased use of birth control and steroid anabolic medications among the Ghanaian populace, for which improper disposal of domestic waste containing these drugs is the worst offender to the pollution in the aquatic environment.

The model results also showed that the mix hospital and industrial waste sources loaded significantly ($p < 0.05$) for BPA ($r = 0.82$), primidone ($r = 0.70$), $\text{IE}_2$ ($r = 0.69$) and 4-t-OP ($r = 0.50$) in the order of predictor importance (Fig. 5b). The model predicted 99.7% accuracy in the ensemble for mix hospital and industrial waste sources. Among the signature PSHXEs for this source, BPA and 4-t-OP are reported to be part of industrial chemical wastes [103,127] whereas primidone and $\text{IE}_2$ are more of the hospital waste contaminants [73,129].

Again, the APCS-MLR model suggested that agricultural wastes sources, which mainly included livestock and aquaculture waste loaded significantly ($p < 0.05$) for Chloramphenicol ($r = 0.89$) and 4-t-OP ($r = 0.51$). The pictorial model, APCS-MLR conducted with enhanced accuracy predicted 98.8% in the ensemble for agricultural waste sources. The predictor importance shown in Fig. 5c confirmed the results from the APCS-MLR. Though chloramphenicol has been banned for use in livestock production and aquaculture, McCubbin et al. [130] and Abdalla et al. [131] recently reported the possible use of the drug in livestock production in Uganda and South Africa respectively. Other studies have earlier reported the use of chloramphenicol in livestock production and aquaculture [132-134]. In Ghana, Agoba et al. [135] reported the use of chloramphenicol in aquaculture by some farmers suggesting the menace is still ongoing and has contributed to the pollution levels. Bina et al. [136] reported higher levels of 4-t-OP in livestock wastewater sources than in other sources suggesting livestock production waste as a source of 4-t-OP in the aquatic environment which is in good agreement with the source apportionment in this study.

4. Conclusion

The results and discussion showed elevated levels of the PSHXEs analytes in the Ghanaian aquatic environment. Sediment samples generally had relatively higher levels of PSHXEs, than water samples of the same sites. These elevated levels of PSHXEs in the Ghanaian aquatic environment posed an acute high risk (RQ > 1) to aquatic organisms in the three trophic levels for most of the waterbodies studied. The elevated risks recorded may have resulted in the alarming decrease in fish population and biodiversity in most Ghanaian aquatic systems, especially for Korle and Chemu lagoons, as well as the significant deterioration of these aquatic systems. The source apportionment predicted three significant sources designated according to the signature PSHXEs as domestic (Major), mix hospital and industrial, and agricultural waste sources. The results also suggested increased domestic use of birth control and anabolic drugs among the Ghanaian populace.

CRediT authorship contribution statement

Joseph K. Adjei: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision. Alberta D. Dayie: Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing, Supervision. Justice K. Addo: Conceptualization, Validation, Investigation, Resources, Writing – review & editing, Supervision. Anita Asamoah: Conceptualization, Validation, Investigation, Resources, Writing – review & editing, Supervision. Ernest O. Amoako: Methodology, Validation, Formal analysis, Resources, Investigation, Writing – original draft. Benedicita Y. Egoh: Methodology, Validation, Formal analysis, Investigation, Resources. Ebenezer Bekoe: Methodology, Validation, Investigation, Writing – original draft. Resources. Nathaniel O. Ofori: Methodology, Validation, Investigation, Writing – original draft, Resources. George A. Adjei: Conceptualization, Methodology, Validation, Writing – review & editing, Resources. David K. Essumang: Conceptualization, Methodology, Formal analysis; Validation, Writing – review & editing, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

We wish to express our utmost appreciation to the School of Physical Sciences, University of Cape Coast, Ghana, for making available their facilities to make this work a success. We also wish to thank Dr Thomas Ahenguah for his contributions in getting us the standards and reagents.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2022.06.011.

