Marine Pollution Bulletin 138 (2019) 49–57

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Biological toxicity estimates show involvement of a wider range of toxic compounds in sediments from Durban, South Africa than indicated from instrumental analyses

Tash Vogt[a,*], Rialet Pieters[a], John Giesy[b,c,d,e,f], Brent K. Newman[g,h]

[a] Unit for Environmental Sciences and Management, North-West University, Private Bag X1290, Potchefstroom 2520, South Africa.
[b] Department of Veterinary Biomedical Sciences, Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B4, Canada,
[c] Department of Zoology, Centre for Integrative Toxicology, Michigan State University, East Lansing, MI 48824, USA
[d] Department of Biology and Chemistry, State Key Laboratory in Marine Pollution, City University of Hong Kong, Kowloon, Hong Kong, China
[e] School of Biological Sciences, University of Hong Kong, Hong Kong, China
[f] State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210093, Jiangsu, China
[g] Coastal Systems Research Group, Council for Scientific and Industrial Research (CSIR), Durban, South Africa
[h] Nelson Mandela University, P.O. Box 77000, NMU, Port Elizabeth 6031, South Africa

ARTICLE INFO

Keywords:
Africa
Harbour
Aryl hydrocarbon receptor
H4IIE-*luc*
SQGs
Dioxin

ABSTRACT

The toxic equivalences (TEQs) of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) from sediment of aquatic systems in Durban, South Africa were determined in two ways: 1) TEQs of PAHs and PCBs were determined by instrumental analyses and converted to 2,3,7,8-tetrachlorodibenzo-*para*-dioxin equivalence (TCDDeq). 2) Bioassay equivalences (BEQs) of aryl hydrocarbon receptor (AhR) ligands were analysed using the H4IIE-*luc* bioassay. TEQs of PCBs ranged from below limit of detection (< LOD) to 57 pg TCDDeq·g^{-1} while PAHs ranged from < LOD to 790 pg TCDDeq·g^{-1}. BEQs were 100- to 1000-fold greater than TEQs. Potency-balance revealed < 10% of the BEQs were explained by instrumentally analysed compounds. Sediment quality guidelines indicated *di minimis* risk relating to TEQs, however had potential risk due to BEQs. The results reveal that far more AhR ligands were present in the sediments than what was instrumentally analysed and capable of causing AhR-mediated toxicity.

1. Introduction

eThekwini municipality contains the third largest city in South Africa, Durban, with 3.6 million inhabitants (eThekwini Municipality, 2015), many living in large, dense informal settlements, some of which lack basic services such as piped water, sanitation services, and adequate health care (Kumalo et al., 2012). eThekwini, which is situated on the Indian Ocean coast of South Africa, is highly industrial, containing the second largest manufacturing base in the country and is the largest municipality in the province of KwaZulu-Natal. The municipality encompasses the busiest harbour on the continent and it is the foremost container handling port in South Africa, handling two thirds of the country's container traffic (EDGE, 2014).

Anthropogenic activities, such as industry, petrochemical processing, and vehicular traffic in the region can release halogenated aromatic hydrocarbons (HAHs) (Godefroy et al., 2005; Masood et al., 2016; Zhu et al., 2017). These compounds, such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), typically accumulate in sediment. Although sediment provides a long-term sink for these chemicals they can also contain contaminants from natural sources (Barakat et al., 2002; Kilemade et al., 2004). These residues can become bioavailable through remobilisation events, such as floods or dredging or through bioturbation by benthic organisms (Mzoughi and Chouba, 2011). Remobilisation may result in exposure of benthic and associated aquatic organisms to these compounds. Numerous adverse outcomes have been reported in humans and wildlife as a result of exposure to these compounds, including toxic responses such as development of porphyria, immunotoxicity, developmental and reproductive toxicity, disruption of endocrine pathways, chloracne, and carcinogenesis (Denison et al., 2004; Hecker and Giesy, 2011; Safe, 2001).

One of the key proteins in vertebrate bodies for mediating both toxicological and physiological effects of exogenous and endogenous chemicals is the aryl hydrocarbon receptor (AhR) (Tian et al., 2015).

* Corresponding author.
E-mail address: tash.vogt@gmail.com (T. Vogt).

https://doi.org/10.1016/j.marpolbul.2018.11.019
Received 10 July 2018; Received in revised form 2 October 2018; Accepted 11 November 2018

The AhR is a ligand-dependant, nuclear transcription factor. Physiological responses mediated by the AhR include cell differentiation, immunoregulation, and homeostasis (Tian et al., 2015). However, the best known function of the AhR is its role in up-regulation of RNA coding for enzymes such as CYP1A1, which can transform many ligands of the AhR (Giesy and Kannan, 1998; Vallack et al., 1998). CYP enzymes belong to the P450 enzyme series responsible for phase I detoxification (Newman, 2010). The toxicity of a xenobiotic is directly proportional to its affinity to bind to the AhR (Behnisch et al., 2001). It is especially compounds that are about 10 by 30 Å, and that can attain a planar configuration that are capable of binding to the AhR. These compounds include PAHs, PCBs, polychlorinated naphthalenes, polychlorinated dibenzo-*p*-dioxins and -furans (PCDD/Fs) (Giesy and Kannan, 1998; Hilscherova et al., 2000), and brominated flame retardants (BFRs) (Behnisch et al., 2003). Pesticides such as hexachlorobenzene have also been demonstrated to bind to the AhR (Behnisch et al., 2003). Some naturally occurring compounds, like indoles, tryptophan-derived products, oxidised carotenoids, and heterocyclic amines, have also been shown to bind, but have a weak affinity for the AhR and are degraded rapidly (Hecker and Giesy, 2011; Hilscherova et al., 2000).

Instrumental analysis, usually gas chromatography coupled to mass spectrometry (GC–MS), is used to quantify AhR ligands. However, all compounds and degradation products of contaminants cannot be analysed exhaustively because often no analytical standards or methods exist, analyses are expensive, and specialised equipment and highly trained personnel are required (Giesy et al., 2002; Hilscherova et al., 2000). All of these are often unavailable to regulatory agencies and research institutions in developing countries (De Vos et al., 2013; Lee et al., 2013). One of the advantages of biological analysis is that it can respond to the total effect of the complex mixture of compounds from the environment. Chemical analysis assumes additivity of compounds, however interactions of the compounds in complex mixtures could result in synergism or antagonism (Hilscherova et al., 2000). What might also happen when relying on instrumental analysis only is that biologically active chemicals below the instrumental detection limit might not be detected (Qiao et al., 2009). Compounding the problem is the need for a priori information to determine which compounds would be worth analysing (Newsted et al., 1995). Even if concentrations of all organic and inorganic residues were available, it still is difficult to predict biological effects of mixtures.

In an attempt to predict the toxic potential of AhR ligands when their concentrations are known, the World Health Organisation developed the concept of toxic equivalence factors (TEFs). The toxicities of the most potent AhR ligands, PCDD/Fs and dioxin-like PCBs, have been ranked in relation to 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD), the most potent and toxic AhR agonist (Van den Berg et al., 2006). By multiplying each compound's concentration (Ci) with its respective TEF (TEFi), the toxic equivalence (TEQ) of AhR-ligands in a mixture can be derived from instrumental analysis of the sample: $TEQ = \Sigma Ci \times TEFi$. These WHO TEF values are useful in gauging the relative potency of the cocktail of AhR ligands in environmental samples. They are not useful in potency balance calculations when comparing the results of a bioassay, such as the H4IIE-*luc* reporter gene assay to the TEQ (Lee et al., 2013). To use the bioassay response to verify whether all AhR ligands in a complex mixture have been accounted for in instrumental analysis, bioassay specific relative potencies (RePs) are needed (Lee et al., 2013). However, other environmental contaminants are not well characterised and TEF or ReP values do not exist for these compounds, confusing risk estimates (Hong et al., 2012). Although TEFs and RePs correct for relative potency of AhR agonists at the receptor level, they do not compensate for differential solubility, sorption, bioaccumulation, or biotransformations (Sanderson et al., 1996). Additionally, the ingredients of complex mixtures, such as occur in sediments, potentially interact in a non-additive manner (synergism or antagonism) (Eichbaum et al., 2016; Hecker and Giesy, 2011; Hilscherova et al., 2000; Kannan et al., 2000; Larsson et al., 2013; Wang et al., 2014; Whyte et al., 2004). This effect of mixtures of the compounds at varying concentrations potentially modulates the toxic potential, complicating the risk assessment of xenobiotics (Hilscherova et al., 2000). Performing instrumental analysis alone often leads to an inaccurate estimation of potential risks and other important, but unknown, contaminants are overlooked (Hilscherova et al., 2000).

