

Endocrine disrupting chemicals (phenol and phthalates) in the South African environment: a need for more monitoring

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Abstract

There has been increasing concern about the impacts of exposure to chemical compounds with endocrine disrupting activities in the environment, especially aquatic environments, to wildlife and humans. South Africa is known to have used and abused most chemicals listed by developed and developing countries as endocrine-disrupting chemicals. Endocrine-disrupting chemicals have been reported in water, sediment and serum, as well as in fish tissue samples, at a level that could trigger endocrine disruption in humans and wildlife. Although some monitoring has been reported, particularly in water systems within the country, information on EDCs in other environmental matrices is scanty. The water systems monitored so far are very few. The strongest economy in Africa, and an emerging world economy depending on agriculture, mining, manufacturing and industry, needs to focus more on monitoring and to strengthen government organs and institutions to monitor and ensure environmental safety.

Keywords: Endocrine disrupting chemicals (EDCs), phenols, phthalates, monitoring, South Africa

Introduction

The endocrine system, also called the hormonal system, is one of the main systems for communicating, controlling and coordinating the body's activities in mammals (Ying et al., 2004). It works with the nervous system to regulate essential body functions. These body functions include energy metabolism, reproduction, growth and development, osmoregulation and homeostasis. The endocrine system also regulates reproductive processes and skeletal development (Bornman et al., 2007; Burger and Moolman, 2006; Ying et al., 2004; Vogel, 2004).

Endocrine disrupting chemicals (EDCs) consist of many natural and synthetic organic compounds, but are mostly man-made products such as alkylphenols, alkylphenols-ethoxylates, and polychlorinated biphenyls (PCBs). Others include polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, organochlor pesticides, dichlorodiphenyl, dichloroethylene, nonylphenols, steroid hormones and phthalates (Arditsoglou and Voutsas, 2008; Hjelmborg et al., 2006; Mauricio et al., 2006). EDCs are able to cause abnormalities in invertebrate, fish, avian, reptilian, and mammalian species (Arditsoglou and Voutsas, 2008; Ferraz et al., 2007; Hjelmborg et al., 2006; Mauricio et al., 2006; Moder et al., 2007; Peng et al., 2006).

There has been debate for and against the occurrence of EDCs in humans. However, there are increased incidences of abnormalities in human sexual and cognitive development in some societies with speculation that these are being caused by EDC exposure (Campbell et al., 2006; Falconer et al., 2006; Matthiessen, 2000; Suliman et al., 2006; Solomon and Schettler, 2000). These abnormalities include low sperm count

and decrease in sperm quality (Van Wyk et al., 2003), and immunological and neurological effects (Patandin et al., 1999).

Over the last 2 decades there has been increasing concern about the likely impacts of exposure to chemical compounds with endocrine-disrupting activity in the environment (Game et al., 2006; Hecker and Giesy, 2008; Mauricio et al., 2006; Moder et al., 2007; Segner, 2005; Shin et al., 2007; Sumpter, 2005; Xue and Xu, 2006). Endocrine-disrupting effects of chemicals were first documented in the 1930s but research was only accelerated in the late 1970s and early 1980s along 2 initially isolated pathways, i.e. human health effects and wildlife biology (Matthiessen, 2000; Moder et al., 2007; Trenholm et al., 2006; Vogel, 2004). The endocrine system's glands, hormones and their respective functions are listed in Table 1; Table 2 shows some EDCs with their common utilisations, and the target hormones and animals affected.

Considering that many of these compounds can elicit estrogenic responses at very low concentrations (parts per billion to parts per trillion), there is need for concern as many of the phthalate esters and phenolic compounds have been found at measurable concentrations in wastewater, surface waters, sediments, groundwater, and even drinking water in many countries (Cai et al., 2003; Cortazar et al., 2005; Huang et al., 2008; Sha et al., 2007).

Mechanisms of endocrine disruption

Endocrine disruptors initiate their disruption activities using one or more of the following mechanisms:

- By binding to receptors and mimicking or antagonising the effects of the endocrine hormones (Barcelo and Kettrup, 2004; Burger and Moolman, 2006; Jiao and Cheng, 2008; Sumpter, 2005; Vogel, 2004)
- By affecting the concentration of hormones through the altering of their synthesis or metabolism of natural

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Gland	Hormones	Functions
Hypothalamus	Releasing hormones	Stimulate pituitary activity
Pituitary	Trophic (stimulating) hormones	Stimulate thyroid, adrenal, gonadal and pancreatic activity
Thyroid	Thyroid	Regulate metabolism, growth and development
Adrenal	Corticosteroid hormones Catecholamines	Regulate metabolism and behaviour
Pancreas	Insulin and glucagon	Regulate blood sugar levels
Gonads	Sex steroid hormones (estrogens and androgens)	Regulate development and growth, reproduction, immunity, onset of puberty and behaviour

Adapted from Bornman et al., 2007

Compound	Common usage	Target hormone	Animals affected
Industrial chemicals			
Bisphenol A ^b	Plasticiser	Thyroid, cortisol	Mammals, birds, fish
PCBs, Dioxins, PCP, PCDFs	Flame retardants, unintended by-products during incineration	Estrogens	Reptiles, amphibians
Phthalates^a			
BBP, DEHP, D-n-BP	Plasticiser	Estrogens	Mammals, birds, fish, reptiles, amphibians
Alkylphenols^b			
p-Nonylphenol	Plasticiser	Estrogens	Mammals, birds, fish, reptiles, amphibians
Organochlorine Pesticides^b			
DDT, DDE, Chlordane Dieldrin, Heptachlor, Lindane, Endosulfan Oxychlordane, etc.	Insecticides	Estrogens and androgens	Mammals, birds, fish, reptiles, amphibians
Heavy metals^b			
Cadmium, mercury, lead	Batteries, paints	Adrenaline, estrogens	Mammals, birds, fish

^a Hill et al., 2001

^b Zala and Penn, 2004

hormones (Bradlow et al., 1995; Rice et al., 2003; Sumpter, 2005; Toppari et al., 1996; Ying et al., 2004)

- By interfering with the signal between the different components of the hypothalamus-pituitary-endocrine gland axes (Clotfelter et al., 2004; Dawson, 2000; Kitano et al., 2006)
- By modifying the number of hormone receptors in a cell (Lascombe et al., 2000; Rajapakse et al., 2001; Soto et al., 1995; Welch et al., 1969).

