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Developmental toxicity of hexachloronaphthalene in Wistar rats. A role of CYP1A1 expression

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ABSTRACT

Hexachloronaphthalene (HxCN) is one of the most toxic congeners of polychlorinated naphthalenes (PCNs). This study assesses the prenatal toxicity of HxCN after daily administration at doses of 0.1–1.0 mg/kg b.w. to pregnant Wistar rats during organogenesis. We evaluated also the expression of CYP1A1 mRNA and protein in the livers of dams and fetuses, as well as the placenta. The results indicate that 0.3 mg/kg b.w. was the lowest HxCN toxic dose for dams (LOAEL) while a dose of 0.1 mg/kg b.w. was sufficient to impair the intrauterine development of embryos/fetuses without maternal toxicity. Regardless of the applied dose, HxCN generated embryotoxic effects. Dose-dependent fetotoxic effects were associated with HxCN exposure. HxCN was found to be a strong inducer of maternal and fetal CYP1A1. Expression of CYP1A1 mRNA in the placenta appears to be the most sensitive marker of HxCN exposure.

1. Introduction

The group of polychlorinated naphthalenes (PCNs) comprise 75 possible congeners in eight homologue groups, with one to eight chlorine atoms substituted around a planar aromatic naphthalene molecule. In the past, congener mixtures of PCNs were produced in several countries under the trade names Halowax, Nibren and Seekay waxes and Cerifal Materials [1]. PCNs were used mainly as flame retardants and dielectric fluids for capacitors. They also found an array of applications in industry, such as dye-making, fungicides in the wood, textile and paper industries, plasticizers, oil additives, casting materials for alloys and lubricants for graphite electrodes [1–3]. Despite the fact that the manufacture and application of PCNs have been formally restricted in the majority of countries, these compounds are still released into the environment, for example, as a result of thermal processing of plastics containing polychlorinated biphenyls (PCB), in which PCNs also occur as trace contaminants [1–4].

Owing to their chemical structure and physicochemical properties, *i.e.* their durability, weak water solubility and accumulation in all elements of the natural environment and total ecosystems, PCNs are candidates for inclusion in the Stockholm Convention on persistent organic pollutants (POPs) [5]. They can be transported over long distances, and have been detected in the air, water, and biota of many global locations, including the Arctic and Antarctic [1,3,6–9]. The consequence of contamination of the natural environment by PCNs is their presence in food. Higher chlorinated congeners of PCNs (especially from tetra- to heptaCN isomers) have been found in food of animal origin (milk, meat, eggs and fish) [10–16]. The consumption of even small, trace amounts of such lipophilic substances as PCNs results in their inevitable accumulation in the body. General population studies carried out in various regions of the globe have revealed the highest concentrations of ΣPCNs (from tetra- to heptachloronaphthalene isomers) mainly in the adipose tissue [2,17,18]. Various pentachloronaphthalene isomers, as well as two major HxCN congeners, 1,2,3,4,6,7-hexachloronapththalene (PCN 66) and 1,2,3,5,6,7-hexachloronaphthalene (PCN 67), have been identified as dominant congeners in the adipose tissues of the general population [17]. As PCN66 and PCN67 cannot be readily separated in biological samples, their exposure assessments in food and human adipose tissue are usually estimated as a combined

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PCN66/PCN67 value [18,19]. The impact of PCNs on the human body in the context of potential health effects induced by environmental exposure, by ingestion, has not been practically elucidated. The only information concerning the toxicity of these compounds in humans applies to occupational exposure by inhalation. Symptoms associated with occupational exposure to PCN include, among others eye irritations, headache, anemia, impotency, anorexia and hepatotoxicity [2,6].

Scant experimental data also exists concerning the toxicity of PCNs. Due to their structural similarity to TCDD and affinity to the aryl hydrocarbon receptor (AhR), PCNs are frequently compared to dioxins, TCDD in particular, and are often referred to as dioxin-like compounds [20,21]. It was also shown that highly chlorinated naphthalenes (hepta- and oktaCNs) exert androgenic and anti-estrogenic properties and affect steroidogenesis in porcine ovarian follicles. These effects of PCNs in the ovary are similar to those caused by PCDDs [22,23]. Moreover, the similarity of the toxicities of the hexachloronaphthalenes (PCN66 and PCN67) and TCDD has been also confirmed by a number of studies, including that of Hooth et al. [19]. They noted that after repeated administration at very low doses to rats, both PCN congeners cause, characteristic of TCDD, hepatocellular hypertrophy and fatty change in the liver, together with the CYP1A1 induction, as well as histopathological changes in the thymus including atrophy, and changes in the lungs. Our own earlier studies carried out on rats indicate that PCNs induce anorectic effects such as wasting syndrome and neurotoxic effects, as well as hepatotoxicity; characteristic of TCDD [24–26].

