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# Photoperiod modulates access of 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB153) to the brain and its effect on gonadotropin and thyroid hormones in adult ewes

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ABSTRACT

The effects of photoperiod on the cerebrospinal fluid (CSF) concentration of six *ortho*-substituted polychlorinated biphenyls (PCBs: PCB28, PCB52, PCB101, PCB138, PCB153, and PCB180), the effects of an orally administered low dose of PCB153 (0.3 mg/kg, three times a week for three weeks) on PCBs and thyroid hormones (THs) concentrations in the CSF and plasma, and the release of luteinizing hormone (LH) were determined in ovariectomized, estradiol-implanted ewes (2.5 years old) maintained indoors under artificial long day (LD, 16L: 8D) and short day (SD, 8L: 16D) conditions. Concentrations of two PCBs (PCB28 and PCB153) in the plasma and four PCBs in the CSF (PCB101, PCB138, PCB153, and PCB180) were significantly higher during LD than SD. Following PCB153 treatment, its concentration in the plasma was higher in SD ($1.2 \pm 0.3$ ng/ml) than LD ($0.2 \pm 0.05$ ng/ml), but similar in the CSF ($10.2 \pm 3.7$ pg/ml vs. $13 \pm 0.7$ pg/ml) under both photoperiods. During SD, the concentration of PCB153 in the CSF was higher in treated animals than controls, while no differences were noted under LD. These findings indicate that in ewes, exposure of the brain to more highly chlorinated, *ortho*-substituted PCBs may be modulated by photoperiod. PCB153 treatment had no effect on plasma THs, but reduced total triiodothyronine concentration during LD and free thyroxine during SD in the CSF. Under both photoperiods, PCB153 reduced basal plasma LH and reinforced the inhibition of pulsatile LH release during LD. As PCB153 reduced LH and THs (which are involved in the seasonal control of reproduction in ewes), it may have a braking effect on seasonal transitions between active and inactive phases of reproduction.



## 1. Introduction

Polychlorinated biphenyls (PCBs) are chemicals that comprise a group of 209 congeners with varying degrees of chlorination, which determines their physical, chemical, and biological properties (Safe, 1994). Structurally, there are at least two distinct classes of PCBs: coplanar (non-*ortho*-substituted) and non-coplanar (*ortho*-substituted) congeners. Coplanar congeners, like dioxin, exert their toxic effect mainly by binding to the aryl-hydrocarbon receptor (Okey et al., 1994), while non-coplanar congeners, initially regarded as less-toxic to animals, have since been shown to be endocrine disruptors (McKinney and Waller, 1994). *Ortho*-substituted PCBs constitute a large part of the PCB residue found in the environment and in animal tissues (Kodavanti et al., 1998). Although the brain appears to be better protected against PCBs than other tissues because of the blood–brain and blood–cerebrospinal fluid (CSF) barriers (Skipor and Thiery, 2008), *ortho*-substituted PCBs have been reported to accumulate preferentially in the brain and CSF compared to other PCBs (Kodavanti et al., 1998; Montie et al., 2009; Takasuga et al., 2004). Interestingly, Atlantic dolphin CSF contained PCB138, PCB149, PCB153, PCB180, and PCB187 that altogether represented about 64–67% of the total PCBs in the CSF (Montie et al., 2009). Among them, PCB153 was the most abundant congener (Montie et al., 2009). Of note, PCB138 and PCB153 were also predominant

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congeners detected in different brain regions of rats treated with Aroclor 1254 (Kodavanti et al., 1998).

Access of endogenous molecules, such as dopamine metabolites (Skipor et al., 2001, 2004), leptin (Adam et al., 2006), and gonadal steroids (Thiéry et al., 2003, 2006), to the brain is modulated by photoperiod in sheep. For gonadal steroids, we have demonstrated the involvement of the pineal gland, suggesting the possibility of melatonin control of mechanisms responsible for photoperiodically induced increase in steroids in the CSF (Thiéry et al., 2006). This result led us to hypothesize that access of *ortho*-substituted PCBs to the brain in sheep may vary with photoperiod and may have photoperiod-specific effects. Studies in bank voles indicated photoperiod-dependent effects of PCBs and cadmium on kidney and liver function (Wlostowski et al., 2008). In sheep, photoperiod synchronizes the endogenous annual rhythm of reproduction and affects several physiological functions (Thiéry et al., 2002). Despite evidence for *ortho*-substituted PCB-mediated disruption of the level of thyroid hormones (THs) (e.g. Craft et al., 2002; Kobayashi et al., 2009) and role of THs in the central mechanisms governing seasonal reproduction in sheep (Hanon et al., 2008; Karsch et al., 1995), data about the effect of photoperiod on access of *ortho*-substituted PCBs to the brain and further action on the endocrine axis controlling reproduction in sheep are limited. *Ortho*-substituted PCBs may also disturb estradiol-regulated central mechanisms of seasonal reproduction in sheep (Bosc et al., 1982) because the most abundant highly-chlorinated PCBs in the brain (i.e. PCB138, PCB153, and PCB180) have been reported to have anti-estrogenic effects in MCF-7 cells (Bonnefeld-Jorgensen et al., 2001).