Routes of exposure of EDCs to humans and animals

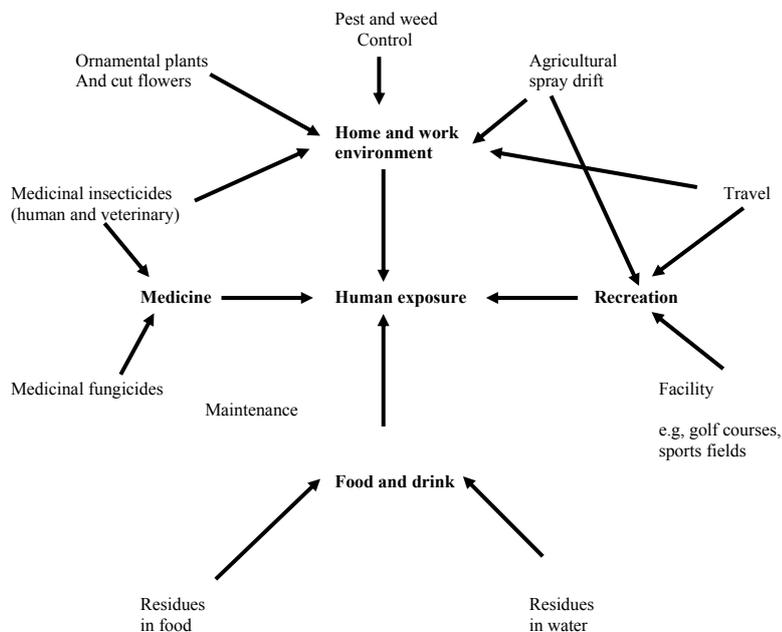
Human beings can be exposed to endocrine-disrupting chemicals via water, air, soil or food, through ingestion (i.e. oral), inhalation, and dermal absorption (Rice et al., 2003; US EPA, 1992; WHO, 2003), as with every other environmental contaminant. The major route of exposure to EDCs for young infants and children is via the oral route by direct ingestion of the chemicals, breast milk, infant formula, cow's milk, contaminated media like water, food, surface and carpet dust, toys and medical devices (Huang et al., 2008; Rice et al., 2003; Sathyanarayana, 2008).

Animals are also exposed to EDCs in the air, water and in their food (McKinlay et al., 2008; Clotfelter et al., 2004). EDCs enter animal bodies through the skin, gill, and even via the mother *in utero* or *in ovo*. EDCs, because they are

lipid-soluble, tend to accumulate in animal's fat tissues. This problem is further aggravated by the process of biomagnification, in which chemical concentrations increase at higher trophic levels (Huang et al., 2008; McKinlay et al., 2008). In aquatic birds, contaminant concentrations are often 100 times greater in body tissue than in the surrounding water. In the case of marine animals, significant bioaccumulation has been observed in several species (Alatrisme-Mondrageon et al., 2003). Top predators are essential for maintaining the integrity of food webs; thus, biomagnification of EDCs can affect entire ecosystems by harming species at the highest trophic levels (Clotfelter et al., 2004). The most common routes of human and animal exposure to EDCs are depicted in Fig. 1.

Some of these chemicals used as pesticides remain as residues in fresh and processed food (Bornman et al., 2007; Vogel, 2004). The effects of EDCs on the human body differ substantially from poisoning or toxic exposure which could cause cancer, physiological birth defects, gene mutation, cell damage or acute health effects (Campbell et al., 2006; Sharpe and Skakkabaek, 1993; Vogel, 2004). The health effects of EDCs may be pervasive throughout the planet due to fast and universal transport of chemicals through the world's atmosphere and oceans. Endocrine disruption may take longer time spans, or act selectively during certain stages of development or only in later generations for the effects to manifest (Falconer et al., 2006; Juvancy et al., 2008; Vogel, 2004).

Figure 1
Exposure routes of humans to EDCs
(Adapted from: McKinlay et al., 2008)



Routes of exposure of EDCs to the aquatic environment

There is growing concern regarding water quality; consequently industries around the world are faced with the challenge of ensuring a sustained and safe supply of drinking water from various sources. However, population growth, urbanisation, industrial development and associated changes in agricultural and land-use practices have contributed significantly to reducing water quality through naturally occurring and anthropogenic contamination (Falconer et al., 2006; Suliman et al., 2006).

Surface water contaminants include metals, carcinogens, synthetic chemicals, pharmaceuticals, veterinary and illicit drugs. Others are ingredients in cosmetics, personal care products, food supplements together with their respective metabolites and transformed products (Brasher and Wolff, 2004; Cortazar et al., 2005; Falconer et al., 2006). Some of these compounds and their metabolites are endocrine-disrupting chemicals and get into water through direct discharge of pharmaceuticals, chemicals, households, agricultural and industrial wastes. EDCs also get into water through accidental spills and indirect sources such as storm-water runoff (Falconer et al., 2006; Huang et al., 2008).

Drinking water sources can be contaminated due to flow of water through agricultural areas where agrochemicals are extensively used to improve crop yield (Falconer et al., 2006). EDCs are not only concentrated in the environment through biogeochemical processes but are also scavenged from water through sorption onto suspended materials, and deposited to be part of the bottom substrate. Aquatic wildlife appears to be particularly at risk since the aquatic environment is usually a sink for many hormonally-active chemicals, including industrial chemicals, pesticides, organochlorides, pharmaceuticals, natural and synthetic estrogens or phytoestrogens (Alatrisme-Mondragon et al., 2003; Li et al., 2006; Luks-Betlej et al., 2001; Segner, 2005; Suliman et al., 2006; Xue and Xu, 2006; Yuan et al., 2002).