Recently, more attention has been paid to the potential influence of the accumulation of lipophilic toxins such as PCNs in the adipose tissue of the maternal body on the safety of developing fetuses. However, only two studies regarding the potential prenatal toxicity of PCNs in mammals have been performed to date. Omura et al. [27] report that hexachloronaphthalene (HxCN) administered to pregnant female rats at a single dose not toxic to dams and developing fetuses (1 μg/kg b.w.) on days 14–16 of gestation accelerated the onset of spermatogenesis in male offspring. Another study notes that a mixture of different PCN congeners from tetraCN to heptaCN, approximately corresponding to Halowax 1013 and 1014, given to pregnant rats by gavage in the period of organogenesis, *i.e.* days 6–15 of gestation, generated embryo- and fetotoxic effects and teratogenicity that occurs in the absence of maternal toxicity [28].

In the present study, HxCN was given to pregnant female Wistar rats at three different daily doses during organogenesis, *i.e.* on days 6–15 of gestation. CYP1A1 expression was then assessed in dam and fetus livers and the placenta. The maternal toxicity, embryotoxic, fetotoxic and teratogenic effects were also assessed.

2. Materials and methods

2.1. Chemicals

Hexachloronaphthalene was synthesized according to Auger et al. [29] and obtained from the Institute of Radiation, Faculty of Chemistry, Technical University of Lodz (Poland) as a mixture of HxCN isomers. The purity of the HxCN sample was over 94% and it contained mainly 1,2,3,5,6,7-hexaCN (~81%). An Agilent-6890 gas chromatograph, with an electronic pressure programmer and a split/splitless injector was used to analyze the HxCN sample. Isotope Dilution HRGC/HRMS was used to analyze the tetra- through octa-chlorinated dioxin and furan content of the HxCN administered to the animals [26,30]. The analysis demonstrated that the content of dioxins and furans was <0.1 pg/100 μg of the investigated sample. All chemicals used for preparation and staining of fetuses were obtained from Sigma (St. Louis, MO).

2.2. Animals exposure

Wistar rats were obtained from the breeding colony of the Nofer Institute of Occupational Medicine, Lodz, Poland (Imp: WIST rats). They were housed with controlled temperature (22 ± 1 °C), relative humidity (45–55%), a 12-h light/dark cycle and maintained on commercial pelleted chow (Food Factory, Motycz, Poland) and tap water. Food and water were supplied *ad libitum* throughout the study. Nulliparous female rats, aged approximately 10 weeks and weighing 185–220 g, after 10 days of acclimatization, were mated (2:1) overnight with 14-week-old males and examined by vaginal smear for the presence of sperm the following morning. The day on which sperm was observed in the vaginal smear was designated as day 0 of gestation. Mated females were assigned to four experimental groups (19–21 dams per group). The animals were kept in cages (two females in each) in the same quarters as mentioned above.

The HxCN preparation was dissolved in sunflower oil and administered to females by stomach tube at single daily doses of 0.1, 0.3 or 1.0 mg/kg body weight (b.w.) at the same time for 10 consecutive days during organogenesis (days 6–15 of gestation). The dose given to animals was based on a recent determination of individual body weight, all dams were weighed every day just before administration, hence the absolute dose of HxCN was in agreement with current body weight of pregnant females on a given gestation day.

The animals were given 0.3 ml of test solution per 100 g b.w. Controls received an equivalent volume of oil. The doses of HxCN were chosen on the basis of earlier prenatal toxicity studies [28]. In line with the OECD guidelines and UE law, the doses should be properly chosen so that the highest dose could cause developmental toxicity and/or toxic symptoms in females (body weight loss), at least one medium dose should induce minimum toxic effects, and the lowest dose should not cause any developmental toxicity or toxicity in dams. The choice of two extreme doses was intentional to analyze CYP1A1 mRNA and protein expression as the aim of the study was to investigate the effects in the absence of maternal toxicity (dose 0.1 mg/kg b.w.), and in the presence of maternal toxicity manifested by a significant decrease in body weight gain during pregnancy (dose 1.0 mg/kg b.w.).

The studies were performed in accordance with Polish law on the protection of animals and with the permission of the Local Ethical Committee for Experimentation on Animals of Lodz, Poland (No 42/LB478/2009 and 28/LB478/211).

2.3. Maternal and fetal toxicity

The general behavior of the dams was observed daily. The pregnant females were weighed every day from 0 to 20 gestation days (GD) always at the same time of the day. Based on weights determined on days 0, 6, 10, 15 and 20 of gestation; body weight gain was calculated.

Food and water consumption were measured on days 1, 6, 10, 15 and 20 of gestation.

Final GD 20 body weights were corrected for gravid uterine weights (adjusted final body weight), and gestation body weight change and adjusted body weight change from day 0 (final body weight GD 20 minus gravid uterine weight minus GD 0 body weight) were calculated.

All females were sacrificed by decapitation on day 20 of gestation. The maternal liver, kidneys, adrenals, ovaries, spleen, brain and gravid uterine were removed and weighed. Live fetuses from each litter were weighed, sexed, and tagged. Maternal blood, placenta, whole maternal and fetal liver were collected for assays.

The following indicators of maternal toxicity were used: the concentrations of reduced glutathione (GSH) and malondialdehyde (MDA) in the liver and the activity of alanine aminotransferase