

Endocrine-disrupting chemicals and male reproductive health: a review

Kemični povzročitelji endokrinih motenj in reproduktivno zdravje pri moških: pregled literature

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Ključne besede:

kemični povzročitelji endokrinih motenj; endogeni hormoni; okolje; reproduktivno zdravje pri moških

Key words:

endocrine-disrupting chemicals; endogenous hormones; environment; human male reproductive health

Prispelo: 20. 11. 2016

Sprejeto: 18. 12. 2017

Abstract

Balanced functioning of the endocrine system is essential for preservation of human species by providing normal growth and development, reproduction, and normal functioning of all other organ systems. In the last decades, emerging area of interest is the impact of environmental exposures to human health. Important environmental pollutants are endocrine-disrupting chemicals (EDCs), which can have adverse effects on the living organism due to their interference with the endocrine system. The group of known EDCs embraces ubiquitous synthetic substances used as industrial lubricants and solvents, with their by-products, incomplete combustion remains, pharmaceuticals and personal care products, pesticides and plasticizers. Natural compounds such as genistein, a phytoestrogen, and heavy metals can also have endocrine effects. Endocrine disruption is a serious public health problem. EDCs among other health problems generate reproductive disorders in males, such as decreases in sperm count and quality, increases in testicular germ cell numbers, prostate and breast cancers, cryptorchidism and hypospadias, impaired fertility, and infertility. This paper critically reviews the current knowledge of the impact of EDCs on reproductive disorders in human males.

Izveček

Uravnoteženo delovanje hormonskega sistema zagotavlja rast in razvoj, razmnoževanje in normalno delovanje vseh drugih organskih sistemov v telesu. Zato je ključnega pomena za ohranitev človeške vrste. V zadnjih desetletjih narašča število raziskav, povezanih z izpostavljenostjo ljudi različnim kemičnim snovem iz okolja in njihovim vplivom na zdravje. Kemični povzročitelji endokrinih motenj (KPEM) sodijo med pomembna okoljska onesnažila z negativnim učinkom na organizem zaradi poseganja v endokrini sistem. V skupino znanih KPEM sodijo povsod prisotne sintezne snovi, ki se uporabljajo kot industrijska maziva in topila ter njihovi stranski produkti, ostanki nepopolnega zgorevanja, zdravilne učinkovine, izdelki za osebno nego, pesticidi, mehčala in drugo. Naravne spojine, kot so genistein, fitoestrogenske snovi in težke kovine lahko prav tako moteče delujejo na endokrini sistem ljudi in živali. Motnje endokrinega sistema so resen problem javnega zdravja. KPEM med drugim povzročajo reproduktivne motnje pri moških, kot so zmanjšano število spermskih celic in njihovo nižjo kakovost, rak prostate in dojk, kriporhizem in hipospadijo, zmanjšano plodnost in neplodnost. V prispevku je predstavljen kritičen pregled trenutnega znanja o vplivih KPHM na reproduktivne motnje pri moških.

Citirajte kot/Cite as: Balabanič D, Krivograd Klemenčič A. Endocrine-disrupting chemicals and male reproductive health: a review. *Zdrav Vestn.* 2018;87(1–2):69–80.

DOI: 10.6016/ZdravVestn.2456

1. Introduction

In the last decades, numerous studies have reported that certain chemicals called endocrine-disrupting chemicals (EDCs) may cause disruption of the endocrine system and can affect reproduction and development of exposed humans and wildlife (1-5). An "Endocrine-disrupting chemical" (EDC) has been broadly defined as "an exogenous substance or a mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations" (6). EDCs can disrupt the normal actions of endogenous hormones by altering normal hormone levels, inhibiting or stimulating the production of hormones, or changing the way hormones travel through the body, thus affecting the functions that these hormones control (4).