Many aquatic ecosystems are faced with spatially or temporally alarming levels, and persistent complex mixtures, of EDCs, as a result from pollution with industrial

chemicals (Schmidt et al., 2005). The aquatic environment is susceptible to pollution partly because there is very considerable intentional release of chemicals into rivers, lakes and the sea and partly because it receives a lot of accidental releases of chemicals through spills, runoff, wastewater from waste treatment plants and atmospheric deposition (Luks-Betley et al., 2001; Sanchez-Avila et al., 2009; Sumpter, 2005).

The main sources of endocrine-disrupting chemicals in rivers and lakes of Europe and North America are sewage effluents and agricultural chemical runoff. In developing countries, such as those in Africa and Asia, uncontrolled domestic and industrial discharge to waterways contributes tremendously to high levels of EDCs in aquatic environments (Falconer et al., 2006; Peng et al., 2006). The pressure of waste dumping or accidental spills has recently been on the increase with growing populations. The increasing use of water by people has contributed to the spatial and temporally alarming levels and complex mixtures of these chemicals (Falconer et al., 2006; Sumpter, 2005; Schmidt et al., 2005).

EDCs are introduced into the environment as byproducts of various technological processes. In the aquatic environment, the mobility of EDCs is increased when associated with fulvic or humic acids and particulates, which are often deposited in sediments, which usually determines their rates of transformation (Huang et al., 2008; Sun et al., 2006). They are transported through the food chain via benthic algae and invertebrates, which can be eaten by fish or birds. They may also undergo a series of processes such as biodegradation, dilution and photolysis once in waterways; activities that contribute to their elimination from the environmental water (Brasher and Wolff, 2004; Cortazar et al., 2005; Czaplicka, 2001; Petrovic et al., 2001). However, this creates potential routes of exposure of endocrine-disrupting chemicals to terrestrial and aquatic wildlife.

Phthalates, phenols and phenol derivatives have been included in the list of priority pollutants by the US Environmental Protection Agency (EPA) and the European Union (Llompart et al., 2002) due to their activities on aquatic and terrestrial animals.

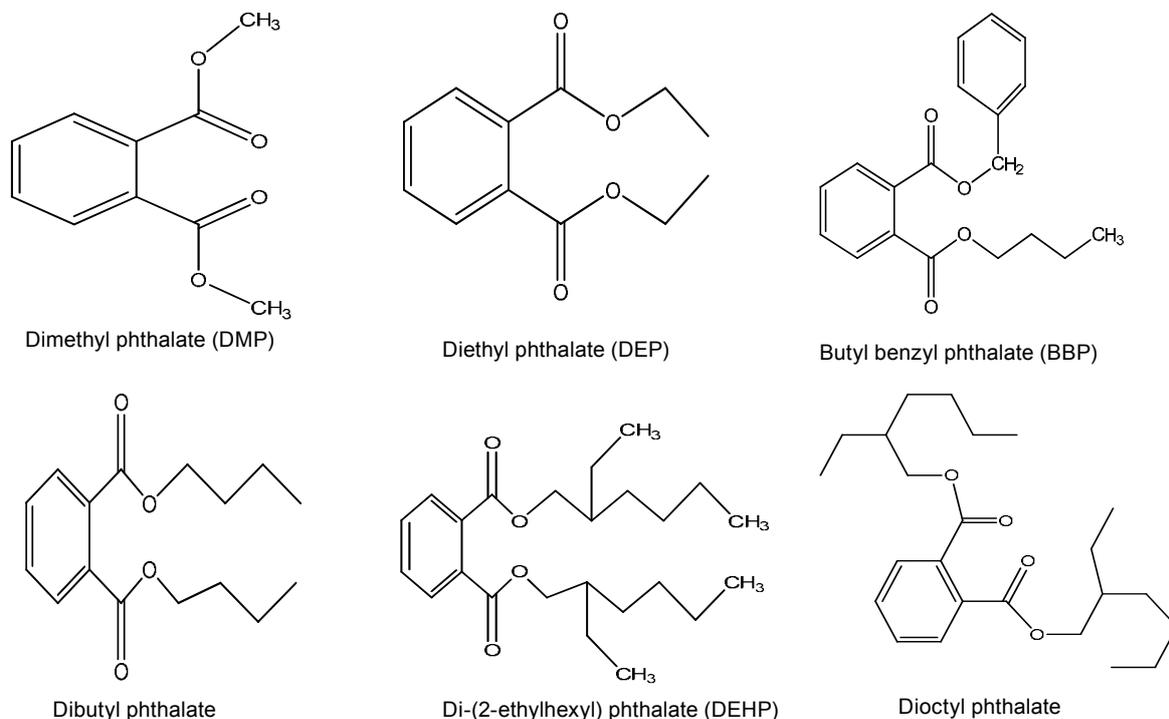


Figure 2
Chemical structures of phthalates

Phthalates

Background information on phthalic acid esters (PAEs)

Phthalates or phthalic acid esters (PAEs) are di-alkyl or alkyl esters of 1,2 benzene carboxylic acid (Adeniyi et al., 2008; Alatrisme-Mondragon et al., 2003; Luks-Betlej et al., 2001). They are formed when methanol, ethanol or other alcohols react with the carboxyl groups on the benzene ring of phthalic acids. The corresponding esters are formed with different alkyl chains, e.g., dimethyl phthalate (DMP), dibutyl phthalate (DBP) and di(2-ethylhexyl) phthalate (DEHP). PAEs are hydrophilic in nature and vary in structure (Fig. 2) and composition. Generally, the molecular weight of phthalates ranges between 194 and 396 with low melting points ranging from -4.6°C to 5.5°C (Table 4).