References

[1] USEPA, 2010. Pharmaceuticals and Personal Care Products (PPCPs). United States Environmental Protection Agency. http://www.epa.gov/ppcp/faq.html#othersec.
[2] H.P.H. Arp, Emerging contaminants, Environ. Sci. Technol. 46 (8) (2012) 4259–4260, https://doi.org/10.1021/es301074a.
[3] (a) N.J. Diepens, A.A. Koelmans, H. Baveco, P.J. van den Brink, M.J. van den Heuvel-Greve, T. Brock, Prospective environmental risk assessment for sediment-bound organic chemicals: a proposal for tiered effect assessment, Rev. Environ. Contam. Toxicol. 239 (2017) 1–77; (b) A.J. Ebele, M. Abou-Elwafa Abdallah, S. Harrad, Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment, Emerg. Contam. 3 (1) (2017) 1–16, https://doi.org/10.1016/j.emcon.2016.12.004.
[4] J.K. Adjei, D.K. Essumang, E. Twumasi, E. Nyame, I. Muah, Levels and risk assessment of residual phthalates, polyacryllic aromatic hydrocarbons and semi-volatile chlorinated organic compounds in toilet tissue papers, Toxicol. Rep. 6 (2019) 1263–1272, https://doi.org/10.1016/j.toxrep.2019.11.013.
[5] J.K. Adjei, A. Ofori, H.K. Mgebenu, T. Ahenguah, et al., Health risk and source assessment of semi-volatile phenols, p-chloroaniline and plasticizers in plastic packaged (sachet) drinking water, Sci. Total Environ. 797 (2021), 149008, https://doi.org/10.1016/j.scitotenv.2021.149008.
[6] Y. Xiang, H. Wu, L. Li, M. Ren, H. Qie, A. Lin, A review of distribution and risk of pharmaceuticals and personal care products in the aquatic environment in China, Ecol. Toxicol. Environ. Saf. 213 (2021), 112644.
[7] EU, 2013. Directive 2013/39/EU: Of the European Parliament and Of the Council of 12 August 2013- amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. Official Journal of the European Union.
[8] Negro De Carvalho R., Ceriani L., Ippolito A., Lettieri T., 2015. Development of the First Watch List under the Environmental Quality Standards Directive. EUR 27142. Luxembourg (Luxembourg): Publications Office of the European Union. JRC95018. 10.2788/101576.
[9] H. Inadera, Neurological effects of bisphenol A and its analogues, Int. J. Med. Sci. 12 (12) (2015) 926.
[10] M. Perez-Alvarez, F. Wandosell, Stroke and neuroinflammation: role of sexual hormones, Curr. Pharm. Des. 22 (10) (2016) 1334–1349.
[11] T. Porseryd, et al., Persistent effects of developmental exposure to 17α-ethynylerstradiol on the zebrafish (Danio rerio) brain transcriptome and behavior, Front. Behav. Neurosci. 11 (2017) 69.
[12] M. Preciacos, C. Yoo, D. Roy, Estrogenic endocrine disrupting chemicals influencing NRF1 regulated gene networks in the development of complex human brain diseases, Int. J. Mol. Sci. 17 (12) (2016) 2086.
[13] M.F. Rossetti, et al., Dioxenogens and proestogenes: synthesis and action in the brain. J. Neuroendocrinol. 28 (2016) 7.
[14] G.-E. Seralini, G. Jungers, Endocrine disruptors also function as nervous disruptors and can be renamed endocrine and nervous disruptors (ENd), Toxicol. Rep. 8 (2021) (2021) 1538–1557, https://doi.org/10.1016/j.toxrep.2021.07.014.
[15] B. Castro, P. Sánchez, J.M. Torres, E. Ortega, Bisphenol A, bisphenol F and bisphenol S affect differently 5α-reductase expression and dopamine-serotonin systems in the prefrontal cortex of juvenile female rats, Environ. Res. 142 (2015) 281–287, https://doi.org/10.1016/j.envres.2015.07.001.
Toxicology Reports 9 (2022) 1398–1409

J.K. Adjei et al.

[82] B.M. Sharma, J. Be, W.J. Sim, J.W. Lee, E.S. Lee, S.K. Shin, S.R. Hwang, J.E. Oh, Occurrence and treatment of pharmaceuticals in wastewater from households, livestock farms, and fisheries in the Pearl River estuary, Environ. Pollut. 158 (2010) 1058–1065.

[89] D.J. Fairbairn, M.E. Karpuzcu, W.A. Arnold, B.L. Barber, E.F. Kaufenberg, et al., Pharmaceuticals and personal care products in the mainstream of urban stormwater discharges and anaerobic wastewater treatment plants in south Florida, Environ. Sci. Technol. 56 (2020) 851–861.

