Toxicology Letters 204 (2011) 57–63

Contents lists available at ScienceDirect

Toxicology Letters

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

Neurobehavioral and physiological effects of low doses of polybrominated diphenyl ether (PBDE)-99 in male adult rats

Stéphanie Daubié[a,b,*], Jean-François Bisson[b], Robert Lalonde[c], Henri Schroeder[a], Guido Rychen[a]

[a] URAFPA, INRA UC340, Nancy Université, BP 172, 54505 Vandoeuvre-lès-Nancy Cedex, France
[b] ETAP-Ethologie Appliquée, Plate-Forme des Biotechnologies des Sciences du Vivant & Santé, Technopôle de Nancy-Brabois, 13 rue du Bois de la Champelle, 54500 Vandoeuvre-lès-Nancy, France
[c] Centre Hospitalier Universitaire de Montréal/St-Luc, Centre de Recherche en Sciences Neurologiques, 1058 rue St-Denis, Montréal, PQ, Canada H2X 3J4

ARTICLE INFO

Article history:
Received 12 January 2011
Received in revised form 8 April 2011
Accepted 11 April 2011
Available online 15 April 2011

Keywords:
PBDE-99
Locomotor activity
Learning
Memory
Anxiety
Adult rats

ABSTRACT

Polybrominated diphenyl ethers (PBDEs) are flame retardants. Because of their high lipophilicity and persistence, PBDEs bioaccumulate in all abiotic and biological matrices. The aim of this study was to investigate the long-term neurobehavioral and physiological effects of exposure to environmental doses of PBDE-99 in adult rats. Rats received a daily administration of PBDE-99 for 90 days by oral gavage at 0.15, 1.5 and 15 μg/kg, doses which are relevant of human exposure. Before and after the 90 days of exposure, behavioral tests including the open-field and the elevated plus-maze tests for locomotor activity and anxiety, and the Morris water maze for spatial learning were conducted. Physiological measures such as body weight, food and water consumption, organs weight, hepatic enzymes levels and PBDE-99 concentration in adipose tissue were also evaluated at the end of exposure. There was no effect on body weight, food and water consumption, organs weight, hepatic enzymes levels despite rising PBDE-99 concentration in adipose tissue with the doses tested. Moreover, there was no effect on locomotor activity and exploration, and spatial learning. Deleterious effects of PBDE-99 at high doses have often been highlighted in many studies after an acute dose whereas exposure during 90 days at realistic doses would have no significant effect in adult rats.

1. Introduction

Brominated flame retardants are a group of industrial compounds used to retard, suppress or inhibit combustion processes to reduce fire risks (IPCS, 1994). Polybrominated diphenyl ethers (PBDEs) are used as additive flame retardants in electrical and electronic appliances, building materials, polyurethane foams, coatings, textiles (Birnbaum and Staskal, 2004; Darnerud et al., 2001). Their chemical formula is $C_{12}H_{(10-n)}Br_nO$ ($1 < n < 10$) with the sum of H and Br atoms always equal to 10. The theoretical number of possible congeners is 209, varying in number and position of Br atom, and divided into 10 congener groups (mono to decabromodiphenyl ethers) (Birnbaum and Staskal, 2004; Darnerud et al., 2001). Being added to polymers as additive, PBDEs are not chemically bound to the material and can be released and dispersed into the surrounding environment.

Because of their high lipophilicity and persistence, PBDEs bioaccumulate in all abiotic samples (soil, water, air, sewage sludge, sediments), terrestrial ecosystems including humans, freshwater and marine ecosystems (Birnbaum and Staskal, 2004). Humans may be exposed to these compounds through food of animal origin, particularly fish (Domingo, 2004; Jones-Otazo et al., 2005) or ingestion and inhalation of dust (Jones-Otazo et al., 2005; Wilford et al., 2005). Since their introduction in 1970s, the levels of PBDEs have exponentially increased in the environment, wildlife and human (adipose tissue, serum and breast milk) (Costa and Giordano, 2007; Meironyté et al., 1999; Rayne et al., 2003). Moreover, the environmental PBDE concentrations still increase each year (He et al., 2009; Hites, 2004) in contrast to other environmentally persistent contaminants such as polychlorinated biphenyls (PCBs), dioxins and furans which concentrations in human decreased in the last 20 years (Schecter et al., 2005; Schuhmacher et al., 2009). PBDE tissue levels in humans differ from one continent to another. Indeed, PBDE concentrations in breast milk, serum or adipose tissue appear to be 10–70 times higher in the United States than in Europe (Schecter et al., 2003; Sjödin et al., 2008). These differences would be related to usage of PBDEs and to a less stringent regulation in the United States (Darnerud et al., 2001).