The group of known EDCs is extremely heterogeneous and includes different groups of chemicals such as steroids (ethinyl estradiol, 17 β -estradiol, estrone, mestranol and diethylstilbenstrol), alkylphenols (nonylphenol, nonylphenol ethoxylate, octylphenol and octylphenol ethoxylate), polyaromatic compounds (polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and brominated flame retardants), organic oxygen compounds (bisphenol A (BPA) and phthalates), pesticides (atrazine, chlordane, demeton-S-methyl, dichlorvos, dieldrin, dimethoate, endosulfan, hexachlorobenzene, lindane, linuron, pentachlorophenol, permethrin, simazine and trifluralin) and others (tributyltin, dioxins and furans) as well as certain heavy metals (arsenic, cadmium, lead and mercury) (4,7,8). The $\log K_{ow}$ (octanol/water partition coefficient) values for some EDCs indicate a

high degree of lipophilicity (9) and thus bioaccumulation. Human exposure to EDCs may result from the ingestion of contaminated food and water, inhalation of air and absorption of the EDCs through the skin. However, in most cases, human exposure to EDCs is through the ingestion of contaminated food. The EDCs that are most commonly present in contaminated food are bisphenol A (BPA), nonylphenol, phthalates, and heavy metals. BPA is used as a source material for the production of epoxy resins, polyacrylates and polyesters, but mainly for the production of epoxy resins and polycarbonate plastics (10). Epoxy resins are used as food contact surface lacquer coatings for cans, protective coatings and finishes, adhesives and as coatings for polyvinyl chloride (PVC) pipes (10). Polycarbonate plastics are used in the manufacture of household appliances, food packaging and plastic bottles because they have high impact strength, hardness, toughness, transparency, and resistance to temperatures, many acids and oils (10). In food packaging, nonylphenol originates from oxidation of the antioxidant additive trisnonylphenyl phosphite (11). Levels of nonylphenol in different packaging materials were recently assessed and were found to range from below 0.03 $\mu\text{g/g}$ in a polyethylene terephthalate (PET) water bottle to 287 $\mu\text{g/g}$ in PVC cling film (12). Nonylphenol was also detected in different types of retail-purchased foods: up to 78 ng/L were found in PET-bottled mineral water (13), up to 40 $\mu\text{g/kg}$ in beverage cartons of ultra-high temperatures (UHT) whole milk and up to 32.3 $\mu\text{g/kg}$ in high density polyethylene (HDPE)-bottled milk (in bottle sterilization) (14). Dimethyl phthalate, Diethyl phthalate, Diisobutyl pht-

halate, Di-*n*-butyl phthalate, Benzylbutyl phthalate, Di(2-ethylhexyl) phthalate, Dicyclohexyl phthalate, Di-*n*-octyl phthalate, Diisononyl phthalate and Diisodecyl phthalate were detected in different fruits and vegetables, sport drinks, artificial juice drinks, meat and meat products, fish and fish products, milk and nutraceutical products (15,16). Lead (Pb) in food originates mainly from atmospheric deposition and adherence of Pb-rich soil particles to fruits and vegetables (17). Exposure to excess Arsenic (As), principally from contaminated drinking water, is considered one of the top environmental health threats worldwide (18). Most of this exposure is from natural geological sources of As that contaminate groundwater (18). It has been established that fish and seafood can accumulate organic As from their environment (19,20). The concentrations of As in fruits and vegetables depends on the soil content, water contamination, air pollution and the usage of fertilizers (19). The general human population is exposed to mercury (Hg) (usually in an inorganic form and at very low concentrations) primarily through the diet with contaminated fish, dental amalgam and water consumption (4,21). The level of Hg in foods is inconsistent and reflects the level of pollution of the local environment (22).

2. Impact of endocrine-disrupting chemicals on male reproduction health

Epidemiological data has revealed an increase in male reproductive function disorders over the past 50 years, suggesting a correlative relationship with the increasing amounts of EDCs in the environment (4,8). In the context of male

