Reproductive Toxicology 43 (2014) 1–7

Contents lists available at ScienceDirect

Reproductive Toxicology

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

Exposure to polybrominated diphenyl ethers and male reproductive function in Greenland, Poland and Ukraine

Gunnar Toft[a,*], Virissa Lenters[b], Roel Vermeulen[b], Dick Heederik[b], Cathrine Thomsen[c], Georg Becher[c,d], Aleksander Giwercman[e], Davide Bizzaro[f], Gian Carlo Manicardi[g], Marcello Spanò[h], Lars Rylander[i], Henning S. Pedersen[j], Paweł Struciński[k], Valentyna Zviezdai[l], Jens Peter Bonde[m]

[a] Danish Ramazzini Center, Department of Occupational Medicine, Aarhus University Hospital, Aarhus, Denmark
[b] Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands
[c] Division of Environmental Medicine, Norwegian Institute of Public Health, Oslo, Norway
[d] Department of Chemistry, University of Oslo, Oslo, Norway
[e] Reproductive Medicine Centre, Skåne University Hospital, Lund University, Malmö, Sweden
[f] Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy
[g] Department of Life Science, Università di Modena e Reggio Emilia, Reggio Emilia, Italy
[h] Laboratory of Toxicology, Unit of Radiation Biology and Human Health, ENEA Casaccia Research Center, Rome, Italy
[i] Division of Occupational and Environmental Medicine, Lund University, Lund University Hospital, Lund, Sweden
[j] Centre for Arctic Environmental Medicine, Nuuk, Greenland
[k] Department of Toxicology and Risk Assessment, National Institute of Public Health–National Institute of Hygiene, Warsaw, Poland
[l] Department of Social Medicine and Organization of Public Health, Kharkiv National Medical University, Kharkiv, Ukraine
[m] Department of Occupational and Environmental Medicine, Bispebjerg University Hospital, Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 22 May 2013
Received in revised form
19 September 2013
Accepted 8 October 2013
Available online 26 October 2013

Keywords:
Semen quality
Reproductive hormones
Inhibin B
DNA damage
Apoptosis
PBDE
BDE-47
BDE-153

ABSTRACT

Animal and a few human studies suggest that polybrominated diphenyl ethers (PBDEs) may affect male reproductive function. The aim of the present study was to evaluate if male reproductive function was associated with serum level of PBDEs. We evaluated, in a cross-sectional study, the effects of environmental exposure to BDE-47 and BDE-153 on reproductive hormones and semen quality, including markers of DNA damage and apoptosis, in 299 spouses of pregnant women from Greenland, Poland and Ukraine. Adjusted linear regression models indicated no strong associations between BDE-47 or BDE-153 exposure and markers of male semen quality or reproductive hormones. In the largest study to date we demonstrate that BDE-47 and BDE-153 exposure was not associated with altered semen characteristics or reproductive hormones, indicating that male reproductive function is not affected by the exposure level of these compounds in fertile European or Arctic populations.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) have been used since the 1970s as additive flame retardants in a variety of consumer products including polyurethane foam used in mattresses and upholstered furniture and thermoplastics used in electronic equipment [1]. As they are mixed into polymers, and not chemically bound to the plastics or textiles, they are able to separate or leach from the products into the environment [2]. PBDEs are lipophilic compounds with a biological half-life up to 7 years [3]. Important sources of exposure include fatty fish and mammals from higher trophic levels, and therefore health risks for Arctic populations are a special concern [4]. House dust has also been indicated as an important contributor to human PBDE exposure, especially for toddlers and small children [5,6]. Of the 209 PBDE congeners, BDE-47 is usually the compound detected in the highest concentration in human biological material, followed by BDE-153 [5]. In addition to effects on the thyroid hormones [7], several PBDEs have shown the

* Corresponding author at: Danish Ramazzini Center, Department of Occupational Medicine, Aarhus University Hospital, Norrebrogade 44, Building 2C, 8000 Aarhus C, Denmark. Tel.: +45 78 46 4251; fax: +45 7846 4260.
E-mail address: guntof@rm.dk (G. Toft).

0890-6238/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.reprotox.2013.10.002

potential to interfere with reproductive hormone signaling through anti-androgenic, estrogenic or anti-estrogenic activity in *in vitro* models [8–10]. *In vivo* subacute (28 day) toxicity tests on adult male rats exposed to a commercial pentaBDE mixture in a dose range from 0.27 to 200 mg/kg body weight/day have shown dose-dependent decreased weight of the epididymis, seminal vesicles and prostate, and an increase in sperm head deformities with lower confidence bound of the estimated benchmark dose in the range of 10–50 mg/kg body weight/day [11]. Exposure of the fish Fathead Minnows (*Pimephales promelas*) to 14 μg BDE-47/day for 25 days indicated specific effects on male reproductive function, including a reduction in sperm concentration [12]. Furthermore, exposure *in ovo* of American kestrels (*Falco sparverius*) to the commercial pentaBDE mixture, DE-71, measured at 1 μg/g wet weight in the eggs, reduced testosterone levels and altered testis structure in the male offspring [13]. Some earlier toxicological evaluations of PBDEs did not indicate adverse effects on reproductive function in rodents after exposure in adulthood [14], but more recent experiments in mice have reported that, whereas neonatal exposure to 500 or 1500 mg/kg BDE-209 per day could induce weak effects on a few aspects of sperm function [15], *in utero* exposure to BDE-209 induced testicular changes together with a significant increase of sperm head abnormalities and sperm chromatin damage in the male progeny [16], and some of these effects were observed at a much lower concentration (10 mg/kg/day BDE-209).