Application of phthalates

PAEs have been widely used as plasticizers in the production of polyvinyl chloride (PVC) based plastics which include rubber, cellulose film, styrene, adhesives, coatings, pulp and paper manufacturing (Cortazar et al., 2005; Huang et al., 2008; Yuan et al., 2002; Rudel and Perovich, 2009). Other important usage of PAEs are in plumbing, pesticide formulations, non-ionic surfactants, construction materials, vinyl upholstery, table cloths, and shower curtains, to improve the mechanical properties of the plastic resin, particularly its flexibility and softness (Alatrisme-Mondragon et al., 2003; Cortazar et al., 2005; Huang et al., 2008; Kayali et al., 2006; Ling et al., 2007; Luks-Betlej et al., 2001; Yuan et al., 2002; Rudel and Perovich 2009; Yuan et al., 2008).

The PAE component makes up 10 to 60% of plastic product to provide flexibility; the phthalate plasticizers

are not chemically bonded to resin and therefore are easily released or leached during the life cycle of plastic products (Adeniyi et al., 2008; Yuan et al., 2008). Phthalates occur as components of plastics that are used for major domestic and industrial purposes. This includes teething rings, pacifiers, soft squeeze toys, plastic bottles, food containers and medical equipment. They are also parts of laboratory products (tubes, caps, gas chromatography septa, vinyl gloves), cosmetics and industrial solvents that are made from plastics (Adeniyi et al., 2008; Alatrisme-Mondragon et al., 2003; De Jager et al., 1998; Huang et al., 2008; Kayali et al., 2006).

Globally, several millions of phthalate esters are produced and used annually as primary additives to polyvinyl chloride (PVC) based plastics (Adeniyi et al., 2008; Alatrisme-Mondragon et al., 2003; Ling et al., 2007; Rudel and Perovich, 2009; Yuan et al., 2008). Phthalate esters migrate into environmental components during production and distribution processes, usage and disposal. Ways of entering the environment include aqueous leaching from plastics and waste, incineration of plastic waste, volatilization from resin matrices and wet deposition from the atmosphere (Muszkat et al., 1997; Ling et al., 2007; Luks-Betlej et al., 2001; Polo et al., 2005; Rudel and Perovich, 2009).

Phthalates as EDCs

Since PAEs are ubiquitous in our environment, the major sources of their exposure to man are shown in Table 3. Studies have revealed detectable levels of phthalate esters in samples of foodstuff, human mother's milk, dust, environmental samples (water, soil, sediment) and textiles with di (2-ethylhexyl) phthalate (DEHP) and di-n-butyl phthalate (DBP) recorded as the most abundant. Generally, phthalates are of low acute toxicity with short biologic half-lives

Phthalate parent compound	Potential sources of exposure
Di-2-ethylhexyl phthalate (DEHP)	PVC-containing medical tubing, blood storage bags, medical devices, food contamination, food packaging, indoor air, plastic toys, wall coverings, tablecloths, floor tiles, furniture upholstery, shower curtains, garden hoses, swimming pool liners, rainwear, baby pants, dolls, some toys, shoes, automobile upholstery and tops, packaging film and sheets, and sheathing for wire and cable
Diethyl phthalate (DEP)	Cosmetics, nail polish, deodorant, perfumes/cologne, lotions, aftershave, pharmaceuticals/herbal coating, insecticide
Di-butyl phthalate (DBP)	Nail polish, make-up, aftershave, perfumes, pharmaceuticals/herbal coating, chemiluminescent glow sticks
Di-n-octyl phthalate (DnOP)	Children's toys
Butyl benzyl phthalate (BBzP)	Vinyl flooring, adhesives, sealants, food packaging, furniture upholstery, vinyl tiles, carpet tiles, and artificial leather; also used in certain adhesives
Di-methyl phthalate (DMP)	Insecticides, indoor-air, adhesives, hairstyling products, shampoo, aftershave

Sources: Swan, 2008; Rudel and Perovich 2009

	Dimethyl phthalate	Diethyl phthalate	Dibutyl phthalate	Butylbenzyl phthalate	Di-n-octylphthalate	Di-(2-ethylhexyl) phthalate
Abbreviation	DMP	DEP	DBP	BBP	DNOP	DEHP
CAS RN	131-11-3	84-66-2 ^a	84-74-2	85-68-7 ^a	117-84-0 ^a	117-81-7
Molecular weight	194.1886	222.24 ^a	278.3474	312.36 ^a	390.6 ^b	390.5618
Molecular formula	C ₁₀ H ₁₀ O ₄	C ₁₂ H ₁₄ O ₄	C ₁₆ H ₂₂ O ₄	C ₁₉ H ₂₂ O ₄	C ₂₄ H ₃₈ O ₄	C ₂₄ H ₃₈ O ₄
Density	1.19	1.12 ^a	1.043	1.0 ^a		0.9732
Melting point	5.5°C ^b	-40.5°C ^b	-35°C	61.3°C ^a	-46°C ^b	-50°C
Boiling point	283.7°C	295°C ^a	340°C	92.5°C ^a	390 ^b	386.9°C
Water solubility	4 200 mg·ℓ ⁻¹ at 20°C	1 100 mg·ℓ ⁻¹ at 25°C	11.2 mg·ℓ ^{-1b}	2.7 mg·ℓ ^{-1b}	0.0005 mg·ℓ ^{-1b}	0.003 mg·ℓ ^{-1b}
Physical state	Colourless, oily liquid with a slight ester odour	Colourless, oily liquid	Colourless, oily liquid with a very weak aromatic odour			Colourless, oily liquid with almost no odour

^a Luks-Betlej et al., 2001

^b Huang et al., 2008

of approximately 6-12 hours (Duty et al., 2005). They are metabolised and eliminated within 48 h of exposure in vertebrates and are therefore not highly bioaccumulative in the system (van den Berg et al., 2003). However, the frequent use of phthalate-containing personal care and consumer products, along with the frequent detection of metabolites in random population samples, suggests that phthalate exposure is continuous and ubiquitous. Consequently, the endocrine-disrupting nature of some phthalates is potentially responsible for adverse effects on human reproduction and development.