One way of addressing this gap in risk assessment is to estimate overall biological effects of mixtures of AhR ligands on biota using bioassays (Garrison et al., 1996; Giesy et al., 1994; Murk et al., 1996). It is also sensitive and more cost effective than a full-range instrumental determination (Behnisch et al., 2002; Hilscherova et al., 2000; Hong et al., 2012). Bioassays are able to provide an estimation of total biological activity of all compounds that mediate a response through a similar mechanism of action, such as the AhR, and in doing so provide an integrated biological response (Koh et al., 2006). An advantage of in vitro bioassays above in vivo bioassays it that they involve fewer ethical issues. Limitations, however, are that bioassays cannot identify nor determine the concentrations of chemicals present because their response is not only a function of concentration but also potency and interactions (Koh et al., 2006). Ideally, risk assessment should be conducted by screening environmental samples using assays such as the H4IIE-*luc* assay, and only if the assay results indicate evidence of risk, instrumental analysis should be conducted to determine compounds and concentrations causing the assay responses.

The H4IIE-*luc* bioassay makes use of a mechanistically recombinant luciferase reporter gene that has been stably transfected into rat hepatoma carcinoma cells for the detection and semi-quantification of AhR-ligands. Toxicity of the complex mixture is expressed in terms of the reference compound 2,3,7,8-TCDD (Aarts et al., 1993). Complex mixtures in extracts can produce a variety of concentration-dependant induction responses in H4IIE-*luc* cells, and induction is directly proportional to the compounds present. These effects are created through a common mechanism of action (i.e., activating the AhR). This mechanism of action allows for normalisation of toxicity results and sample potency determination (Baston and Denison, 2011). In addition, organic compounds that do not bind to the AhR with sufficient affinity to cause activation can affect chemical activities of other AhR ligands and thus reduce their apparent potencies (Sanderson and Giesy, 1998).

The H4IIE-*luc* assay has been used widely as a screening tool for AhR mediated potency of various complex matrices, such as sediments, soils, and biological samples, and has been used extensively for risk assessment (Behnisch et al., 2003; Giesy et al., 2002; Hong et al., 2012; Khim et al., 1999; Koh et al., 2004; Larsson et al., 2018; Murk et al., 1996; Tuyen et al., 2014; USEPA, 2014b). Results of the bioassay, indicating presence of AhR ligands are associated with adverse outcomes caused by dioxin-like compounds (Tillitt et al., 1991). Most of the compounds for which TEFs have been derived have been tested on the H4IIE-*luc* cells and resulting relative potencies (RePs) (Behnisch et al., 2003; Larsson et al., 2018; Lee et al., 2013; Scippo et al., 2004; Villeneuve et al., 2002) can be used as surrogates for TEFs for a comparison between predicted toxicity based on analytical concentrations (TEQs) and measured toxicity from bioassays (BEQs).

While there have been studies in the eThekwini area that report on water quality, these have been focused on conventional inorganic and/or microbiological indicators. Organic contaminants, including PAHs, PCBs and organochlorine pesticides (OCPs), have been detected in sediments in the region (Batterman et al., 2009; Godefroy et al., 2005; Newman et al., 2015; Vogt et al., 2018) and concentrations of these compounds were found to be in excess of sediment quality guidelines (SQG) derived by Long et al. (1995) and MacDonald et al. (2000) at some locations, and even the highest limit was exceeded at some sites. These exceedances suggest that organisms exposed to concentrations could experience detrimental effects. However, there is limited information available on the actual effects these organic pollutants can cause to biota.

The aims of this research were to: i) determine the concentrations of

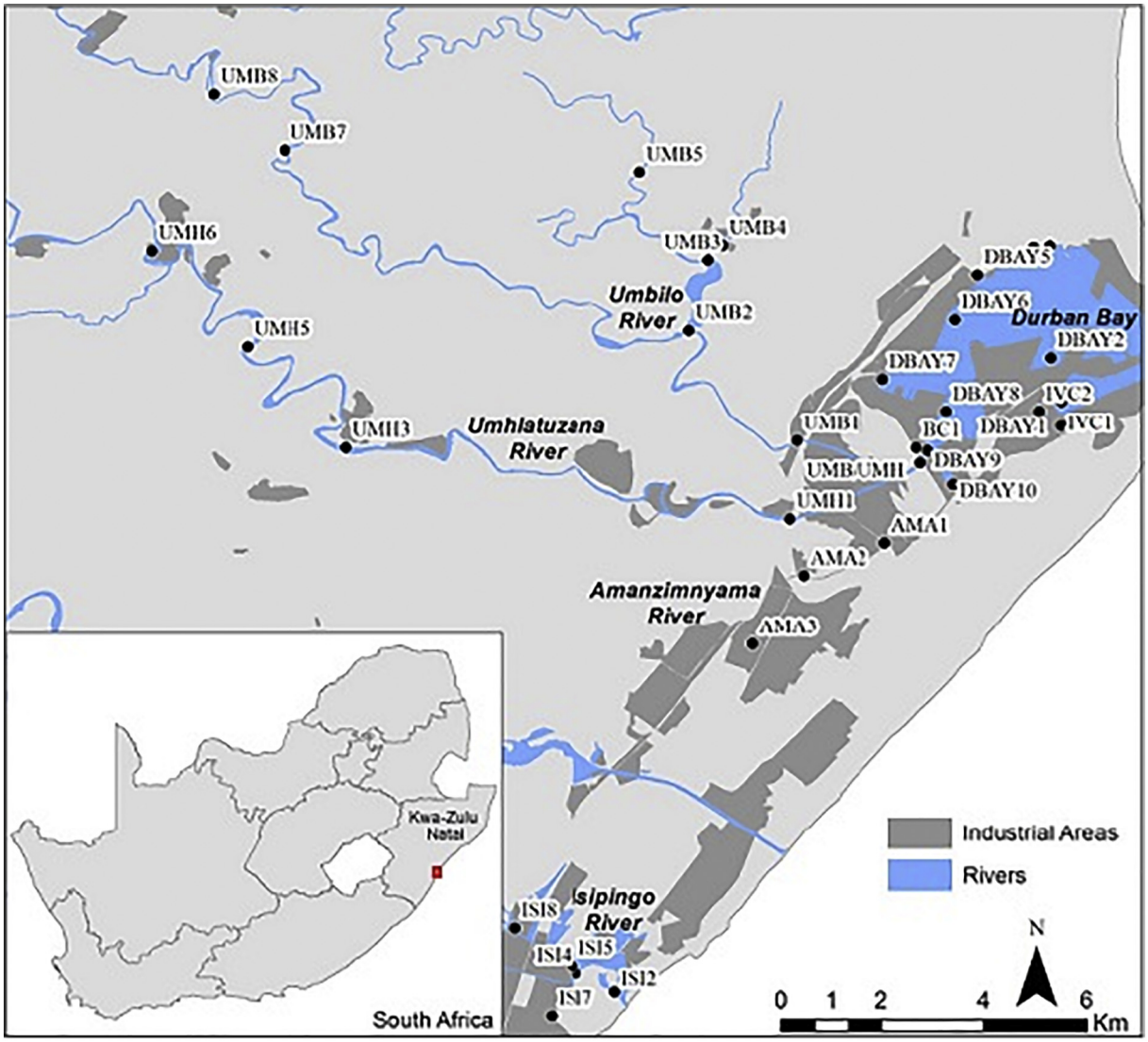

Fig. 1. Overview of Durban, KwaZulu-Natal, showing the positions where sediment was collected.

PAHs and PCBs in the sediment by instrumental analysis; ii) predict their toxic equivalence using previously published RePs (based on the H4IIE-*luc* assay) to calculate a TEQ from the concentrations of the congeners (from aim i); iii) determine the BEQs of the actual sediment extracts using the H4IIE-*luc* bioassay; and iv) determine relative contributions of the instrumentally targeted compounds to total BEQ of the assay by potency balance.

2. Methods

2.1. Study area

Sampling sites were identified within three rivers, the Amanzimnyama, Umbilo, and Umhlatuzana, two canals that drain surface runoff into Durban Bay, the Island View and Bayhead Canals, and in Durban Bay in which the Port of Durban is situated (Fig. 1). These natural and man-made waterways flow through and drain industrialised and urbanised areas in the City of Durban entering the Bay. A fourth river to the south of the Bay, the Isipingo River, was also sampled. This system contains numerous canals lined with cement, allowing sediment to accumulate. Both the Bay area's rivers and Isipingo River had been reported to have poor water quality (Forbes and Demetriades, 2008; Moodley et al., 2015).

2.2. Sample collection and processing

Sediment was collected at 33 sites in the Umbilo (UMB), Umhlatuzana (UMH), Amanzimnyama (AMA) and Isipingo (ISI) Rivers, Durban Bay (DBAY), and in Island View (IV) and Bayhead Canals (BC) (Fig. 1). Three sediment sub-samples were collected, each about 1 m apart, at every site using a Van Veen grab. Samples were composited in a glass bowl until homogenous. Aliquots were transferred to glass jars. The samples were kept on ice in the field and stored at −20 °C in the laboratory until analysis. All equipment that came into contact with the sediment was pre-cleaned with acetone and hexane (Honeywell, Burdick and Jackson, UV-grade).