reproductive health, EDCs have been linked to disrupted reproductive function (such as reduced semen quality and infertility), altered fetal development (such as urogenital tract abnormalities, including hypospadias and cryptorchidism) and testicular germ-cell, prostate and breast cancers (4,5,23). As previously mentioned, the potential *lag* between exposure to EDCs and the manifestation of a clinical reproductive disorder is of critical concern. In humans, this period may be years or decades post exposure because sexual maturity and fertility cannot be assessed until the exposed individual has attained a certain age (24). In 1993, Sharpe and Skakkebaek (25) suggested fetal exposure to environmental oestrogens such as synthetic oestrogens, phytoestrogens, oestrogens in milk and oestrogenic chemicals (e.g. chlorinated hydrocarbon (TCDD)) to be the common aetiological factor for the observed rise in the incidence of testicular cancer, cryptorchidism and the downward trend in sperm quality (25). Skakkebaek et al. (26) have suggested that the incidences of cryptorchidism, hypospadias and poor semen quality are risk factors for one another and that they are all predictive of testicular germ-cell cancer development. Cryptorchidism, hypospadias, poor semen quality, and testicular germ-cell cancers are defined as the testicular dysgenesis syndrome (TDS). They propose that the aetiology of TDS lies in the diminished androgen action in fetal developmental periods and has a negative impact on the proper functioning of Sertoli cells (the cells supporting germ cells) and Leydig cells (where androgen synthesis occurs). This hypothesis proposes a strong association between environmental exposures (e.g., to phthalates, polychlorinated biphenyls (PCBs), dioxins and non-resistant pesticides) and

development of TDS (27). Identifying environmental causes of TDS in humans is difficult because developing fetal tissues are inaccessible for examination. Thus, the majority of mechanistic evidence linking EDCs to TDS comes from animal experiments. It is possible to experimentally induce all the elements of TDS, except for germ-cell cancer, by exposing pregnant rats to di-2-ethylhexyl phthalate, di-n-butyl phthalate and butyl benzyl phthalate and other chemicals that block androgen action (28). This model is referred to as the “phthalate syndrome” model, and it comprises cryptorchidism, hypospadias, poor semen quality, and malformations of other sex organs (29). Certain pesticides (dichlorodiphenyltrichloroethane (DDT), alachlor, atrazine, diazinon) are able to block the androgen receptor, or interfere with the conversion of testosterone into dihydrotestosterone, thus producing effects similar to phthalate syndrome. Androgen action is also essential for proliferation and development of Sertoli cells, which are necessary for sperm production. Altogether, EDC-mediated disruption of androgen action during fetal development results in reduced fertility later in life (30). A study of dioxin exposure conducted by Mocarelli et al. (31), suggests that timing of exposure has a significant impact on semen quality. This study was based on men who were exposed to high levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) as a result of a chemical plant explosion in 1976 in Italy. Men who were exposed prepubertally (1–9 years of age) demonstrated poor semen quality as adults (27). Interestingly, men who were exposed between 10–17 and 18–27 years of age showed slightly positive or no differences in semen quality, respectively (27).

2.1. Cryptorchidism and hypospadias

Several studies have shown that sons of mothers treated with diethylstilbestrol (DES) during pregnancy are at increased risk of cryptorchidism and hypospadias (5,32). Exposure of fathers to dioxins in the Seveso accident in 1976 resulted in an increased incidence of hypospadias among their sons (3). In exposure studies, the risk for cryptorchidism was higher in sons of women, working with pesticides (33), while significantly higher concentrations of hexachlorbenzene and heptachlorepoxyde were found in adipose tissues of boys with cryptorchidism, compared to those of a control group (34). The aetiology of this entity is unknown and thus prenatal exposure to exogenous oestrogens is advised (34). Moreover, these findings do not prove a direct causality between the accumulation of hexachlorbenzene and heptachlorepoxyde and undescended testes since they reflect the total accumulation until the time of sampling, and a conclusion regarding exposure during a specific period of development cannot be made. Comparison of registry-based information on cryptorchidism and hypospadias may have pitfalls because of underreporting, differences in registration systems, and inclusion criteria (35). Therefore, prospectively designed cohort studies have been performed in Denmark and Finland to assess the incidence of these disorders during the years 1997 and 2001. The results of these studies showed that the rate of congenital cryptorchidism at birth was significantly higher in Denmark as compared to Finland (9.0 % in Denmark and 2.4 % in Finland) (36).

2.2. Decreasing sperm counts and quality

Based on a meta-analysis of 61 studies, it has been suggested that the human sperm count and quality have decreased in the past 50 years (37). It was suggested that some common prenatal influences could be responsible for the decline in sperm density. Several studies have shown that sons of mothers treated with DES during pregnancy are at increased risk of decreased sperm count (5,32). A recent study found an inverse correlation between the concentration of PCB metabolites in blood and seminal plasma and sperm motility as well as concentration (38). Guo et al. (39) concluded that heavy exposure to PCBs resulted in negative effects on sperm morphology and motility, but not on sperm concentration.