* Corresponding author at: ETAP-Applied Ethology, Technopôle de Nancy-Brabois, 13 rue du Bois de la Champelle, 54500 Vandoeuvre-lès-Nancy, France. Tel.: +33 0 383 444 635; fax: +33 0 383 446 441.
E-mail address: sdaubie@etap-lab.com (S. Daubié).

0378-4274/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.toxlet.2011.04.009

PBDE-99 (2,2′,4,4′,5-pentabromodiphenylether) is one of the most persistent congeners. It is one of the most frequently found and at highest levels in almost all environmental samples including human tissues (Darnerud et al., 2001; de Wit, 2002; Inoue et al., 2006; Schecter et al., 2003, 2006). PBDE-99 is commonly found in human milk and cord blood (Norén and Meironyté, 2000; Schuhmacher et al., 2009). This compound was the main component of the commercial pentaBDE mixture, used as additive in polyurethane foam for cushioning in upholstery (Birnbaum and Staskal, 2004). Although this commercial mixture has been banned in European Union since 2004 by the directive 2003/11/EC, many materials containing this mixture are still into circulation and continually emit congeners in the environment. Consequently, the rate of these pollutants is increasing in different biological matrices.

Since the chemical structure of PBDEs and their metabolites are similar to other environmentally persistent contaminants such as PCBs, dioxins and furans, their toxicological properties are predicted to be comparable (Coburn et al., 2007; Kodavanti and Ward, 2005). PBDEs are known to induce neurobehavioral toxicity in rodents, particularly in case of developmental exposure (Branchi et al., 2002, 2003,2005; Cheng et al., 2009; Costa and Giordano, 2007; Eriksson et al., 2001). Indeed, prenatal or perinatal exposure to PBDE-99 in Wistar rats or CD-mice treated with a single oral dose of 60 μg/kg or 300 μg/kg or at a dose of 18 mg/kg caused hyperactivity in the offspring (Branchi et al., 2005; Kuriyama et al., 2005). Learning and memory functions were affected in rats having received a dose of 2 mg/kg of PBDE-99 (Cheng et al., 2009) or 300, 600 and 1200 mg/kg of PBDE-209 (Wu et al., 2008), during gestation and lactation. Postnatal exposure to PBDE-99 in NMR1 mice or Sprague-Dawley rats at the age of 10 days with a single oral dose of 0.8 mg/kg, 8 mg/kg or 12 mg/kg resulted in aberrations in spontaneous behavior (hypoactivity at the beginning of the test and hyperactivity at the end of the test) (Eriksson et al., 2001; Viberg et al., 2004, 2005) with effects being more pronounced with increasing age (Eriksson et al., 2001). In addition, NMR1 mice or Sprague-Dawley rats exposed on PND-10 at doses of 1 mg/kg, 5 mg/kg or 10 mg/kg of PBDE-47 or at dose of 12 mg/kg of PBDE-99 exhibit learning and memory alterations (Eriksson et al., 2001; He et al., 2009).

Neurotoxic effects of PBDE-99 have indeed been observed but at very high doses (some mg/kg). Actually, doses of human exposure to this pollutant were much lower and there is no information available on its effects at environmental levels. Moreover, PBDEs are generally administered in rodents in high acute doses which do not reflect pollution to which humans are exposed. The aim of the present study was therefore to investigate in adult rats the long-term neurobehavioral and physiological effects of exposure to environmental doses of PBDE-99 for 90 days according to the recommendations of the Organization for Economic Cooperation and Development (OECD) test guideline 408 (OECD, 1998). The tested doses of 0.15, 1.5 and 15 μg/kg, which are relevant to human exposure levels (Jones-Otazo et al., 2005; USEPA, 2002), were used in evaluating open-field and elevated plus-maze tests for locomotor activity and anxiety, and water maze test for spatial memory adapted from Morris (1984). The rats were also assessed for body weight, food and water consumption, organs weight, hepatic enzymes levels and PBDE-99 concentration in adipose tissue.

2. Materials and methods

2.1. Animals

Thirty-two male Sprague-Dawley rats (Harlan, the Netherlands) weighing 160–180 g at the start of the experiments were housed in groups of two inside 48 cm × 27 cm × 20 cm polycarbonate cages (UAR, Epinay-sur-Orge, France) under stable conditions with respect to temperature (22 ± 2 °C) and humidity (50 ± 10%). The rats were maintained on a 12 h light/12 h dark cycle (lights on at 9.00 pm–9.00 am). Food pellets (Harlan, Gannat, France) and tap water were provided *ad libitum*. After a 7-day adaptation period, the rats were weighed and randomly assigned to one of the four treatment groups ($n = 8$): control-vehicle or PBDE-99 at 0.15, 1.5 and 15 μg/kg (BDE 0.15, BDE 1.5 and BDE 15, respectively).