2.3. Target compound extraction and analysis

Instrumental analyses were performed at a commercial laboratory in the USA using methods described elsewhere (Vogt et al., 2018). In brief, PAHs (Table 1) were Soxhlet extracted with acetone/hexane and underwent an alumina and silica gel clean-up before being analysed on a gas chromatograph coupled to a mass spectrometer (GC–MS). The 16 PAHs listed by the USEPA on their pollutant priority list were analysed. These were naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, benz[*a*]anthracene,

Table 1
Concentrations of PAHs and PCBs quantified by analytical methods, and the resulting TEQs. BEQs (resulting from ReP_{25}) quantified by H4IIE-luc cells exposed to sediment extracts targeting for fractions containing PAH, and PCBs. Bold BEQs indicate viability below 70%. All concentrations are expressed in terms of dry mass.

Station	ΣPAH ($ng{\cdot}g^{-1}$)	ΣPAH_{16} ($ng{\cdot}g^{-1}$)	TEQ_{PAH} ($pg{\cdot}g^{-1}$)	$BEQ_{PAH\text{-}CF}$ ($pg{\cdot}g^{-1}$)	ΣPCB ($ng{\cdot}g^{-1}$)	Σdl PCB ($ng{\cdot}g^{-1}$)	TEQ_{PCB} ($pg{\cdot}g^{-1}$)	$BEQ_{PCB\text{-}CF}$ ($pg{\cdot}g^{-1}$)
AMA1	1500	1000	3.0	200 ± 14	15	1.4	0.1	35 ± 8
AMA2	1000	730	1.6	200 ± 10	3.7	0.6	0.1	32 ± 11
AMA3	160	140	0.7	200 ± 63	5.1	0.6	0.1	9 ± 4
BC1	7200	5200	28	380 ± 120	11	1.5	0.2	< LOD
DBAY1	700	520	2.1	200 ± 5	27	2.2	0.2	< LOD
DBAY2	530	350	1.1	100 ± 9	22	0.6	0.1	< LOD
DBAY3	5100	4700	16	660 ± 130	110	11	1.0	16 ± 7
DBAY4	1400	1300	4.5	210 ± 22	30	1.9	0.2	< LOD
DBAY5	3100	2800	11	780 ± 340	9.8	2.2	0.2	12 ± 6
DBAY6	1100	840	4.9	290 ± 24	26	2.2	0.2	< LOD
DBAY7	2700	2300	13.	790 ± 96	110	13	0.8	9 ± 1
DBAY8	990	740	4.0	230 ± 6	24	4.4	0.2	< LOD
DBAY9	1400	1200	7.8	150 ± 2	7.7	0.6	0.1	< LOD
DBAY10	920	750	2.8	650 ± 26	35	5.1	0.3	9 ± 7
ISI2	450	310	1.6	110 ± 5	3.7	0.6	0.1	**9 ± 5**
ISI4	360	260	1.1	230 ± 110	72	15	1.0	31 ± 1
ISI5	2400	2100	12	220 ± 40	8.7	1.7	0.2	47 ± 2
ISI7	820	700	4.1	120 ± 22	3.7	0.6	0.1	< **LOD**
ISI8	1700	1400	8.3	< **LOD**	48	6.3	0.3	13 ± 4
IVC1	4800	3900	17	650 ± 53	22	2.5	0.2	57 ± 13
IVC2	750	450	1.2	**180 ± 7.0**	3.7	0.6	0.1	< LOD
UMB1	290	190	0.7	82 ± 22	3.7	0.6	0.1	14 ± 9
UMB2	180	150	0.8	**42 ± 1.0**	3.7	0.6	0.1	< LOD
UMB3	85	66	0.2	37 ± 4.0	3.7	0.6	0.1	< LOD
UMB4	210	190	1.2	< LOD	4.1	0.6	0.1	2.6 ± 30
UMB5	120	110	0.5	53 ± 11	3.7	0.6	0.1	< LOD
UMB7	100	89	0.5	25 ± 5.0	3.7	0.6	0.1	< LOD
UMB8	320	280	1.0	58 ± 3.0	3.7	0.6	0.1	< LOD
UMB	160	120	0.5	5.0 ± 1.0	3.7	0.6	0.1	< LOD
UMH1	110	90	0.4	< LOD	3.7	0.6	0.1	< LOD
UMH3	62	48	0.2	18 ± 2.0	3.7	0.6	0.1	< LOD
UMH5	140	110	0.5	54 ± 4.0	3.7	0.6	0.1	< LOD
UMH6	71	59	0.2	2.0 ± 2.0	3.7	0.6	0.1	< LOD

chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*g,h,i*]perylene, indeno[1,2,3-*c,d*]pyrene, and dibenz[*a,h*]anthracene. Other PAHs included are: 1-methylnaphthalene, 2-methylnaphthalene, 1-methylphenanthrene, 2,3,5-trimethylnaphthalene, 2,6-dimethylnaphthalene, benzo[*e*]pyrene, and perylene. PCBs (congeners # 1, 8, 18, 28, 44, 52, 66, 77, 101, 105, 118, 126, 128, 138, 153, 169, 170, 180, 187, 195, 206, and 209) (Table 1) were extracted in an ultrasonic bath with dichloromethane acetone/hexane and analysed with GC–MS (Vogt et al., 2018). The limits of detection (LOD) for PAHs were 1 $ng{\cdot}g^{-1}$ and 1–5 $ng{\cdot}g^{-1}$ for PCBs. All data is expressed in terms of dry mass (dm) unless otherwise stated.

For biological analyses, two sediment extracts were prepared per site: after extraction and clean-up the one extract would contain mostly persistent AhR-ligands, including, but not exclusively, the dl-PCBs. This fraction was denoted the "PCB-containing-fraction" (PCB-CF). The second extract would contain mostly non-persistent AhR-ligands, including, but not exclusively, the 16 USEPA priority PAHs, and is referred to as the "PAH-containing-fraction" (PAH-CF). The sediment was mixed with anhydrous sodium sulphate and extracted in a 3:1 mixture of dichloromethane (DCM) and hexane (Burdick and Jackson; UV-grade) at 100 °C and 11,032 kPa using an accelerated solvent extractor (Dionex ASE 1.0) (Hilscherova et al., 2000; Kannan et al., 2001). The extractor was set to ten-minute static time and five minute heat intervals. The extraction process was repeated once more. The extract containing most of the persistent AhR-ligands was treated with 98% sulphuric acid (1:1 v:v) to digest the majority of non-persistent compounds such as PAHs. Although, some non-persistent AhR-ligands such as smaller PAHs can resist degradation, their contribution would be negligible compared to the persistent AhR-ligands in the bioassay (Behnisch et al., 2001; Lam et al., 2018; Lamoree et al., 2004).

All extracts were fractionated by size exclusion chromatography using two Envirogel gel permeation chromatography (GPC) clean-up columns (19 × 150 mm and 19 × 300 mm) in series. The columns were connected to a Waters 717 plus auto-sampler, Waters 1515 isocratic high pressure liquid chromatography pump, Waters dual λ absorbance detector, and a Waters fraction collector III. A reference mixture containing five compounds of different sizes was used to calibrate the system. The components of the mixture were corn oil, phthalate, methoxychlor, perylene, and sulphur. All were from PESTANAL except the corn oil, which was from Sigma-Aldrich. The PCB-CF was collected in the time period immediately from injection until just before the sulphur would elute. The PAH-CF was collected after verifying the elution time of the single 16 USEPA priority PAHs. It was established that benzo(*g,h,i*)perylene eluted first and fluorene last, which were all before sulphur.

Each of these extracts was further cleaned-up by solid phase extraction (SPE) using dual layer Superclean silica/Florisil columns (LC-Si, 2 g/2 g, Supelco). The target compounds were eluted with a 1:1 DCM:hexane mixture, followed by DCM (USEPA, 2014a). The solvents were replaced by hexane for bioassay analysis.