Based on previous human and animal studies, phthalates have been classified according to their impact strength. DEHP has been included in Class B2 and has been shown to be embryotoxic and teratogenic (Altriste-Mondragon et al., 2003; Kayali et al., 2006; Latini et al., 2009), while butyl-benzyl phthalate (BBP) is in Class C (possible human carcinogen) and di-n-butyl phthalate (DBP), di-ethyl phthalate (DEP) and dimethyl phthalate (DMP) were included in Class D (not yet classified as human carcinogens (Altriste-Mondragon et al., 2003).

Phenols

Background information on phenol

Phenol and its derivatives are aromatic molecules containing hydroxyl (OH), methyl (CH₃), amide (NH₂) or sulphonic group (McNeely et al., 1979) attached to the benzene ring structure (Huang et al., 2007; Toniolo et al., 2007). Phenols are widely present in the environment and occur in nature as building blocks for plants (Baltussen et al., 1999; Santana et al., 2009; Santana et al., 2005). They are formed naturally from decomposition of leaves and wood as well as through human activity like water purification processes (Santana et al., 2009; Santana et al., 2005; Schmidt-Baumler et al., 1999).

Phenol and its methyl derivatives show a stronger tendency to adsorb onto solid matrices and some have been found to be toxic to fish and other aquatic life. The compounds at very low concentration have adverse effects on taste and odour of water and fish (Baldwin and Debowski, 1988; Czaplicka, 2001; Schmidt-Baumler et al., 1999). The compounds are introduced into rivers, groundwater and soil directly through industrial

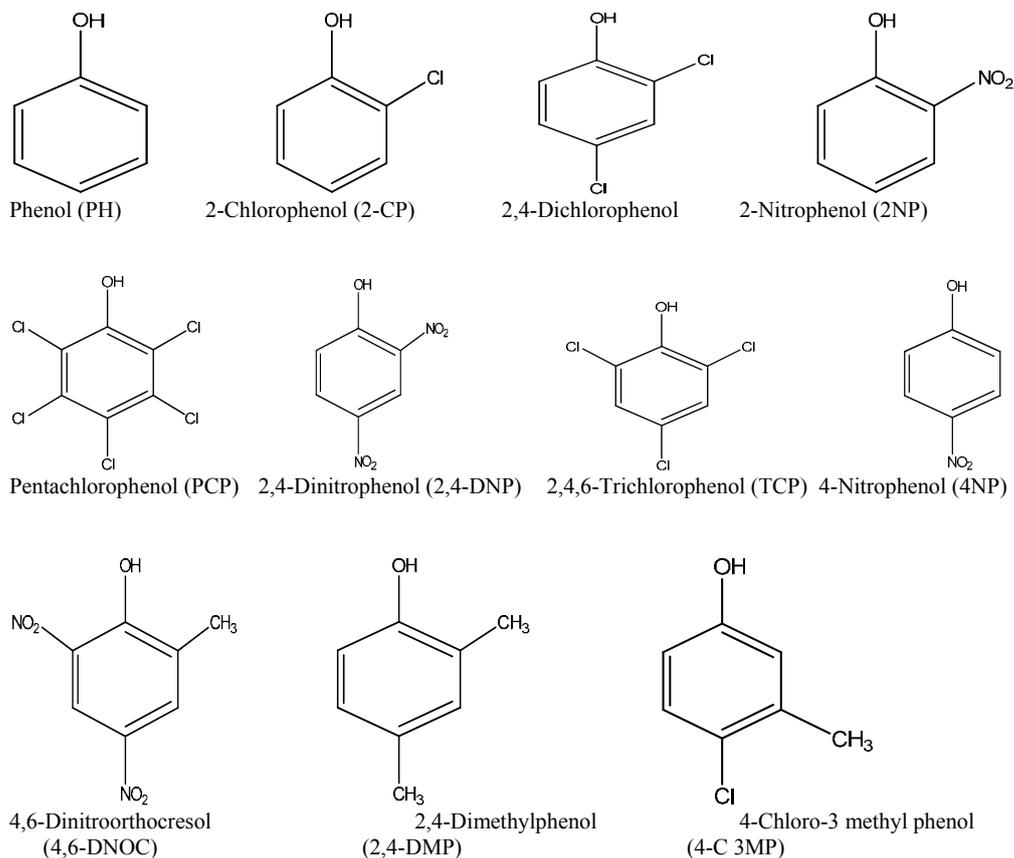


Figure 3
Chemical structures of some phenolic compounds classified as priority pollutants

effluents or indirectly through natural or synthetic chemicals (Bagheri et al., 2004; Ribeiro et al., 2002; Suliman et al., 2006). They are considered major environmental risks, with speculations of suspected endocrine disruptor or carcinogen effects (Bagheri et al., 2004; Suliman et al., 2006).

Application of phenol

Industrially, phenols and their derivatives are used in several useful products. They are used to manufacture chemicals such

as pesticides, explosives, dyes, for pulp bleaching with chlorine, in wood preservatives, insecticides, herbicides, antioxidants, adhesives, plastics and synthetic intermediates (Baldwin and Debowski, 1988; Cledera-Castro et al., 2006; Czaplicka, 2001; Hartung et al., 2007; Ozkaya, 2005; Ribeiro et al., 2002; Santana et al., 2009; Santana et al., 2005; Suliman et al., 2006).