2.3. Cancer

EDCs are based on animal models, human clinical observations, and epidemiological studies suspected of causing various types of human cancer in males, including testicular, prostate and breast cancers (24,40-42). Over the last 50 years, the incidence of prostate cancer in some countries (e.g. U.S., Britain and Denmark) has doubled, while that of testicular cancer has tripled (e.g. U.S. and Britain) or even quadrupled (e.g. Denmark) since 1943 (43,44). EDCs may be accountable for the observed trends (4); since both humans and wildlife are chronically exposed to copious potentially hazardous chemicals that are continuously released into the environment (45). Most evidence depicting cancer risk associated with exposure to EDCs is limited to cellular and animal models (46).

2.3.1. Testicular cancer

During recent decades, there has been a significant increase in the incidence of testicular cancer, albeit with clear racial and geographical differences (the highest incidence is among white men in northern Europe) (47) suggesting that both genetic and environmental factors are important in the development of testicular cancer. The main risk factor for testicular cancer is cryptorchidism, followed by hypospadias (48,49). Testicular cancer is most common among young men between the ages of 15–34 years (50). The origins of testicular cancer are elusive; however, many investigators are exploring the possibility that fetal and early-life EDCs exposures can disrupt the critical hormonal balance during development, and in turn contribute to the formation of testicular cancer later in life (41,51). It was found that men who have some form of gonadal dysgenesis are more likely to develop testicular cancer in conjunction with other male reproductive abnormalities such as hypospadias and cryptorchidism (26,52). The mechanisms behind the development of testicular cancer are still unknown, but both environmental and lifestyle factors have been associated with its development. Although the strength of the association is unclear, some EDCs have been identified that may play a role in testicular carcinogenesis, including certain types of persistent organic pollutants (i.e. organochlorine pesticides) (40), and synthetic hormones, namely oestrogens, gestagens and androgenic anabolics used for meat production (52). Animal studies are helpful in assessing the effects of exogenous oestrogens or anti-androgenic chemicals after *in utero* or early-life exposures. Some examples of highly studied oestrogenic compou-

nds include DES, ethinyl estradiol, and BPA, while anti-androgenic compounds include flutamide and vinclozolin (26). Results from animal studies have shown that *in utero* exposures to selected EDCs (Table 1) can lead to the development of hypospadias, cryptorchidism and reduction in sperm volume in the majority of the animals exposed, and in some severe cases the formation of Leydig cell tumours (26). These initial studies provide some insight on the effects of early life exposures, but the animal models have significant limitations in terms of their relevance to human testicular germ-cell tumour also due to much higher doses used in experiments compared to doses of human exposure. Phthalates (e.g. mono-n-butyl phthalate), which have a wide application in commercial and industrial plastics, have been shown to

alter male reproductive development by acting as anti-androgens within the fetal testis of rats, as described above (26). Phthalates induce multinucleated germ cells (MNGs) following a gestational exposure (53). MNGs have multiple nuclei that are contained within one cytoplasm and are postulated to be formed from the abnormal differentiation of germ cells. Although MNGs and carcinoma *in situ* cells share a putative origin as developmentally dysgenetic germ-line cells, MNGs do not appear to lead to testicular cancer in rodent models (53-55).

2.3.2. Prostate cancer

Prostate cancer remains an important public health concern in Western countries and an emerging malignancy in developing nations (e.g. U.S., New Zealand, Sweden, Norway, Austria

Table 1: Reproduction problems in males and connected endocrine-disrupting chemicals.