2.4. Bioassays

2.4.1. H4IIE-luc bioassay

H4IIE-*luc* cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich D2902) supplemented with 10% foetal bovine serum (FBS, Hyclone) in a humidified environment with 5% CO_2 at 37 °C. Before commencing an assay, the nutrient medium was stripped of hormones to prevent false positives. H4IIE-*luc* cells were seeded (80,000 cells mL^{-1}) in the inner 60 wells of white-walled, clear bottom 96-well microtiter plates and incubated for 24 h before exposure in triplicate to a three times dilution series of the sample extract, four times dilution series of 480 pg/mL TCDD, and hexane as a solvent control. H4IIE-*luc* cells were incubated for a further 72 h before light

produced by luciferase was quantified by lysing the cells and adding luciferase assay reagent containing 20 nM tricine, 1.07 mM $Mg(CO3)_4Mg(OH)_2{\cdot}5H_2O$, 2.67 mM $MgSO_4{\cdot}7H_2O$, 0.1 mM ethylenediaminetetraacetic acid-disodium salt, 33.3 mM dithiothreitol, 270 μM coenzyme A, 530 μM adenosine triphosphate, and 470 μM beetle luciferin (Villeneuve et al., 1999). Luminescence was quantified using a Berthold multi-mode microplate reader (TriStar LB941).

Dose-response curves were generated by expressing the sample responses as a percentage of the maximal induction elicited by the reference compound 2,3,7,8-TCDD (%TCDDmax), against a logarithmic transformed function of the sample volume. Relative effect potency (ReP_{25}) was calculated by dividing the 25% effects concentration (EC_{25}) of TCDD by that of the sample (Sanderson and Giesy, 1998). RePs were back calculated to take into account the amount of sediment that was extracted, and expressed as a TCDDequivalence (TCDDeq) in dry mass. This is further referred to as bioassay equivalence (BEQ).

2.4.2. Cell viability

As a control for dead and dying cells, a viability assay was run in parallel to the luminescence assay: It is important to distinguish between "no luminescence" due to dead cells, and "no luminescence" because of the absence of pollutants binding to the AhR. Therefore, cell viability was measured using the xCELLigence real-time cell analyser (RTCA®) (Roche) (Quereda et al., 2010). xCELLigence plates were treated identical to the luminescence plates, and the cell index (CI) was recorded every 30 min. Viabilities of cells exposed to extracts were expressed as a percentage of the viability of the solvent-controlled wells (Quereda et al., 2010).

2.5. Determination of toxicity equivalence of PCBs and PAHs

To compare potencies estimated from instrumentally determined PAHs and PCBs to that calculated from bioassays, a TEQ approach was followed. Concentrations of PCBs or PAHs were multiplied by their corresponding ReP that was determined from exposing H4IIE-*luc* cells to individual PAH (Larsson et al., 2012) or PCB (Scippo et al., 2004) congeners. These RePs are based on 72 h exposures. These RePs were used instead of the traditional WHO TEFs because they would be more relevant to bioassay responses when comparing potencies. TEQs of individual compounds were summed to provide a ΣPCB_{TEQ} and a ΣPAH_{TEQ} for each site.

2.6. Statistical analyses

Statistical analyses were conducted using GraphPad Prism 5 (GraphPad Software, Inc.), and the level of statistical significance was set at $p < 0.05$. Where concentrations were < LOD, a surrogate value was used, calculated by multiplying the detection frequency of the congener with the congeners' LOD (Verhaert et al., 2013). Normality testing, using the Kolmogorov-Smirnov test, revealed that the data were not normal, thus the non-parametric Spearman-r was used to compute the correlation of TEQs to BEQs.

3. Results & discussion

3.1. AhR activity of sediment extracts

The majority of sediment extracts did not reduce viability of cells to < 70% (which is an arbitrary value that was used to indicate cytotoxicity). However, there were exceptions: the PAH-CF of sites IVC2 and UMB2 were cytotoxic; for site ISI8 cytotoxicity was considerable and only 8% of the cells were viable. The PCB-CF from sites ISI2 and ISI7 also impaired cell viability (< 70%). Low viability could diminish the ability of cells to respond to AhR-ligands, leading to lower BEQs compared to cells with high viability. Reduced viability can be caused by co-extracted toxic compounds, or even high concentrations of the target compounds.

Responses induced from the PAH-CF were, on average, 20-fold greater than that for PCB-CF. This was expected because the PAH-CF contains more AhR-ligands because it did not go through an acid clean-up step like the PCB-CF. The PAH-CF exposed cells expressed a %TCDDmax of 8–150%, which resulted in a mean $BEQ_{PAH\text{-}CF}$ of 209 ± 234 pgTCDDeq·g^{-1} and a range of < LOD–790 pgTCDDeq·g^{-1} (Table 1). Responses > 100% TCDDmax indicate superinduction of the AhR (Baston and Denison, 2011). Cells exposed to the PCB-CF ranged from 3 to 43% TCDDmax, which equated to a $BEQ_{PCB\text{-}CF}$ of < LOD–57 pgTCDDeq·g^{-1} and a mean of 8.9 ± 15 pgTCDDeq·g^{-1} (Table 1). These responses were less than those for sediments tested on H4IIE-*luc* cells in other regions of the world: Liaohne River (89–251 pgTCDDeq·g^{-1}) (Zhang et al., 2017), Haihe River (330–930 pgTCDDeq·g^{-1}), Dagu River (1200–13,900 pgTCDDeq·g^{-1}) (Song et al., 2006) and Wenyu River (8.5–336 pgTCDDeq·g^{-1}) (Luo et al., 2009) in China, and Danube River, Germany (112–1400 pgTCDDeq·g^{-1}) (Keiter et al., 2008). Similar responses were observed for extracts of sediments from Meiliang Bay, China, using the ethoxyresorufin *O*-deethylase activity assay (17–35 pgTCDDeq·g^{-1}) (Qiao et al., 2006).

3.2. Instrumental analysis

Instrumentally quantified PAHs were ubiquitous throughout the study area, although not all congeners were detected at each site. The mean ΣPAH concentration was 1240 ± 1660 ng·g^{-1}. The 16 USEPA priority PAHs (ΣPAH_{16}) made up the greater proportion of the concentrations (Table 1), with the greatest concentrations on average within Durban Bay and rivers and canals discharging into it. Identification of potential sources of PAHs using ratios between congeners indicated that they were largely derived from pyrogenic sources (Vogt et al., 2018), which is typical of urban and industrial regions (Sun et al., 2009; Tao et al., 2010). Relatively high concentrations of PAHs in these areas are attributed to activities in the port and various other industrial activities in the region. PCBs were sporadically at a concentration > LOD. The mean ΣPCB concentration was 19 ± 28 ng·g^{-1}, with the highest concentrations in Durban Bay and associated canals and rivers (Table 1).

3.3. Potency balance

Potency balance can be used to compare contributions of various chemicals to the total AhR-potency, whereby explained versus unexplained contributions can be determined (Khim et al., 1999). The ratio of TEQs to BEQs can explain the contribution of analysed congeners to the overall toxicity of a sample as determined through AhR activation. Here, concentrations of $BEQ_{PAH\text{-}CF}$ were compared to concentrations of TEQ_{PAH}, and concentrations of $BEQ_{PCB\text{-}CF}$ to concentrations of TEQ_{PCB}. Concentrations of TEQ_{PAH} ranged from 0.2–28 pg·g^{-1} with a mean of 4.6 ± 6 pg·g^{-1}. Concentrations of TEQ_{PCB} were less than those of TEQ_{PAH}, between 0.06 and 1 pg·g^{-1}, with a mean of 0.2 ± 0.2 pg·g^{-1} (Table 1). This is likely because many of the PCB congeners were not detected in samples, and ReP values have only been determined for a few PCB congeners, the dioxin-like PCBs, of which our instrumental analysis only targeted for three.

Concentrations of TEQ_{PAH} and $BEQ_{PAH\text{-}CF}$ were significantly correlated ($r = 0.65$, $p < 0.05$), however only between 0.34 and 9.9% of the potencies of the extracts could be explained by the instrumentally quantified PAHs, as indicated by the ratio of $TEQ_{PAH}/BEQ_{PAH\text{-}CF}$. Indicating that the potencies of extracts cannot be explained by instrumentally quantified PAHs. Concentrations of TEQ_{PCB} and $BEQ_{PCB\text{-}CF}$ were poorly correlated ($r = 0.3$, $p > 0.05$), and the ratio of $TEQ_{PCB}/BEQ_{PCB\text{-}CF}$ was small (0.17–9.0%). This therefore implies that in both types of extracts there were more AhR-ligands present than what was instrumentally quantified and subsequently calculated concentrations

of TEQs. Examples of compounds that might have been present in the PAH-CF could also include PAHs not included on the USEPA priority pollutant list (Wölz et al., 2010), other halogenated aromatic hydrocarbons, polychlorinated naphthalenes (Hong et al., 2012), polycyclic aromatic compounds (PACs), such as hydroxylated or alkylated PAHs (Lam et al., 2018), and natural occurring compounds like indoles, tryptophan-derived products, oxidised carotenoids, and heterocyclic amines. Although these have a weak affinity for the AhR (Hecker and Giesy, 2011; Hilscherova et al., 2000; Otte et al., 2013), they nevertheless have been shown to be AhR agonists. The PCB-CF could also have contained PCDD/Fs, polybrominated biphenyls and –diphenylethers (Behnisch et al., 2003; Hong et al., 2012). Due to a lack of resources, and not knowing specifically which other compounds to target, additional AhR ligands were not analysed.

