Environmental Pollution 243 (2018) 1026–1035

Contents lists available at ScienceDirect

Environmental Pollution

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

Hexachloronaphthalene (HxCN) as a potential endocrine disruptor in female rats☆

Joanna Stragierowicz, Elżbieta Bruchajzer, Adam Daragó, Marzenna Nasiadek, Anna Kilanowicz*

Department of Toxicology, Faculty of Pharmacy, Medical University, Muszynskiego 1, 90-151, Lodz, Poland

ARTICLE INFO

Article history:
Received 4 July 2018
Received in revised form
5 September 2018
Accepted 7 September 2018
Available online 17 September 2018

Keywords:
Hexachloronaphthalene
Estrous cycle
Estradiol
Progesterone
Thyroid hormones

ABSTRACT

Hexachloronaphthalene (HxCN) is one of the most toxic and most bioaccumulative congeners of polychlorinated naphthalenes (PCNs) known to be present in animal and human adipose tissue. Unfortunately, little data is available regarding the negative effect of PCNs on endocrine function. The aim of the study was to investigate the direct influence of subacute (two and four-week) and subchronic (13-week) daily oral exposure of female rats to 30, 100 and 300 μg kg b.w.$^{-1}$ HxCN on ovarian, thyroid function and neurotransmitters level. The levels of selected sex hormones (progesterone: P and estradiol: E2) in the serum and uterus, regularity of estrous cycle, levels of thyroid hormones (fT3 and fT4), TSH, γ-aminobutyric acid and glutamate levels in selected brain areas and the activity of CYP1A1 and CYP2B in the liver were examined. Estrogenic action (elevated E2 concentration in the uterus and serum) was observed only after subacute exposure, and antiestrogenic activity (decreased E2 level and uterus weight) after 13 weeks administration of 300 μg kg b.w.$^{-1}$ day^{-1}. Subchronic administration of HxCN significantly lengthens the estrous cycle, by up to almost 50%, and increases the number of irregular cycles. In addition, increased TSH and decreased fT4 serum levels were observed after all doses and durations of exposure to HxCN. Only subacute exposure led to a significant decrease in the level of examined neurotransmitters in all analyzed structures. Additionally, exposure to low doses of HxCN appears to lead to strong induction of CYP1A1 in a liver. It can be hypothesized that HxCN produces effects which are very similar to those caused by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and dioxin-like compounds (DLCs), particularly concerning endocrine and estrous cyclicity disorders. Therefore, HxCN exposure may exert unexpected effects on female fecundity among the general population.

1. Introduction

Despite data on the toxicity of PCNs (polychlorinated naphthalenes) being limited, the 2015 update of the Stockholm Convention on Persistent Organic Pollutants (POPs) included di– to octachlorinated naphthalenes in Annex A and C, which prohibits their production, use and import/export, and reduces or eliminates their release (UNEP, 2015). PCNs include 75 congeners that were widely used in a range of technical mixtures in various industries until the end of the 1980s, mainly as flame retardants, dielectric fluids for capacitors, engine oil additives and cable insulation additives, as well as for wood and paper impregnation (IPCS, 2001). Nowadays, exposure to PCNs is most commonly acquired through environments contaminated by old technical products incorporating PCNs and other similar compounds such as polychlorinated biphenyls (PCBs) contaminated with PCNs; in addition, exposure can occur via unintentional by–products generated in various thermal industrial processes (Falandysz, 1998).

PCNs are widespread in various media such as air, water, soil, sediment and food (Falandysz, 1998, 2003; Marti-Cid et al., 2008; Li et al., 2015); however, the most common source of exposure to the general population is though food of animal origin, such as meat, eggs and dairy products, which can often contain higher chlorinated naphthalenes (from tetra– to heptaCN congeners) (Kannan et al., 2000; Hanari et al., 2004; Marti-Cid et al., 2008; Kim et al.,

☆ This paper has been recommended for acceptance by Eddy Y. Zeng.

* Corresponding author. Department of Toxicology Faculty of Pharmacy Medical University, Muszynskiego 1, 90-151, Lodz, Poland.
E-mail addresses: joanna.stragierowicz@umed.lodz.pl (J. Stragierowicz), elzbieta.bruchajzer@umed.lodz.pl (E. Bruchajzer), adam.darago@umed.lodz.pl (A. Daragó), marzenna.nasiadek@umed.lodz.pl (M. Nasiadek), anna.kilanowicz@umed.lodz.pl (A. Kilanowicz).

https://doi.org/10.1016/j.envpol.2018.09.045
0269-7491/

2017). Due to their lipophilic properties, the consumption of even small amounts of PCNs results in their accumulation in the body. This has been evidenced by the presence of various PCNs throughout the general population, particularly in the liver and adipose tissue, (Schiavone et al., 2010), serum (Park et al., 2010), human milk (Lunden and Noren, 1998) and umbilical cord blood (EWG, 2009; Kim et al., 2015). Of all PCNs, HxCN isomers are considered one of the most toxic groups of congeners (Blankenship et al., 2000; Fernandes et al., 2017), as they demonstrate the highest accumulation (Hanari et al., 2004) both in the natural environment and in human tissues. The amount of PCNs in some cases (serum and cord blood) has been found to reach up to 25% of the total Toxic Equivalent (TEQ) value, with the dominant congeners in these studies being mostly penta– and hexachloronaphthalenes (Park et al., 2010; Kim et al., 2015).