Male reproduction problems	Endocrine-disrupting chemicals	References
Reduced semen quality and infertility	Phthalates (di-butyl, di-(2-ethylhexyl) and butyl benzyl phthalates); TCDD; PCBs and their metabolites; DES; ethinyl estradiol; BPA; pesticides (flutamide, vinclozolin, DDE, DDT)	25, 26, 29, 31, 38, 39
Hypospadias	Phthalates (di-butyl, di-(2-ethylhexyl) and butyl benzyl phthalates); DES; dioxins; ethinyl estradiol; BPA; pesticides (flutamide, vinclozolin, DDE, DDT)	3, 5, 26, 29, 32
Cryptorchidism	Phthalates (di-butyl, di-(2-ethylhexyl) and butyl benzyl phthalates); DES; ethinyl estradiol; BPA; pesticides (hexachlorobenzene, heptachlorepoxyde, flutamide; vinclozolin, DDE, DDT)	5, 25, 26, 29, 32, 33, 34
testicular cancer	DES; ethinyl estradiol; BPA; pesticides (hexachlorobenzene, heptachlorepoxyde, flutamide; vinclozolin, DDE, DDT)	25, 26, 40, 52
prostate cancer	DES; PCB; BPA; UV filters; heavy metals (cadmium, arsenic); pesticides (beta-hexachlorohexane, gamma-hexachlorohexane, p,p-DDE, vinclozolin)	4, 42, 60, 61, 62, 63
breast cancer	Phthalates (diethyl and butyl benzyl phthalate); TCDD; insecticide (heptachlor); pesticides (hexachlorobenzene, organochlorine pesticides, organophosphorus pesticides, malathion, parathion); PCBs; BPA; cosmetics (benzyl salicylate, benzyl benzoate and butylphenyl methylpropional); cadmium	69, 70, 71, 72, 73, 74, 75, 76, 77, 78

BPA – bisphenol A, DES – diethylstilbestrol, DDE – 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene, DDT – 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane, TCDD – 2,3,7,8-tetrachlorodibenzo-p-dioxin.

(Tyrol), Canada, Switzerland, Australia, Iceland) (56). In the United States prostate cancer has the highest incidence among cancers and is second leading cause of cancer death among men (57-59). Despite the common occurrence of prostate tumours, its aetiology remains largely unknown. Prins (60), who contributed importantly to the study of prostate carcinogenesis, found out that there is substantial evidence from animal models about the influence of specific EDCs (Table 1) to prostate cancer development or progression. In large part, these effects appear to be linked to interference with oestrogen signalling, either through interacting with ERs or by influencing steroid metabolism and altering oestrogen levels within the body. Studies in animal models show augmentation of prostate carcinogenesis with several environmental EDCs including DES, PCBs, cadmium, UV filters, BPA, and arsenic (4,60-62). Importantly, sensitivity of the prostate to these EDCs appears to be heightened during the critical developmental windows including *in utero* and neonatal time points as well as during puberty. Thus infants and children may be considered a highly susceptible population for EDCs exposures and increased risk of prostate cancers with aging (60). BPA, a plastic component that can be considered a model agent for endocrine disruption, was shown to induce changes in differentiation patterns, cell proliferation at a BPA dose of $\sim 10^{-7}$ M or 23 ppb, and size of the prostate in male offspring after feeding pregnant mice 2 or 20 $\mu\text{g}/\text{kg}/\text{day}$ BPA, changes that are probably associated with an increase in cancer risk (61). Exposure to DES, especially prenatal and early in life, has been associated with prostate abnormalities, including prostatic squamous neoplasia (60). UV filters, compounds of sunscreens, have been reported to alter prostate

gland development and oestrogen target gene expression in rats (63). Especially 4-methylbenzylidene and 3-benzylidene camphor are ER-beta ligands (62-64). Cadmium is a known ER ligand (65). *In vitro* work has shown proliferative action of cadmium in human prostate cells, and in rats prostatic tumours have been induced by oral cadmium exposure or by injection (60,66,67). According to Benbrahim-Tallaa and Waalkes (68), arsenic can induce malignant transformation of human prostate epithelial cells and also appears to impact prostate cancer cell progression by precipitating events leading to androgen independence *in vitro*. Vinclozolin, a fungicide, has known anti-androgenic properties. On the one hand, prostate gland growth in rats was reduced by vinclozolin, but on the other hand, prenatal exposure leads to aging-associated prostatitis in the next four generations of offspring. Kumar et al. (42) studied 70 newly diagnosed prostate cancer patients and 61 age-matched healthy male controls. They found significantly higher levels of beta-hexachlorohexane (5.64 ± 3.06 ng/mL vs. 4.27 ± 2.74 ng/mL), gamma-hexachlorohexane (7.37 ± 6.97 ng/mL vs. 5.33 ± 4.22 ng/mL), and *p,p*-DDE (4.16 ± 2.67 ng/mL vs. 3.02 ± 2.33 ng/mL) in blood of prostate cancer patients compared to controls (42).