Another explanation for the difference between concentrations of TEQ and BEQ is that the AhR agonists present in complex environmental mixtures may have synergistic effects and therefore account for the greater BEQ concentrations (Eichbaum et al., 2016; Hecker and Giesy, 2011; Hilscherova et al., 2000; Larsson et al., 2013; Whyte et al., 2004). Synergism has been observed using H4IIE-*luc* cells exposed to PAHs (Larsson et al., 2012), which was attributed to superinduction of AhR by the PAHs. Synergism has also been suggested as an explanation for BEQs being greater than TEQs by Hong et al. (2012), who reported that only 17% of the concentrations of BEQ determined by H4IIE-*luc* assay could be explained by TEQ concentrations. TEQs, based on 7 dL-PAHs and 12 dL-PCBs, explained < 10% of the AhR potency measured by the H4IIE-*luc* (Xia et al., 2014). While < 26% of the concentration of BEQ was explained by concentrations of TEQs based on PAHs, PCBs and PCDD/Fs (Zhang et al., 2017).

3.4. Comparison to sediment quality guidelines

South Africa has not defined sediment quality guidelines for organic chemicals. Thus, sediment quality guidelines derived by the Canadian Council of Ministers of the Environment (CCME) were used to assess relative risks posed by AhR-mediated compounds in the sediments. The Canadian guideline is based on TEQs for fish (Van den Berg et al., 1998) and is defined by two limits: interim sediment quality guideline (ISQG) of < 0.85 pg TCDD·g^{-1} dm, below which detrimental effects are rarely expected, and probable effects level (PEL) of > 21.5 pg TCDD·g^{-1} dm, above which detrimental effects are expected to be frequent. Concentrations between these limits are estimated to cause occasional adverse effects, and have been regarded expectant to experience moderate risk (CCME, 2001).

All $BEQ_{PAH\text{-}CF}$ concentrations exceeded the ISQG—with the exception of the two sites that were < LOD (Fig. 2a), and the $BEQ_{PAH\text{-}CF}$ were also greater than the PEL at 82% sites. The TEQ_{PAHs} concentration at 67% of the sites exceeded the ISGQ, and the PEL was exceeded at one site (BC1). Concentrations of BEQs were greater than those of TEQs because there are more AhR ligands present in environmental extracts that are capable of mediating a response in the cells compared to the amount of TEF-possessing compounds analysed instrumentally. It has been reported that contributions to potency of a mixture is greater for less potent AhR ligands, which are those that would be present in the PAH-CF because they are omnipresent and generally in much higher concentrations than the persistent compounds (Eljarrat and Barceló, 2003). It is also possible that there are mixture effects, causing synergism (Eichbaum et al., 2016; Hecker and Giesy, 2011; Hilscherova et al., 2000; Larsson et al., 2013; Whyte et al., 2004).

Concentrations of TEQ_{PCBs} exceeded the ISQG at two sites (DBAY3 and ISI4) (Fig. 2b). This is a cause for concern because this TEQ was based on only three PCB congeners for which RePs were available. If more PCB congeners and other persistent compounds (e.g. PCDD/Fs and BFR) were to be included in the analysis, the number of sites with TEQ concentrations in excess of the SQG would likely have been greater. However, the general trend is that PCBs are observed at greater concentrations in the environment, from 10-fold greater, compared to PCDD/Fs (Eljarrat and Barceló, 2003). Concentrations of BEQs at 45% of the sites exceeded the ISQG, and 15% of the sites had BEQ concentrations in excess of the PEL. Although many of the sites exhibited BEQ concentrations < LOD, all detectable BEQs (at 15 sites) were in excess of the guidelines. The exceedance of the ISQG, and more importantly the PEL is concerning considering the compounds causing the response are persistent (PCBs, PCDD/Fs) and are known to be far more toxic than PAHs (Hilscherova et al., 2000).

Applying the Canadian SQG it would be probable that organisms at sites where AhR mediated toxicity levels (BEQs and TEQs) exceeded the ISQGs would experience detrimental effects. Predicted AhR-mediated toxicity of PAHs (TEQ_{PAH} and $BEQ_{PAH\text{-}CF}$) revealed that only 61% of the sites were commonly identified as being a moderate risk (in excess of the ISQG), as opposed to the 94% of the sites in this risk category when only $BEQ_{PAH\text{-}CF}$ are considered. When the TEQ_{PCB} and $BEQ_{PCB\text{-}CF}$ were compared for the PCBs exceeding ISQG, only 18% of the sites were commonly identified to experience moderate risk (Fig. 3). Therefore, the likelihood of detrimental effects at these commonly identified sites would be much higher considering both toxicity prediction models identified them as moderate risk. Additionally, two sites (DBAY3 and ISI4) were inclusively predicted to have toxic effects to aquatic life based on exposure to PAHs and PCBs—concentrations of both $BEQ_{PCB\&PAH\text{-}CF}$ and $TEQ_{PCB\&PAH}$ were in excess of the ISQG.

Instrumentally analysed PAHs and PCBs were compared to SQGs derived by Long et al. (1995) for marine and estuarine systems. This SQG was chosen because most of the sites in the study area are estuarine. The guideline had been developed based on a variety of biological effects due to exposure to compounds, and not only activation of the AhR. Two guideline levels are set for different compound classes; the Effects Range Low (ERL) below which detrimental effects are unlikely to be observed, and the Effects Range Median (ERM) above which detrimental effects are likely to be observed, and between the two detrimental effects are expected occasionally—and organisms exposed to these compounds are at medium risk.

Using this approach, 9% of the sites had PAH concentrations and 27% of the sites had PCB concentrations in excess of the ERL (Fig. 3a and b). At only one site was both the PAH and PCB concentration in excess of the ERL (DBAY3). No PAH or PCB concentrations exceeded the ERM. Comparing sites that are predicted to experience toxicity from these compounds through the AhR (exceedance of ISQGs by BEQs and TEQs) to those sites that were identified as expected to have detrimental effects due to exposure to the PAHs and PCBs (exceedance of ERL from instrumental analysis), only one site was commonly identified to experience moderate risk, namely DBAY3.

It is clear that the different approaches to predict risk do not coincide on the selection of sites to be at risk. The fact that there was a single site that was identified by all risk assessment approaches to be at risk, proves the limitations to any risk assessments employed and researchers and regulators should be aware of these limitations. The risk prediction depends on the mode through which the toxic effect is mediated, and different sites are identified to be at risk due to the compounds present. Therefore, in instances where resources are limited, using biological measures of toxicity is useful, especially given the fact that numerous compounds have the ability to mediate toxicity through the AhR, and analysis for all will be challenging.

4. Conclusions

BEQ and TEQ concentrations calculated for sediment in urbanised and industrialised parts of Durban in South Africa indicated that there were AhR agonists present, including persistent compounds (as indicated by concentrations of $BEQ_{PCB\text{-}CF}$ and the TEQ_{PCB}). A potency balance showed that only a small portion of the analysed compounds contributed to the responses elicited from the cells (BEQs). This suggests there were many unidentified compounds present in the sediment