Globally, the production of phenolic compounds is over 300 000 tons per annum. They have been widely used in the last 40 years as components of detergents, emulsifiers, dispersants and anti-foamers (Gabriel et al., 2007; Petrovic et al.,

Table 5
Physical properties of selected phenolic compounds

	Molecular formula	Molecular formula	Boiling point (°C)	Melting point (°C)	pKa	Log Kow (Octanol/water)	Water solubility gl-1 at 20°C
Phenol	C ₆ H ₅ OH ^b	94.11 ^a	181.7 ^c	40.5 ^c	10.0 ^b	1.46 ^a	83 ^b
2-Methyl 4,6-dinitrophenol	C ₈ H ₁₀ O ^d	122.17 ⁱ	211 ^c	22-23	10.58 ^d	2.30 ^a	0.05 ^c
4-Chloro-3-methyl phenol	C ₇ H ₇ ClO ^a	142.58 ^b	235 ^b	63-65 ^c	9.6 ^c	3.10 ^c	4 ^c
2-Chlorophenol	C ₆ H ₅ ClO	128.6 ^c	175 ^c	9.3 ^c	8.56 ^c	2.15 ^a	28.5 ^c
2,4-Dichlorophenol	C ₆ H ₄ Cl ₂ O	163 ^c	210 ^c	45 ^c	7.85 ^b	3.06 ^a	4.5 ^b
2-Nitrophenol	C ₆ H ₅ NO ₃	139.1 ^c	216 ^c	45-46 ^d	7.23 ^b	1.89 ^c	2.5 ^b
4-Nitrophenol	C ₆ H ₅ NO ₃	139.11 ^d	279 ^d	113-114 ^e	7.08 ^c	2.04 ^c	11.6 ^c
2,4-Dinitrophenol	C ₆ H ₄ N ₂ O ₅	184.106	113 ^d	108	3.94 ^c	1.67 ^c	5.45
2,4,6-TCP	C ₆ H ₃ Cl ₃ O ^e	197.45 ^c	197.45 ^c	246 ^c	69 ^c	6.15 ^c	3.69 ^a
Pentachlorophenol (PCP)	C ₆ HCl ₅ O ^b	266.34 ^b	309-310 ^b	190-191 ^c	4.7 ^b	5.12 ^a	0.01 ^a

^a Montero et al., 2005

^b Fiamegos et al., 2003

^c Castillo et al., 1997

^d Cledera-Castro et al., 2005

^e Krijgsheid and Van der Gen, 1986

2002; Schmidt-Baumler et al., 1999). Phenol is also used in the pharmaceutical industry for the production of aspirin (Suliman et al., 2006). Sixty percent of phenolic compounds that are introduced into sewage are released into the environment with 85% being in the form of potentially estrogenic degradation products (Petrovic et al., 2002). Phenolic derivatives are among the most important pollutants that are widely present in the environment. These compounds are used in several industrial processes.

Methods for detection and monitoring of EDCs

Established analytical methods are available for many of the compounds designated as EDCs. Most developed countries like the US and the members of the EU have established regulatory authorities and requirements for chemical and biological analytical procedures for testing pesticides, metals, industrial chemicals and PCBs in food and environmental matrices. Despite the institutional frameworks for the analysis of the designated compounds (hormones, drugs and personal care

products), suitable instrumental techniques or standard methods of analysis are lacking. Different methods used to assess the occurrence, distribution and characterisation of phthalates and phenolic compounds in the environmental matrices and human samples in different countries around the world are summarised in Table 6.

Extraction of EDCs in liquid samples

Extraction of EDCs from liquid samples is based on liquid-liquid extraction (LLE) which is often followed by column chromatography cleanup and gas chromatography (GC) or high performance liquid chromatography (HPLC) determination using array of detectors (Cai et al., 2003; Ling et al., 2007; Zhou et al., 2005). LLE is a well developed, efficient and precise method; it requires time and consumption of large volumes of organic solvents (Santana et al., 2009; Santana et al., 2005; Zhou et al., 2005). EDCs often vary in concentration with different ecological risks in different environmental compartments. LLE has remained the preferred extraction

Table 6
Matrix, methods and concentration of endocrine disrupting chemicals (phenols and phthalate esters)