2.3.3. Male breast cancer

Male breast cancer is a rare disease, showing an increasing incidence trend rising along with that of female breast cancer. The 2009 Endocrine Society scientific statement entails considerable evidence indicating that EDCs contribute to the risk of male breast cancer occurrence (24). From the clinical and biological point of view, male and female breast cancer differ mainly in the frequency of their histological type and in the

expression of hormone receptors and of epidermal growth factor receptor 2. The major risk factor related to male breast cancer is a positive family history for breast cancer, which indicates a relevant genetic component (5).

Biological effects favouring malignant transformation were observed on human breast cells for pesticide hexachlorobenzene (69), organochlorine pesticides (70), organophosphorus pesticides, malathion and parathion (71), PCBs (72), BPA (73), butyl benzyl phthalate (74), cosmetics benzyl salicylate, benzyl benzoate and butylphenyl methylpropional (75) and also cadmium (76,77).

Experiments on animals showed that *in utero* exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induce alterations in breast development and increase susceptibility for mammary cancer development (78). Mice and rats exposed perinatally to BPA showed preneoplastic lesions (intraductal hyperplasias) in mammary tissue and such rats developed carcinoma *in situ* (79,80). Rats exposed perinatally or through lactation to BPA showed an increased susceptibility to neoplastic development (81,82). According to Markey et al. (83) perinatal exposure of mice to environmentally relevant levels of BPA (25 and 250 µg/kg body weight) induce in the mammary gland an increase in the number of terminal end buds, the structures in which mammary cancer originates in both rodents and humans (84). The experiment performed by Markey et al. (83) showed that by 6 months of age, the mammary glands of exposed mice demonstrated a dramatic expansion of the ductal network with a significant increase in terminal ducts and alveolar structures relative to the control.

2.4. Poor semen quality

Although several studies have shown that EDCs may operate through hormonal pathways to affect spermatogenesis (85), the decline in semen quality in men is still a controversial issue. The debate began when a Carlsen et al. (37) review highlighted a decrease in sperm counts, of up to 50 % during the period 1940–1990 (mean sperm count from 113 millions/mL in 1940 to 66 millions/mL in 1990) and continued with several meta-analyses reporting a decline in semen quality around the world (4,23,31,49,86,87).

It is known that the environmental EDCs might affect the development of the male reproductive system during fetal or childhood life (25). However, several studies on occupational exposure to EDCs showed that spermatogenesis can also be corrupted by exposure of the adult man to various EDCs (88,89).

3. Conclusion

The preservation of adequate reproductive capacity is crucial for the human species maintenance, while also being one of the most important determinants of “good health” for the individual. In the last decades, numerous studies have reported an increasing incidence of human reproductive diseases and a consequent decline in reproductive function (90). Because of the short time frame, it is not likely that this could be explained by genetic changes. More likely explanation for the observed trends is exposure of humans to copious potentially hazardous chemicals called endocrine-disrupting chemicals (EDCs), which can interact with the endocrine system and are continuously released into the environment. Exposure of humans to

environmental EDCs, which are potentially posing a serious health hazard, is among the most pressing environmental issues facing modern toxicology in recent years. Despite the biological plausibility of the effects of EDCs on animals demonstrated in numerous laboratory studies, it is not clear yet to what extent human health is adversely impacted by EDCs' exposure. There are similarities between results obtained from animal studies and adverse effects seen in humans, but there is no direct evidence for possible effects of EDCs on human fertility. Nevertheless, the obvious regional differences in male abnormalities (such as testicular cancer and reduced sperm

counts) suggest the influence of environmental factors, as opposed to genetic predisposition. Linking environmental factors with health factors is of paramount importance for the identification of the causes of the growing number of reproductive diseases in humans possibly caused by exposure to various environmental factors. Identification of relationships between exposure to specific EDCs in the environment and detrimental fertility measures in humans could lead to preventive strategies and policies aimed at limiting the production of these chemicals, as well as at favouring removal of these substances from the environment.

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