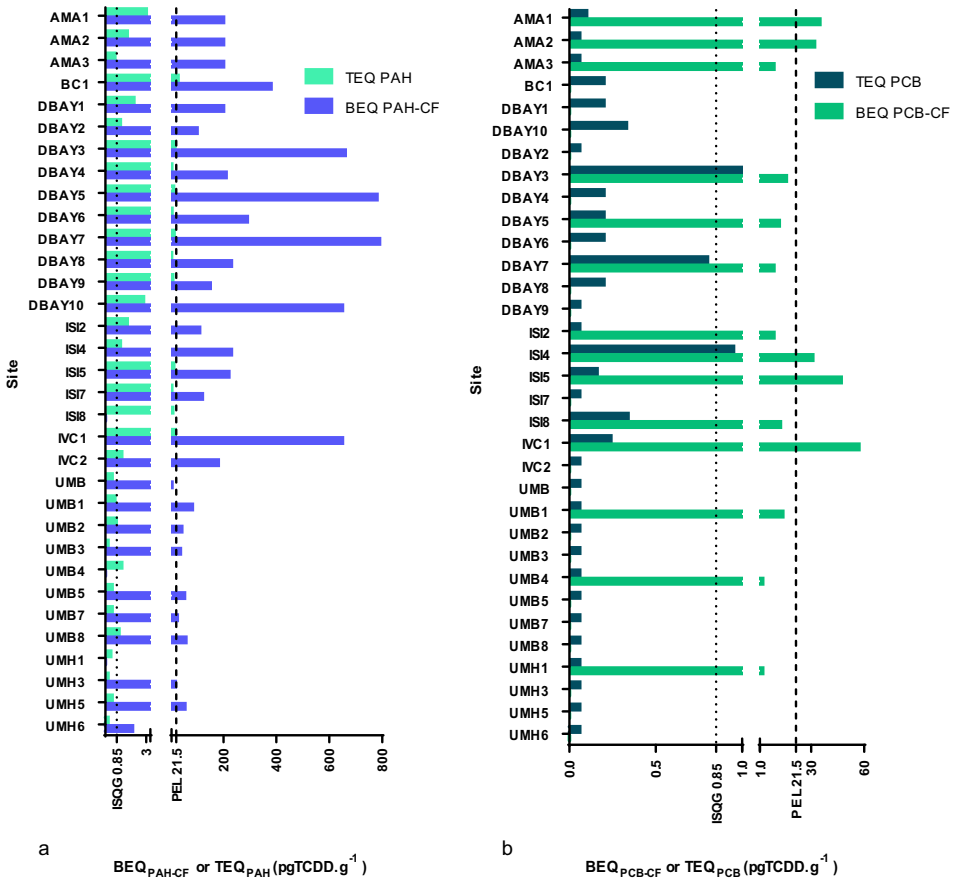

Fig. 2. BEQ and TEQs for (a) PAHs and PAH-CF and (b) PCBs and PCB-CF compared to the Canadian SQGs for the protection of aquatic life as indicated by the ISQG (0.85 pgTCDD·g^{-1}) and PEL (21.5 pgTCDD·g^{-1}).

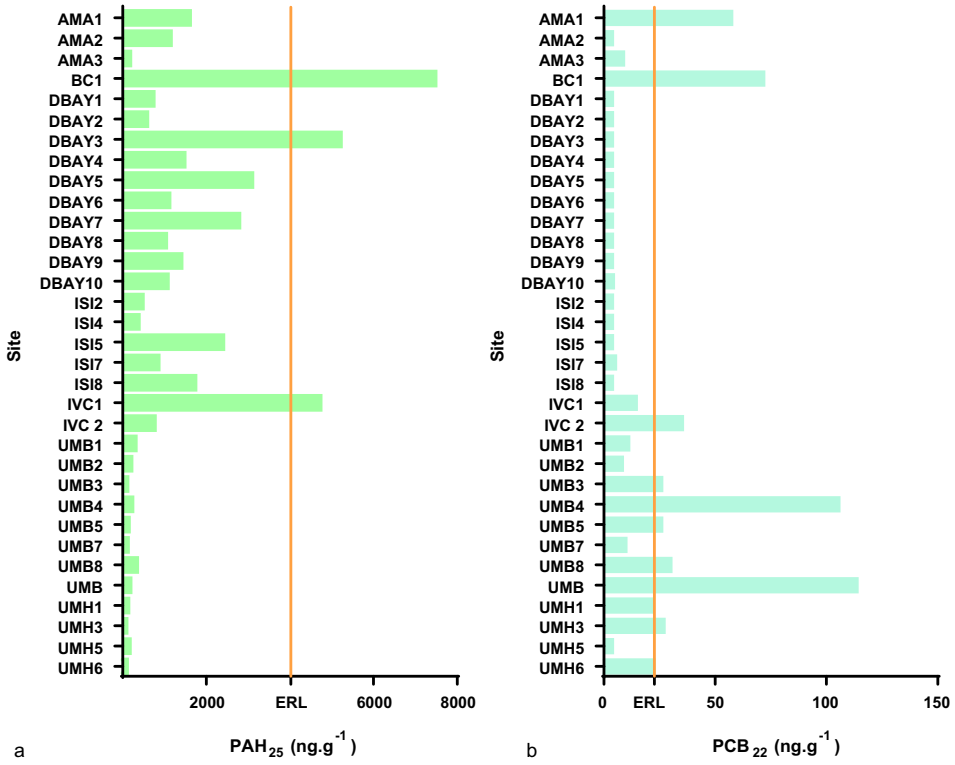

Fig. 3. Concentrations of (a.) ΣPAH$_{25}$ and (b.) ΣPCB$_{22}$ compared to the lower limit SQG (ERL) described by Long et al. (1995).

contributing to these responses, although synergism between determined compounds cannot be ruled out. Furthermore, the bioassay approach provides a more comprehensive assessment of AhR agonists in environmental matrices, providing biological evidence of possible harmful effects to the biota. It is especially useful in instances where funds and appropriate expertise for instrumental analysis are often limited, such as in developing countries. The assay can be used for frequent screening of AhR ligands, and to verify whether mitigation efforts were successful Instrumental analysis can then be selectively used to identify and quantify compounds from sites that did show biological effects probably saving time and money.

Acknowledgments

Colleagues at the CSIR are thanked for assistance in the field and laboratory. Funding was provided by the South African Water Research Commission (WRC) (Project K5/1977) and the National Research Foundation (NRF) (Society, Ecosystems and Change (SEAChange), Project KFD2010083000001). Opinions expressed and conclusions reached are those of the authors and may not necessarily reflect those of the WRC or NRF. Some authors received independent NRF funding: Grant No. 103487. Prof. Giesy was supported by the Canada Research Chairs program.

References

Aarts, J., Denison, M., De Haan, L., Schalk, J., Cox, M., Brouwer, A., 1993. Ah receptor-mediated luciferase expression: a tool for monitoring dioxin-like toxicity. Organohalogen Compd. 13, 361–364.

Barakat, A.O., Moonkoo, K., Yoarong, Q., Wade, T.L., 2002. Organochlorine pesticides and PCB residues in sediments of Alexandria Harbour, Egypt. Mar. Pollut. Bull. 44, 1426–1434.

Baston, D.S., Denison, M.S., 2011. Considerations for potency equivalent calculations in the Ah receptor-based CALUX bioassay: normalization of superinduction results for improved sample potency estimation. Talanta 83, 1415–1421.

Batterman, S.A., Chernyak, S., Gouden, Y., Hayes, J., Robins, T., Chetty, S., 2009. PCBs in air, soil and milk in industrialized and urban areas of KwaZulu-Natal, South Africa. Environ. Pollut. 157, 654–663.

Behnisch, P.A., Hosoe, K., Sakai, S., 2001. Bioanalytical screening methods for dioxins and dioxin-like compounds—a review of bioassay/biomarker technology. Environ. Int. 27, 413–439.

Behnisch, P.A., Hosoe, K., Brouwer, A., Sakai, S., 2002. Screening of dioxin-like toxicity equivalents for various matrices with wildtype and recombinant rat hepatoma H4IIE cells. Toxicol. Sci. 69, 125–130.

Behnisch, P.A., Hosoe, K., Sakai, S., 2003. Brominated dioxin-like compounds: *in vitro* assessment in comparison to classical dioxin-like compounds and other polyaromatic compounds. Environ. Int. 29, 861–877.

CCME (Canadian Council of Ministers of the Environment), 2001. Canadian sediment quality guidelines for the protection of aquatic life: polychlorinated dioxins and furans (PCDD/Fs). In: Canadian Environmental Quality Guidelines, 1999. Canadian Council of Ministers of the Environment, Winnipeg.

De Vos, J., Quinn, L., Roos, C., Pieters, R., Bouwman, H., Gorst-Allman, P., Rohwer, E., Giesy, J.P., 2013. Experience in South Africa of combining bioanalysis and instrumental analysis of PCDD/Fs. Trends Anal. Chem. 46, 189–197.

Denison, M.S., Zhao, B., Baston, D.S., Clark, G.C., Murata, H., Han, D., 2004. Recombinant cell bioassay systems for the detection and relative quantitation of halogenated dioxins and related chemicals. Talanta 63, 1123–1133.

EDGE (Economic Development and Growth in eThekwini), 2014. Port and Logistics, 11th edition. . http://www.durban.gov.za/City_Government/City_Vision/IDP/Documents/Final%202016_17%20IDP%2029052016.pdf, Accessed date: 13 June 2018.

Eichbaum, K., Brinkmann, M., Nuesser, L., Buchinger, S., Reifferscheid, G., Codling, G., Jones, P., Giesy, J.P., Hecker, M., Hollert, H., 2016. Bioanalytical and instrumental screening of the uptake of sediment-borne, dioxin-like compounds in roach (*Rutilus rutilus*). Environ. Sci. Pollut. Res. 23, 12060–12074.

Eljarrat, E., Barceló, D., 2003. Priority lists for persistent organic pollutants and emerging contaminants based on their relative toxic potency in environmental samples. Trends Anal. Chem. 22, 655–665.

eThekwini Municipality, 2015. 2015/2016 integrated development plan. http://www.durban.gov.za/City_Government/City_Vision/IDP/Documents/Final%202016_17%20IDP%2029052016.pdf, Accessed date: 15 July 2018.