[98] H. Chang, S. Wu, J. Hu, M. Asami, S. Kunikane, Trace analysis of androgens and oestrogens in the riverside groundwater of the Beiyun River of Beijing, China, Environ. Toxicol. Pharmacol. 52 (2017) 69–75.

[104] Z. Wang, Y. Du, C. Yang, X. Liu, J. Zhang, E. Li, Q. Zhang, X. Wang, Occurrence and environmental impact of androgenic steroids in surface water and sediment from a Mediterranean coastal area, Sci. Total. Environ. 589 (2017) 46–55.

[109] S. Esteban, et al., Analysis and occurrence of endocrine-disrupting compounds in surface water, sediments and aquatic organisms in a Mediterranean coastal area, Sci. Total. Environ. 585 (2017) 1100–1107.

[110] Environment Canada, Canadian Water Quality Guidelines for the Protection of Aquatic Life, Canadian Council of Ministers of the Environment, 2002.

[115] H. Chang, S. Wu, J. Hu, M. Asami, S. Kunikane, Trace analysis of androgens and oestrogens in the riverside groundwater of the Beiyun River of Beijing, China, Environ. Toxicol. Pharmacol. 52 (2017) 69–75.

[129] ECHA, 2016. Guidance on Information Requirements and Chemical Safety Assessment Chapter R.16: Environmental exposure assessment. ECHA-16-G-03-7698.

[133] H. Chang, S. Wu, J. Hu, M. Asami, S. Kunikane, Trace analysis of androgens and oestrogens in the riverside groundwater of the Beiyun River of Beijing, China, Environ. Toxicol. Pharmacol. 52 (2017) 69–75.

[135] H. Chang, S. Wu, J. Hu, M. Asami, S. Kunikane, Trace analysis of androgens and oestrogens in the riverside groundwater of the Beiyun River of Beijing, China, Environ. Toxicol. Pharmacol. 52 (2017) 69–75.

[138] J.K. Adjei, et al., Health effects of pharmaceuticals and personal care products. In: A. Sharma, G.K. Bharat, et al., Health effects of pharmaceuticals and personal care products in the environment, ECHA-16-G-03-7698.

[139] J.K. Adjei, et al., Health effects of pharmaceuticals and personal care products. In: A. Sharma, G.K. Bharat, et al., Health effects of pharmaceuticals and personal care products in the environment, ECHA-16-G-03-7698.

[140] J.K. Adjei, et al., Health effects of pharmaceuticals and personal care products. In: A. Sharma, G.K. Bharat, et al., Health effects of pharmaceuticals and personal care products in the environment, ECHA-16-G-03-7698.

[141] J.K. Adjei, et al., Health effects of pharmaceuticals and personal care products. In: A. Sharma, G.K. Bharat, et al., Health effects of pharmaceuticals and personal care products in the environment, ECHA-16-G-03-7698.

[142] J.K. Adjei, et al., Health effects of pharmaceuticals and personal care products. In: A. Sharma, G.K. Bharat, et al., Health effects of pharmaceuticals and personal care products in the environment, ECHA-16-G-03-7698.

[143] J.K. Adjei, et al., Health effects of pharmaceuticals and personal care products. In: A. Sharma, G.K. Bharat, et al., Health effects of pharmaceuticals and personal care products in the environment, ECHA-16-G-03-7698.

[144] J.K. Adjei, et al., Health effects of pharmaceuticals and personal care products. In: A. Sharma, G.K. Bharat, et al., Health effects of pharmaceuticals and personal care products in the environment, ECHA-16-G-03-7698.

[145] J.K. Adjei, et al., Health effects of pharmaceuticals and personal care products. In: A. Sharma, G.K. Bharat, et al., Health effects of pharmaceuticals and personal care products in the environment, ECHA-16-G-03-7698.

[146] J.K. Adjei, et al., Health effects of pharmaceuticals and personal care products. In: A. Sharma, G.K. Bharat, et al., Health effects of pharmaceuticals and personal care products in the environment, ECHA-16-G-03-7698.

[147] J.K. Adjei, et al., Health effects of pharmaceuticals and personal care products. In: A. Sharma, G.K. Bharat, et al., Health effects of pharmaceuticals and personal care products in the environment, ECHA-16-G-03-7698.