In humans, only a few studies addressed the issue of environmental PBDE exposure in relation to adult male reproductive function. A Japanese study of 10 young men indicated adverse associations between BDE-153 and sperm concentration and testis size [17], and a Canadian study of 52 men recruited in an infertility clinic indicated adverse associations of BDE-47, BDE-100 and the sum of measured PBDE congeners (BDE-47, BDE-99, BDE-100 and BDE-153) on sperm motility [18], but no relation with other semen parameters. Finally, an American study of 62 men indicated positive associations between house dust penta-BDEs and serum level of estradiol and sex hormone binding globulin (SHBG), and inverse associations with follicle stimulating hormone (FSH). In the same study, house dust octa-BDEs were positively associated with luteinizing hormone (LH) and testosterone and finally deca-BDEs were inversely associated with testosterone [19]. This study was expanding on a previous report from the same group indicating an inverse associations between the measured PBDE congeners (47, 99 and 100) in dust samples and free testosterone, LH and FSH [20].

Thus, although epidemiological studies are only partly supported by experimental studies, animal and human studies suggest that PBDEs may affect human reproductive health. The associations have so far only been evaluated in small studies with divergent endpoints, and confirmation is needed in larger studies before more definite conclusions on effects on male reproductive health can be made.

The aim of the present study was to evaluate if PBDE exposure is associated with male reproductive function in three populations of fertile men with considerable variation in PBDE exposure.

2. Materials and methods

2.1. Study populations

The present study is part of a European study on fertility, the CLEAR study (www.inuendo.dk/clear), using a uniform protocol for data collection in Greenland, Kharkiv in Ukraine, and Warsaw in Poland [21].

Six hundred and two partners of pregnant women provided a semen sample and a blood sample, and filled in a questionnaire on lifestyle, occupation and medical history. All men were 18 years or older at the time of enrolment. In each of the three countries 100 blood samples were selected randomly among the samples with sufficient volume to allow analysis (>4 mL). One sample was lost due to problems during sample preparation before chemical analyses, and therefore the final sample size used to evaluate the effect of lipid adjusted PBDEs on male reproductive outcomes was 299. The 299 included men were not significantly different from the 303 non-included men regarding measures of semen quality or reproductive hormones.

The participation rates in the study varied from 29% in Warsaw and 33% in Kharkiv to 79% in Greenland. Study populations and data collection procedures have been described in detail elsewhere [21].

2.2. Collection of semen samples and basic semen analysis

All semen samples were collected by masturbation. The subject was asked to abstain from sexual activities for at least two days before collecting the sample, and to report the actual abstinence time. The sample was kept close to the body to maintain a temperature close to 37 °C if transport to the local hospital after collection was necessary. Analysis of semen samples was initiated within one hour after ejaculation for 83% of the samples. The samples were analyzed for concentration, motility and morphology according to a manual for the project based on the 1999 World Health Organization (WHO) manual for basic semen analysis [22], which was the most recent version of the manual at the time of sample collection. Briefly, sperm concentration was determined in duplicate using an Improved Neubauer Hemacytometer. Sperm motility was determined by counting the proportion of (a) rapid progressive spermatozoa; (b) slow progressive spermatozoa *versus* (c) non-progressive motile spermatozoa; and (d) immotile spermatozoa among 100 spermatozoa within each of two fresh drops of semen.

Semen samples from Warsaw and Kharkiv were analyzed for concentration and motility at one central hospital in each region, whereas the samples from Greenland were analyzed at the local hospitals or nursing stations spread across the country. One person performed all the semen analyses in each of the three countries. These three persons were previously trained in a quality control program set up specifically for this study [23]. The inter-observer variation as consider concentration was found to be 8% and for motility 11%.

Spermatozoa from all populations were stained and analyzed for morphology centrally at Skåne University Hospital in Malmö, Sweden. Abnormalities were classified as head defects, midpiece defects, tail defects, cytoplasma drop and immature spermatozoa. The morphology was evaluated for at least 200 sperms in each sample by two technicians taking part in the NAFA-ESHRE (Nordic Association for Andrology – European Society of Human Reproduction and Embryology) external quality control program.

2.3. Sperm chromatin structure assay (SCSA)

DNA damage was measured by SCSA following the standardized procedure described by Evenson et al. [24]. Briefly, all coded frozen semen samples from the three study regions were shipped on dry ice to the flow cytometry facility of the ENEA Laboratory of Toxicology (Rome, Italy) for SCSA analysis; a flow cytometric (FCM) technique which identifies the spermatozoa with abnormal chromatin packaging, envisioned by increased susceptibility to acid-induced DNA denaturation *in situ* followed by acridine orange fluorescence staining [24,25]. Adhering strictly to the SCSA protocol as described by Evenson et al. [24], the DNA fragmentation index (%DFI) – representing the percentage of sperm with detectable DNA breaks – together with the percentage of sperm with high levels of high DNA stainability (%HDS) – representing the fraction of sperm with anomalies in the histone-to-protamine transition – were