Forbes, A., Demetriades, N., 2008. Estuaries of Durban, KwaZulu-Natal, South Africa. Report for the Environmental Management Department, eThekwini Municipality. http://www.durban.gov.za/City_Services/development_planning_management/environmental_planning_climate_protection/Publications/Documents/Estuaries%20of%20Durban%20%28technical%20report%29.pdf, Accessed date: 5 July 2018.

Garrison, P., Tullis, K., Aarts, J., Brouwer, A., Giesy, J., Denison, M., 1996. Species-specific recombinant cell lines as bioassay systems for the detection of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-like chemicals. Toxicol. Sci. 30, 194–203.

Giesy, J.P., Kannan, K., 1998. Dioxin-like and non-dioxin-like toxic effects of polychlorinated biphenyls (PCBs): implications for risk assessment. Crit. Rev. Toxicol. 28, 511–569.

Giesy, J.P., Ludwig, J.P., Tillitt, D.E., 1994. Dioxins, dibenzofurans, PCBs and colonial, fish-eating water birds. In: Schecter, A. (Ed.), Dioxins and Health. Springer, Boston, pp. 249–307.

Giesy, J.P., Hilscherova, K., Jones, P., Kannan, K., Machala, M., 2002. Cell bioassays for detection of aryl hydrocarbon (AhR) and estrogen receptor (ER) mediated activity in environmental samples. Mar. Pollut. Bull. 45, 3–16.

Godefroy, S.J., Martincigh, B.S., Salter, L.F., 2005. Measurements of polycyclic aromatic hydrocarbons and genotoxicity in soot deposited at a toll plaza near Durban, South Africa. S. Afr. J. Chem. 58, 61–66.

Hecker, M., Giesy, J.P., 2011. Effect-directed analysis of Ah-receptor mediated toxicants, mutagens, and endocrine disruptors in sediments and biota. In: Brack, W. (Ed.), Effect-Directed Analysis of Complex Environmental Contamination. Springer, Berlin, pp. 285–313.

Hilscherova, K., Machala, M., Kannan, K., Blankenship, A.L., Giesy, J.P., 2000. Cell bioassays for detection of aryl hydrocarbon (AhR) and estrogen receptor (ER) mediated activity in environmental samples. Environ. Sci. Pollut. Res. 7, 159–171.

Hong, S., Khim, J.S., Naile, J.E., Park, J., Kwon, B., Wang, T., Lu, Y., Shim, W.J., Jones, P.D., Giesy, J.P., 2012. AhR-mediated potency of sediments and soils in estuarine and coastal areas of the Yellow Sea region: a comparison between Korea and China. Environ. Pollut. 171, 216–225.

Kannan, K., Villeneuve, D.L., Yamashita, N., Imagawa, T., Hashimoto, S., Miyazaki, A., Giesy, J.P., 2000. Vertical profiles of dioxin-like and estrogenic activities associated with a sediment core from Tokyo Bay, Japan. Environ. Sci. Technol. 34, 3568–3573.

Kannan, K., Hilscherova, K., Imagawa, T., Yamashita, N., Williams, L.L., Giesy, J.P., 2001. Polychlorinated naphthalenes,-biphenyls,-dibenzo-*p*-dioxins, and-dibenzofurans in double-crested cormorants and herring gulls from Michigan waters of the Great Lakes. Environ. Sci. Technol. 35, 441–447.

Keiter, S., Grund, S., van Bavel, B., Hagberg, J., Engwall, M., Kammann, U., Klempt, M., Manz, W., Olsman, H., Braunbeck, T., 2008. Activities and identification of aryl hydrocarbon receptor agonists in sediments from the Danube River. Anal. Bioanal. Chem. 390, 2009–2019.

Khim, J.S., Villeneuve, D.L., Kannan, K., Koh, C.H., Giesy, J.P., 1999. Characterization and distribution of trace organic contaminants in sediment from Masan Bay, Korea. 2. In vitro gene expression assays. Environ. Sci. Technol. 33, 4206–4211.

Kilemade, M., Hartl, M., Sheehan, D., Mothersill, C., Van Pelt, F., O'Brien, N., O'Halloran, J., 2004. An assessment of the pollutant status of surficial sediment in Cork Harbour in the South East of Ireland with particular reference to polycyclic aromatic hydrocarbons. Mar. Pollut. Bull. 49, 1084–1096.

Koh, C.H., Khim, J.S., Kannan, K., Villeneuve, D.L., Senthilkumar, K., Giesy, J.P., 2004. Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) and 2,3,7,8-TCDD equivalents (TEQs) in sediment from the Hyeongsan River, Korea. Environ. Pollut. 132, 489–501.

Koh, C.H., Khim, J.S., Villeneuve, D.L., Kannan, K., Giesy, J.P., 2006. Characterization of trace organic contaminants in marine sediment from Yeongil Bay, Korea: 2. Dioxin-like and estrogenic activities. Environ. Pollut. 142, 48–57.

Kumalo, P., Phewa, P., Thomas, J., 2012. Grey water project starts in Kwa-Mathambo, Durban. http://sasdialliance.org.za/grey-water-project-starts-in-kwa-mathambo-durban, Accessed date: 21 August 2018.

Lam, M.M., Bülow, R., Engwall, M., Giesy, J.P., Larsson, M., 2018. Methylated PACs are more potent than their parent compounds—a study on AhR-mediated activity, degradability and mixture interactions in the H4IIE-*luc* assay. Environ. Toxicol. Chem. 37, 1409–1419.

Lamoree, M., Swart, K., Senhorst, H., van Hattum, B., 2004. Validation of the acidic sample clean-up procedure for the DR-CALUX assay. IVM Report (L-04/02). In: Institute for Environmental Studies. Vrije Universiteit, Amsterdam.

Larsson, M., Orbe, D., Engwall, M., 2012. Exposure time-dependent effects on the relative potencies and additivity of PAHs in the Ah receptor-based H4IIE-*luc* bioassay. Environ. Toxicol. Chem. 31, 1149–1157.

Larsson, M., Hagberg, J., Rotander, A., van Bavel, B., Engwall, M., 2013. Chemical and bioanalytical characterisation of PAHs in risk assessment of remediated PAH-contaminated soils. Environ. Sci. Pollut. Res. 20, 8511–8520.

Larsson, M., Lam, M.M., van Hees, P., Giesy, J.P., Engwall, M., 2018. Occurrence and leachability of polycyclic aromatic compounds in contaminated soils: chemical and bioanalytical characterization. Sci. Total Environ. 622, 1476–1484.

Lee, K.T., Hong, S., Lee, J.S., Chung, K.H., Hilscherova, K., Giesy, J.P., Khim, J.S., 2013. Revised relative potency values for PCDDs, PCDFs, and non-ortho-substituted PCBs for the optimized H4IIE-luc in vitro bioassay. Environ. Sci. Pollut. Res. 20, 8590–8599.

Long, E.R., MacDonald, D.D., Smith, S.L., Calder, F.D., 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environ. Manag. 19, 81–97.

Luo, J., Ma, M., Zha, J., Wang, Z., 2009. Characterization of aryl hydrocarbon receptor agonists in sediments of Wenyu River, Beijing, China. Water Res. 43, 2441–2448.

MacDonald, D.D., Ingersoll, C., Berger, T., 2000. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. Arch. Environ. Contam. Toxicol. 39, 20–31.

Masood, N., Zakaria, M.P., Halimoon, N., Aris, A.Z., Magam, S.M., Kannan, N., Mustafa, S., Ali, M.M., Keshavarzifard, M., Vaezzadeh, V., 2016. Anthropogenic waste indicators (AWIs), particularly PAHs and LABs, in Malaysian sediments: application of aquatic environment for identifying anthropogenic pollution. Mar. Pollut. Bull. 102, 160–175.