The fact that low-dose chemical mixtures of POP compounds accumulate in adipose tissue seems particularly worrying, as there is an increased risk that these compounds will be redistributed throughout the body through the circulation in some cases of obesity or weight loss (Lee et al., 2017). Such redistribution may be particularly hazardous in the case of compounds like PCNs, which have been found to lead to weight loss in studies conducted on animals (Kilanowicz et al., 2009; Kilanowicz and Skrzypińska–Gawrysiak, 2010) and humans (Kleinfeld et al., 1972). The half-life of HxCN in blood samples collected from humans exposed to PCNs from contaminated rice oil in Taiwan has been estimated to be longer than two years, which is very similar to those reported for selected polychlorinated dibenzofurans (PCDFs) (IPCS, 2001). More worryingly, many adverse health effects could result from the additive effects of individual substances, each characterized by a different mechanism of action (Lee et al., 2017).

As few studies, mainly based around animals and occupationally-exposed humans, have proved that the toxic effects induced by PCNs are comparable to those caused by polychlorinated dibenzo-p-dioxins (PCDDs), PCDFs and PCBs (IPCS, 2001), PCNs have often been labelled as dioxin-like compounds (DLCs) (Kimbrough and Jensen, 1989; Blankenship et al., 2000; Villeneuve et al., 2000). Only a few recent *in vitro* studies have examined the potential influence of PCNs on reproductive, fertility, developmental and endocrine disorders, where these abnormalities are very typical of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and DLC exposure (Yonemoto, 2000). Studies using porcine ovarian follicles have shown that PCNs disrupt steroidogenesis through their antiestrogenic and androgenic activities (Barć and Gregoraszczuk, 2014, 2016; Rak et al., 2017). Equally worrying results have also been obtained from studies on pregnant female rats, which have identified embryotoxic, fetotoxic (HxCN administration) and even teratogenic effects (mixture of PCNs similar to Halowax) (Kilanowicz et al., 2011, 2015).

However, although the mechanism behind the developmental toxicity of PCNs is still unknown, it cannot be excluded that this may occur as a result of endocrine disrupting properties. A further study of the organ distribution of HxCN in pregnant female rats and their fetuses and its transplacental transport showed that, in addition to significant accumulation in the maternal liver, HxCN was also found in relatively high concentrations in the reproductive tissues (i.e. the uterus and ovaries), where HxCN levels were three and five times higher than in the blood. In addition, the tested compound easily penetrates through the placental barrier, reaching its highest concentration in the fetal brain (Stragierowicz et al., 2018). Furthemore, scant human environmental exposure data exists concerning the toxicity of PCNs. Studies conducted in North America indicate a relationship between lower IQ, accompanied by delays in psychomotor development in children, and consumption of fish contaminated with organochloride compounds, including PCNs, from the Great Lakes by their mothers during pregnancy (Kannan et al., 2000; Hanari et al., 2004).

The effects of long–term exposure to PCNs in humans and in experimental animals are little known; however, as their chemical structure is very similar to those of PCDDs, it is likely that the two groups of compounds may have similar effects on hormonal homeostasis and the endocrine system. The aim of this study was therefore to determine the effect of subacute (two and four weeks) and subchronic (13 weeks) oral exposure to HxCN on selected hormones (gonadal sex hormones–estradiol: E2 and progesterone: P; thyroid hormones–free triiodothyronine: fT3, free thyroxine: fT4 and thyroid stimulating hormone: TSH) in female rats, as well as their impact on estrous cycle regularity. As HxCN has been previously demonstrated to have various neurotoxic properties, expressed by anorectic effects (Kilanowicz et al., 2009; Kilanowicz and Skrzypińska–Gawrysiak, 2010), behavioral changes (Kilanowicz et al., 2012) and disturbances in the levels of γ-aminobutyric acid (GABA)-metabolizing enzymes (Vinitskaya et al., 2005), the present study evaluates the neurotoxic potential of HxCN by examining the levels of GABA and glutamate in selected rat brain areas (brain stem, cerebellum, and basal ganglia).

2. Materials and methods

2.1. Chemicals

HxCN was synthetized in the Institute of Applied Radiation Chemistry, Technical University of Lodz (Poland) according to Auger et al. (1993). The characteristics and purity of the HxCN has been given elsewhere (Kilanowicz et al., 2012, 2015; Kilanowicz and Skrzypińska-Gawrysiak, 2010; Klimczak et al., 2018). Isotope dilution HRGC/HRMS analysis demonstrated that the PCDDs and PCDFs content was below 0.1 ng mg^{-1} of the investigated sample.

2.2. Animal experimental design

2.2.1. Ethical approval

The studies were performed in accordance with Polish law on the protection of animals and with consent given by the Local Ethical Committee for Experimentation on Animals of Lodz, Poland (No 13/LB703/2014).

2.2.2. Animals and care

The study was carried out in a population of regular cycling female Wistar rats with an average initial body weight 215 ± 9 g, which were obtained from the breeding colony of the Medical University of Lodz. The rats were housed under controlled temperature (22 ± 1 °C), relative humidity (45–55%) and a 12 h light/dark cycle, and were administered a low phytoestrogen–content diet (Ssniff R/M–H low phytoestrogen) and tap water. Food and water were supplied *ad libitum* throughout the study.

2.2.3. Animal treatment

The experiments were conducted on eighty strictly selected (showing at least three consecutive regular estrous cycles) female rats. These were divided randomly into three experimental groups: the first one receiving 300 μg HxCN kg b.w.$^{-1}$ day^{-1} for two weeks, the second receiving 300 μg HxCN kg b.w.$^{-1}$ day^{-1} for four weeks and the third receiving 30, 100 or 300 μg HxCN kg b.w.$^{-1}$ day^{-1} for 13 weeks. Each experimental group comprised a control group (n = 10) and HxCN–exposed group (n = 10 per each dose). Rats from the control group obtained only sunflower oil via intragastric gavage, while rats from the experimental HxCN–exposed group received HxCN dissolved in sunflower oil. The animals were given 0.5 mL of oil or test solution per 100 g b.w. The explanation