Country	Environmental matrix	Method	Concentration/ Detection range	Compound	Reference
China	Water	GC-FID	0.05-3.91 $\mu\text{g}\cdot\text{l}^{-1}$	phthalate esters	Li et al., 2006
Taiwan	Water	GC-MS/ GC-ECD	50 -100 $\mu\text{g}\cdot\text{l}^{-1}$	phthalate esters	Yuan et al., 2002
China	Water	HPLC-UV	3.1-5.8 $\mu\text{g}\cdot\text{l}^{-1}$	phthalate esters	Cai et al., 2003
South Africa	Water	GC-FID	0.03-2309 $\mu\text{g}\cdot\text{l}^{-1}$	phthalate esters	Fatoki and Noma, 2002
Poland and Germany	Water	GC-MS	0.015-0.06 $\mu\text{g}\cdot\text{m}\text{l}^{-1}$	phthalate esters	Luks-Betlej et al., 2001
Spain	Water	SPME/HPLC	9-22 $\mu\text{g}\cdot\text{l}^{-1}$	phthalate esters	Kayali et al., 2006
China	Water	HPLC-UV	1.0-3.8 $\mu\text{g}\cdot\text{l}^{-1}$	phthalate esters	Ling et al., 2007
Spain	Water	DSPME/ HS-SPME/GC-MS	< LOD to 6172 $\text{pg}\cdot\text{m}\text{l}^{-1}$	phthalate esters	Polo et al., 2005
China	Water Sediment Particulate matter	GC-FID GC-FID GC-FID	3.99 x 10 ⁻³ - 45.45 x 10 ⁻³ $\text{mg}\cdot\text{kg}^{-1}$ 40.56 -94.22 $\text{mg}\cdot\text{kg}^{-1}$ 30.52 -63.96 $\text{mg}\cdot\text{kg}^{-1}$	phthalate esters phthalate esters phthalate esters	Sha et al., 2007 Sha et al., 2007 Sha et al., 2007
South Africa	Soil	GC-FID	3.8-48.89 $\text{ng}\cdot\text{kg}^{-1}$	phthalate esters	Adeniyi et al., 2008
Italy	Breast milk	LC/LC – MS/MS	8.4-18.8 $\mu\text{g}\cdot\text{l}^{-1}$	phthalate esters	Latini et al., 2009
Taiwan		GC-MS	0.05-46.5 $\text{mg}\cdot\text{kg}^{-1}$ 1.4-253 $\text{mg}\cdot\text{kg}^{-1}$	phthalate esters phthalate esters	Huang et al., 2008 Huang et al., 2008
Oman	Water Water	RP-HPLC GC-MS	0.1-0.9 $\mu\text{g}\cdot\text{l}^{-1}$ 5-22 $\text{ng}\cdot\text{l}^{-1}$	Phenols Phenol	Suliman et al., 2006; Bagheri et al., 2007
Spain	Water Sediment	HPLC GC-MS	3-76 $\mu\text{g}\cdot\text{l}^{-1}$	Phenol	Cledra-castro et al., 2006 Czaplicka, 2001
Italy	Leachate	SPME/GC-MS	0.005-2.4 $\mu\text{g}\cdot\text{l}^{-1}$	Phenol	Ribeiro et al., 2002
China	Sediment	GC-MS	0.6-664.5 $\text{ng}\cdot\text{g}^{-1}$	Phenol	Peng et al., 2006
China	Wastewater	GC-FID	0.47-9.01 $\mu\text{g}\cdot\text{l}^{-1}$	Phenol	Zhou et al., 2005
China	Water	GC-NCI-MS	17-685 $\text{ng}\cdot\text{l}^{-1}$	Phenol	Zhao et al., 2009
Japan	Soil	GC-MS	1.0 $\text{ng}\cdot\text{g}^{-1}$	Phenol	Helaleh et al., 2001
China	Water	SPE/GC-FID	0.02-0.701 $\mu\text{g}\cdot\text{l}^{-1}$	Phenol	Wang et al., 2006
Germany	Urine	HPLC/MS/MS	0.1-13.6 $\text{ng}\cdot\text{kg}^{-1}\text{b.w}$	Phthalate esters	Seckin et al., 2009
Sweden	Breast milk Blood Urine	GC-MS GC-MS GC-MS	0.06-305 $\text{ng}\cdot\text{m}\text{l}^{-1}$ 0.06-305 $\text{ng}\cdot\text{m}\text{l}^{-1}$ 0.06-305 $\text{ng}\cdot\text{m}\text{l}^{-1}$	Phthalate esters Phthalate esters Phthalate esters	Hogberg et al., 2008 Hogberg et al., 2008 Hogberg et al., 2008
China	Soil	GC-MS	ND-293 $\mu\text{g}\cdot\text{g}^{-1}$	Phthalate esters	Zeng et al., 2009
South Africa	Water	GC-MS	ND-119 $\mu\text{g}\cdot\text{l}^{-1}$	Phthalate esters/p-NP	Mahomed et al., 2008

Key: GC-FID = Gas Chromatography Flame Ionization Detector; GC-MS = Gas Chromatography Mass Spectrometer; GC-ECD = Gas Chromatography Electron Capture Detector; HPLC = High Performance Liquid Chromatography

method for liquid samples because it is cheap compared to other methods like solid phase extraction (SPE), solid phase micro-extraction (SPME) and microwave accelerated extraction (MAE) (Piug and Barcelo, 1996; Polo et al., 2005; Satana et al., 2005). However, use of large amounts of generally toxic and inflammable organic solvents, formation of emulsions and losses during cleanup are still trailing the use of LLE.

The procedure is thus becoming less popular with the introduction of the solid phase extraction (SPE) technique. SPE is currently used in sample preparation for determination of trace EDCs in environmental, drug and biological samples. It offers a faster and easier manipulation, higher concentration factors and requires smaller amounts of organic solvents (Eberlin and Cesar de Silva, 2008; Zhou et al., 2005). Disposable cartridges for SPE have been in use for than 20 years. The desire to decrease the use of organic solvent, especially dichloromethane which is suspected to be carcinogenic, has encouraged the requirement for solvent-free procedures. This has greatly contributed to the rapid growth in the demand for this method at the expense of LLE. Selection of SPE cartridges with particular sorbent materials also plays a key role in the achievement of high and reproducible recovery of analytes in environmental samples. Most sorbents used in SPE are porous silica particles bounded with C-18 or other hydrophobic alkyl groups such as styrene-divinylbenzene. Recovery of organic compounds by SPE is highly dependent on the polarity of the eluent (Patrolecco et al., 2004).

Extraction of EDCs in solid samples

Studies on the determination of EDCs in solid environmental matrices are scarce compared to those carried out on liquid samples. Samples such as sludge and sediments are very complex and can be subjected to various forms of interference during extraction and separation procedures. EDCs are usually present at very low concentrations in these samples. This therefore requires effective sample pre-treatment. Such pre-treatment involves extraction and purification prior to analysis by GC or HPLC. Extraction of the compounds is often performed using Soxhlet extraction, sonication with polar organic solvents or mixtures of them, and most recently by accelerated solvent Soxhlet extraction (Diaz-Cruz et al., 2003; Noppe et al., 2007; Santana et al., 2009; Ternes et al., 2002).

However, prior to their extraction, solid samples, e.g., sludge, soil and sediments, are dried and homogenised by air-drying or lyophilisation (MacDowell and Metcalfe, 2001; Petrovic and Barcelo, 2000) or by mixing with baked sodium sulphate until a free-flowing powder is obtained. Often, solid samples are extracted with dichloromethane (Fatoki et al., 2010), different mixtures of hexane and other solvents (Petrovic et al., 2002) or ethyl acetate. Ethyl acetate is preferred to chlorinated solvent like DCM due to its suspected carcinogenic effect on humans and strong water-polluting properties.