Moodley, K., Pillay, S., Pather, K., 2015. Spatiotemporal characterization of water

chemistry and pollution sources of the Umhlatuzana, Umbilo and Amanzimnyama River catchments of Durban, KwaZulu-Natal, South Africa. Environ. Earth Sci. 74, 1273–1289.
Murk, A.J., Legler, J., Denison, M.S., Giesy, J.P., van de Guchte, C., Brouwer, A., 1996. Chemical-activated luciferase gene expression (CALUX): a novel *in vitro* bioassay for Ah receptor active compounds in sediments and pore water. Toxicol. Sci. 33, 149–160.
Mzoughi, N., Chouba, L., 2011. Distribution and partitioning of aliphatic hydrocarbons and polycyclic aromatic hydrocarbons between water, suspended particulate matter, and sediment in harbours of the west coastal of the Gulf of Tunis (Tunisia). J. Environ. Monit. 13, 689–698.
Newman, M.C., 2010. Fundamentals of Ecotoxicology, 3rd ed. CRC press, Boca Raton, Florida.
Newman, B., Arabi, S., Weerts, S., Pieters, R., Vogt, T., 2015. Prevalence and significance of organic contaminants and metals in aquatic ecosystems in the eThekwini area of Kwazulu-Natal, WRC Report No. 1977/1/15. http://www.wrc.org.za/Knowledge%20Hub%20Documents/Research%20Documents/1977-1-14.pdf, Accessed date: 5 May 2018.
Newsted, J.L., Jones, P.D., Giesy, J.P., Crawford, R.A., Ankley, G.T., Tillitt, D.E., Gooch, J.W., Denison, M.S., 1995. Development of toxic equivalency factors for PCB congeners and the assessment of TCDD and PCB mixtures in rainbow trout. Environ. Toxicol. Chem. 14, 861–871.
Otte, J.C., Keiter, S., Fassbender, C., Higley, E.B., Rocha, P.S., Brinkmann, M., Wahrendorf, D.S., Manz, W., Wetzel, M.A., Braunbeck, T., Giesy, J.P., Hecker, M., Hollert, H., 2013. Contribution of priority PAHs and POPs to Ah receptor-mediated activities in sediment samples from the River Elbe Estuary, Germany. PLoS One 8, 1–11.
Qiao, M., Chen, Y., Zhang, Q., Huang, S., Ma, M., Wang, C., Wang, Z., 2006. Identification of Ah receptor agonists in sediment of Meiliang Bay, Taihu Lake, China. Environ. Sci. Technol. 40, 1415–1419.
Qiao, M., Huang, S.-B., Wang, Z., 2009. Evaluation of chronic toxicities from sediments before and after ecological project in Meiliang Bay, Lake Taihu, using cell bioassays. Ecol. Eng. 35, 1631–1636.
Quereda, J., Martínez-Alarcón, L., Mendoça, L., Majado, M., Herrero-Medrano, J., Pallarés, F., Ríos, A., Ramírez, P., Muñoz, A., Ramis, G., 2010. Validation of xCELLigence real-time cell analyzer to assess compatibility in xenotransplantation with pig-to-baboon model. In: Transplantation Proceedings. Elsevier, pp. 3239–3243.
Safe, S., 2001. Molecular biology of the Ah receptor and its role in carcinogenesis. Toxicol. Lett. 120, 1–7.
Sanderson, J., Giesy, J., 1998. Wildlife toxicology, functional response assays. In: Meyers, R.A. (Ed.), Encyclopedia of Environmental Analysis and Remediation. John Wiley and Sons, New York.
Sanderson, J.T., Aarts, J.M., Brouwer, A., Froese, K.L., Denison, M.S., Giesy, J.P., 1996. Comparison of Ah receptor-mediated luciferase and ethoxyresorufin-*O*-deethylase induction in H4IIE cells: implications for their use as bioanalytical tools for the detection of polyhalogenated aromatic hydrocarbons. Toxicol. Appl. Pharmacol. 137, 316–325.
Scippo, M.L., Eppe, G., De Pauw, E., Maghuin-Rogister, G., 2004. DR-CALUX® screening of food samples: evaluation of the quantitative approach to measure dioxin, furans and dioxin-like PCBs. Talanta 63, 1193–1202.
Song, M., Jiang, Q., Xu, Y., Liu, H., Lam, P.K., O'Toole, D.K., Zhang, Q., Giesy, J.P., Jiang, G., 2006. AhR-active compounds in sediments of the Haihe and Dagu Rivers, China. Chemosphere 63, 1222–1230.
Sun, J.H., Wang, G.L., Chai, Y., Zhang, G., Li, J., Feng, J., 2009. Distribution of polycyclic aromatic hydrocarbons (PAHs) in Henan reach of the Yellow River, Middle China. Ecotoxicol. Environ. Saf. 72, 1614–1624.
Tao, J., Huggins, D., Welker, G., Dias, J., Ingersoll, C., Murowchick, J., 2010. Sediment contamination of residential streams in the metropolitan Kansas city area, USA: part I. distribution of polycyclic aromatic hydrocarbon and pesticide-related compounds. Arch. Environ. Contam. Toxicol. 59, 352–369.
Tian, J., Feng, Y., Fu, H., Xie, H.Q., Jiang, J.X., Zhao, B., 2015. The aryl hydrocarbon receptor: a key bridging molecule of external and internal chemical signals. Environ. Sci. Technol. 49, 9518–9531.
Tillitt, D.E., Ankley, G.T., Verbrugge, D.A., Giesy, J.P., Ludwig, J.P., Kubiak, T.J., 1991. H4IIE rat hepatoma cell bioassay-derived 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents in colonial fish-eating waterbird eggs from the Great Lakes. Arch. Environ. Contam. Toxicol. 21, 91–101.
Tuyen, L.H., Tue, N.M., Suzuki, G., Misaki, K., Viet, P.H., Takahashi, S., Tanabe, S., 2014. Aryl hydrocarbon receptor mediated activities in road dust from a metropolitan area, Hanoi—Vietnam: contribution of polycyclic aromatic hydrocarbons (PAHs) and human risk assessment. Sci. Total Environ. 491, 246–254.
USEPA (U.S. Environmental Protection Agency), 2014a. Method 3630C: silica gel cleanup, part of the test methods for evaluating solid waste, physical/chemical methods. https://www.epa.gov/sites/production/files/2015-12/documents/3630c.pdf, Accessed date: 20 June 2016.
USEPA (U.S. Environmental Protection Agency), 2014b. Method 4435, method for toxic equivalents (TEQs) determinations for dioxin-like chemical activity with the CALUX bioassay. https://www.epa.gov/sites/production/files/2015-12/documents/4435.pdf, Accessed date: 20 June 2016.
Vallack, H.W., Bakker, D.J., Brandt, I., Broström-Lundén, E., Brouwer, A., Bull, K.R., Gough, C., Guardans, R., Holoubek, I., Jansson, B., 1998. Controlling persistent organic pollutants–what next? Environ. Toxicol. Pharmacol. 6, 143–175.
Van den Berg, M., Birnbaum, L., Bosveld, A., Brunström, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ. Health Perspect. 106, 775–792.
Van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., 2006. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol. Sci. 93, 223–241.
Verhaert, V., Covaci, A., Bouillon, S., Abrantes, K., Musibono, D., Bervoets, L., Verheyen, E., Blust, R., 2013. Baseline levels and trophic transfer of persistent organic pollutants in sediments and biota from the Congo River Basin (DR Congo). Environ. Int. 59, 290–302.
Villeneuve, D.L., Richter, C.A., Blankenship, A.L., Giesy, J.P., 1999. Rainbow trout cell bioassay-derived relative potencies for halogenated aromatic hydrocarbons: comparison and sensitivity analysis. Environ. Toxicol. Chem. 18, 879–888.
Villeneuve, D., Khim, J., Kannan, K., Giesy, J., 2002. Relative potencies of individual polycyclic aromatic hydrocarbons to induce dioxinlike and estrogenic responses in three cell lines. Environ. Toxicol. 17, 128–137.
Vogt, T., Pieters, R., Newman, B.K., 2018. PAHs, OCPs and PCBs in sediments from three catchments in Durban, South Africa. Afr. J. Aquat. Sci. 43, 35–49.
Wang, J., Bovee, T.F., Bi, Y., Bernhöft, S., Schramm, K.W., 2014. Aryl hydrocarbon receptor (AhR) inducers and estrogen receptor (ER) activities in surface sediments of Three Gorges Reservoir, China evaluated with in vitro cell bioassays. Environ. Sci. Pollut. Res. 21, 3145–3155.
Whyte, J., Schmitt, C., Tillitt, D., 2004. The H4IIE cell bioassay as an indicator of dioxin-like chemicals in wildlife and the environment. Crit. Rev. Toxicol. 34, 1–83.
Wölz, J., Brack, W., Moehlenkamp, C., Claus, E., Braunbeck, T., Hollert, H., 2010. Effect-directed analysis of Ah receptor-mediated activities caused by PAHs in suspended particulate matter sampled in flood events. Sci. Total Environ. 408, 3327–3333.
Xia, J., Su, G., Zhang, X., Shi, W., Giesy, J.P., Yu, H., 2014. Dioxin-like activity in sediments from Tai Lake, China determined by use of the H4IIE-*luc* bioassay and quantification of individual AhR agonists. Environ. Sci. Pollut. Res. 21, 1480–1488.
Zhang, Y., Ke, X., Gui, S., Wu, X., Wang, C., Zhang, H., 2017. Evaluation of AhR-agonists and AhR-agonist activity in sediments of Liaohe River protected areas, China. Mar. Pollut. Bull. 115, 292–296.
Zhu, Y., Yang, L., Kawamura, K., Chen, J., Ono, K., Wang, X., Xue, L., Wang, W., 2017. Contributions and source identification of biogenic and anthropogenic hydrocarbons to secondary organic aerosols at Mt. Tai in 2014. Environ. Pollut. 220, 863–872.