The animal care unit is authorized by the French Ministries of Agriculture and Research (Government Authorization No. 54-547-1). The experiments adhered to guidelines provided by the ASAB Ethical Committee for the use of animals in research (Anim. Behav. 71 (2006) 245–254) and the Canadian Council on Animal Care (Guide to the Care and Use of Experimental Animals), 2nd ed., vol. 2 (1993); 1st ed., vol. 1 (1984). All procedures were also in compliance with the rules provided by the European Community Council Directive 86/609/EEC on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for scientific purposes (Off. J. L358 (18 December 1986) 0001–0028).

2.2. Chemical and treatment

PBDE-99 (2,2′,4,4′,5-pentabromodiphenylether) was synthesized at the Department of Environmental Chemistry, University of Stockholm, Sweden (Marsh et al., 1999; Örn et al., 1996) and its purity exceeded 98%. The PBDE-99 solution was prepared by mixing the compound with corn oil and then sonicating the mixture at room temperature for 45 min. The substance was daily orally administered (gavage) at a volume of 5 ml/kg BW for 90 days. Control rats received corn oil with the same volume of administration and during the same period.

Due to the necessity to administer exact doses of the studied products by gavages, 16-gauge × 3-in. intragastric feeding needles with a ball tip (Poppers and Sons, Inc., New York, USA) were used by experienced researcher. These needles were used to prevent their introduction into the trachea and to prevent trauma to the oral cavity and to the esophagus. In general, after two oral treatment sessions, the rats habituated to the oral administration procedure.

2.3. Behavioral tests

Behavioral tests were performed before and after the 90 days of exposure to PBDE-99 in compliance with OECD test guideline 408 for repeated dose 90-day oral toxicity study in rodents (OECD, 1998). All the animals underwent the behavioral tests before being sacrificed to allow physiological measures. Two weeks have been necessary to conduct the complete behavioral testing both before and after exposure to PBDE-99. All tests were conducted under red dim light during the first hours of the dark cycle and animal behaviors were recorded using a video camera and a recorder (Sony video system). Then, data were scored from videotapes by a human trained observer unaware of group treatments.

2.3.1. Elevated plus-maze test

Exploration and the level of anxiety of rats were measured 15 days before and 90 days after the start of exposure in the elevated plus-maze. The apparatus was constructed from plasticized wood with two open (50 cm × 20 cm) and two enclosed arms (wall height: 40 cm) placed on opposite sides at a height of 80 cm. The four arms extended from a common central platform (20 cm × 20 cm). The rat was placed in the center with its head turned towards an enclosed arm and was left to explore the apparatus in a single 5-min session. The number of entries and the time spent inside each arm were measured.

2.3.2. Open-field test

Thirteen days before and 92 days after the start of exposure, general exploratory activities were tested in the open-field made of Plexiglas which consisted of a circular arena measuring 60 cm in diameter with 50 cm high walls, divided into 9 equal areas: the central zone (white floor, 10 cm in diameter) and 8 peripheral areas. Each rat was placed in the center of the arena and tested for 3 min. The number of squares crossed (movements in the horizontal plane), the number and the time of rearings (movements in the vertical plane) were recorded. The time spent in the center of the arena was also recorded to estimate anxiety of rats. The floor was cleaned with a diluted alcohol solution and dried before introduction of each animal to reduce odor clues.

2.3.3. Morris water maze test

This test was performed 7 days before and 98 days after the start of exposure. The circular tank measured 150 cm in diameter with 50-cm high walls and was filled with water (25 ± 1 °C) made opaque by the addition of a white powder Blanc d'Espagne (reference 5582, droguerie Lorraine "à l'arc-en-ciel", Nancy, France). The Morris water maze was virtually divided into four equal quadrants. An escape platform (20 cm in diameter), made invisible for rats by opacification of the water, was hidden 1.5 cm below the water surface at the center of the northwest quadrant of the tank. Before starting the PBDE-99 exposure, two daily sessions were conducted that consisted in each session to place the rat in the water facing the wall of the pool at one of the four starting positions (north, south, east, west) for 4 trials with a maximal duration of 60 s. When finding the platform, the rat was allowed to stay on it for 15 s. Whenever unable to find the platform, the rat was guided to it by the experimenter and allowed to stay on it for the same time, and then placed back into the water for the next trial from another starting position. A third session was