Gas chromatography-mass spectrometry (GC-MS) methods

Phenols and phthalate esters have been determined in environmental matrices through the use of different analytical techniques, e.g., high-performance liquid chromatography (HPLC) with ultraviolet, fluorescence, electrochemical or mass spectrometric (MS) detection, as well as gas chromatography (GC) coupled with sensitive and specific detection systems (Huang et al., 2008; Liu et al., 2004; Zeng et al., 2009). GC-MS has been widely used in the determination, analysis

and monitoring of phthalates and phenolic compounds (Liu et al., 2004; Psillakis et al., 2004; Rompa et al., 2003; Zhao et al., 2009). EDCs with hydroxyl groups are usually derivatized for GC analysis.

It is well known that the oxygen atom possessed by hydroxyl has a weak nucleophilicity owing to its low electron density. Therefore, the derivatization procedures for hydroxyl compounds need either high temperatures or longer reaction periods, which makes the derivatized compounds convenient to be analysed on GC with ECD or MS.

The high polarity of phenols affects their chromatographic resolution and usually results in broad and tailed peak heights (Heberer and Stan, 1997; Santana et al., 2009; Schummer et al., 2009; Rompa et al., 2003; Zhao et al., 2009). These problems are often overcome by derivatizing free phenols to less polar compounds, more volatile and thermally stable, and by giving larger and more selective analytical responses through the use of derivatising reagents (Helaleh et al., 2001; Santana et al., 2009; Schummer et al., 2009; Rompa et al., 2003; Zhao et al., 2009).

Phenol and other phenolic compounds are usually derivatized either before or after extraction, or with an on-column reagent in the GC-injector (Helaleh et al., 2001; Rompa et al., 2003). Derivatization reactions used in GC analysis of phenolic compounds are in 4 categories, i.e. acylation, alkylation, esterification, and silylation (Helaleh et al., 2001; Moghadam et al., 2008; Rompa et al., 2003). In derivatization reactions, methylation by diazomethane is slow and carcinogenic while esterification by acetic hydride does not sufficiently improve the separation and derivatization of mono-nitro compounds (Heberer and Stan, 1997). Silylation of all the derivatization has shown to improve chromatographic parameters such as accuracy, reproducibility, sensitivity and resolution. Other advantages include peak tailing suppression, enhancement of thermal stability and product ruggedness (Li and Park, 2001; Saraji and Bakhshi, 2005).

Evidence and monitoring of EDCs (phenol and phthalates) in the South African environment

South Africa has experienced rapid industrial and urban growth especially in the increase in number of small-scale industries (Mahomed et al., 2008). South Africa is known to have used and abused most chemicals listed by developed and developing countries as endocrine-disrupting chemicals (Burger and Nel, 2008). The presence of EDCs has been reported in the South African environment, which therefore exposes humans and wildlife to possible health problems (Aneck-Hahn et al., 2009; Burger and Moolman, 2006; Fatoki and Noma, 2002; Mahomed et al., 2008). Many water bodies in the country receive significant inputs of natural and synthetic chemicals (from both point- and diffuse sources) which often act as EDCs, thus constituting a threat to reproductive health of aquatic organisms (Burger and Moolman, 2006; Govender et al., 2007).

South Africa, as an emerging world economy depending largely on agriculture, chemical industries and mining, still faces challenges in the area of proper waste disposal from a number of industrial and agricultural sources (Aneck-Hahn et al., 2009; Bornman et al., 2007; Fatoki et al., 2002; Mahomed et al., 2008). An audit of wastewater in Pretoria (Mahomed et al., 2008) and water, sediment and serum samples (Bornman et al., 2007) confirmed the presence of EDCs. This weight of evidence gathered on EDCs in the South African environment has posed a huge challenge to water resource management.

South Africa is faced with the problems of limited water resources with most rural and urbanised settlements depending on the available resources for their consumption, industrial and agricultural usage.

In addition, most research in South Africa on EDCs is centred on estrogenic compounds, such as 17- β estradiol, p-Nonyl phenol, PCBs, estrone, estriol, organochlorine pesticides, and heavy metals (Aneck-Hahn et al., 2009; Barnhoorn et al., 2004; Mahomed et al., 2008; Van Wyk et al., 2003). It is evident from various studies that EDCs are capable of initiating various health disorders in aquatic organisms. Van Wyk et al. (2008) reported the testicular effects of some EDCs compound and metals on *Clarias gariepinus*. The impact of 17- β estradiol to stimulate vitellogenin (VTG) on *Xenopus laevis* has also been reported (Barnhoorn et al., 2004).

Though significant concentration of phthalates have been reported in some environmental samples in the country (Adeniyi et al., 2008; Fatoki and Noma, 2002; Fatoki et al., 2010), there has been paucity in research on phenols on the priority list until recently (Abboo and Pletschke, 2010; Baldwin and Debowski, 1988). Concentrations of some of the compounds have been reported in drinking water, lakes and swimming pools; inhibitory effects on β -D-galactosidase and β -D-glucuronidase have also been reported (Abboo and Pletschke, 2010). Assessing the potential ecological and health impacts of EDCs (phenol and phthalate esters) in the country's environment requires investigation of level and distribution in both aquatic and terrestrial systems.

Areas of significant importance that have been neglected in the monitoring of EDCs in the South African environment are treated wastewater from municipal treatment plants and landfill sites. Though wastewaters from households and industries are usually treated before they are released into the river systems, studies have shown that they often contain EDCs. In addition, leachates from landfill sites are treated before onsite application, processes that can result in contamination of the water table by EDCs.

Conclusion

It is of importance to monitor terrestrial and aquatic environments close to agricultural, mining and chemical industries as wastes from these activities contribute immensely to the availability of EDCs in environmental compartments. To cover the vacuum created by the lack of research into these EDCs (phenols and phthalate esters) in the South African environment, future research will focus on the level, distribution, spatial and temporal availability, and possibly the risk assessment, of some of these compounds in South African environments.

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