The aim of the present study was to evaluate the effect of photoperiod on the basal concentrations of several *ortho*-substituted PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, and PCB180) in the blood and CSF of sheep kept under long days (LD) and short days (SD), and to test the impact of PCB153 oral administration during LD and SD on the concentrations of luteinizing hormone (LH) and THs in the plasma and in CSF. For this purpose, we used a method that facilitates the long-lasting sampling of CSF performed in parallel with blood plasma collection.

## 2. Materials and methods

### 2.1. Animals and management

All animal experiments were conducted in accordance with French Authorization No. 37801 for Animal Experimentation and Surgery and approved by the "Val de Loire" Local Ethics committee. Euthanasia was performed by a licensed butcher in a certified slaughterhouse (agreement no. 37175-01). Adult Ile-de France ewes ($n=28$; 2.5 years old) were maintained indoors in separate pens under artificial lighting conditions and fed a constant diet of hay, straw, and commercial concentrates with water and mineral licks available *ad libitum*. About five weeks before starting PCB153 or vehicle treatment, all ewes were implanted with a stainless-steel guide cannula (1.5 mm o.d., 40 mm length) in the third ventricle of the brain under general isoflurane anesthesia, according to the method described by Thiéry et al. (2006). During the surgery, they were also ovariectomized and subcutaneously implanted with E2, as described previously (Thiéry et al., 2006), with an additional subcutaneous injection of morphine chlorhydrate (20 mg, CMD Lavoisier, 75017, Paris, France) before the end of anesthesia to prevent post-operative pain. The E2 implants were made from silastic tubing and allowed to maintain the plasma E2 concentrations of 5–8 pg/ml (Cahill et al., 1981). During the sampling session, ewes were kept in comfortable, individual cages where they could lie down and had access to hay. To prevent the stress of isolation, ewes had visual contact with other sheep.

### 2.2. Experimental design

We used a routine light treatment that induced a neuroendocrine status corresponding to a LD or SD photoperiod. Under these conditions, ewes showed inhibition or stimulation of the pulsatile LH secretion after 30 to 60 day for LD and SD, respectively (Legan and Karsch, 1979; Thiéry et al., 2003). For the LD experiments (LD Exp.), 14 ewes (mean weight $52.5 \pm 3.0$ kg) were maintained under SD (8L: 16D) from the beginning of July 2008 to the end of September and were then transferred to LD (16L: 8D) according to the schedule in Fig. 1. The ewes were then randomly divided into groups of 7 control-LD or 7 PCB153-LD and kept in two separate pens to prevent contamination. After 55 day, control-LD ewes were subjected to the first session (pre-treatment) of blood sampling followed by vehicle (comestible sunflower oil) treatment from D62-77. The second session of blood sampling (D75) and CSF sampling were performed from D75-77, followed by euthanasia at D79. The PCB153-LD group underwent the same experimental schedule with a 3-day delay.

For the SD experiments (SD Exp.), 14 ewes (mean weight $57.4 \pm 1.7$ kg) were maintained under LD from the end of July 2009 to the end of September and were then transferred to SD conditions. The ewes were then randomly divided into groups of 7 control-SD and 7 PCB153-SD. After 59 day, control-LD ewes were subjected to the first session (pre-treatment) of blood sampling followed by vehicle treatment from D64-79. The second session of blood sampling (D77) and CSF sampling was performed from D77-79. Furthermore, due to the reduced LH concentration observed in the LD Exp., we tested the responsiveness of the gonadotroph cells to gonadotropin releasing hormone (GnRH) by intravenously injecting 125 ng of GnRH (Sigma, L'Isle d'Abeau Chesnes, France) at 1:00 pm on D80 and measuring LH 15 min, 45 min, and 90 min after injection. Ewes from control-SD were euthanized at D81. The PCB153-SD group underwent the same experimental schedule with a 3-day delay.

After euthanasia, samples of several tissues including the brain were collected and stored at $-80$ °C.

### 2.3. PCB administration

PCB153 (2,2′,4,4′,5,5′-hexachlorobiphenyl) from AccuStandard (New Haven, CT, USA, 99.9% pure) was purchased from Interchim (Montluçon, France) and dissolved in sunflower oil. Following the results of a preliminary experiment, the PCB153 was administered *per os* at a dose of 0.3 mg/kg of body mass. Nine doses of PCB153 were administered within 16 day, as shown in Fig. 1. This timeframe was chosen according to a study from Costera et al. (2006) showing that the time to reach a steady-state concentration of PCB in the milk of goats exposed to contaminated hay (with a PCB153 concentration of about 460 ng/kg of dry mass) was 15 day for most PCB congeners.

### 2.4. Collection of blood and CSF samples

Blood was collected via an indwelling cannula every 15 min for 10 h, totaling 41 samples in the LD Exp., and every 7 min 30 s for 7 h 30 min, totaling 60 samples in the SD Exp. during both the first and second session of blood sampling. Blood samples were then centrifuged and stored at $-20$ °C for further analysis of PCBs and hormones. CSF was collected from the third ventricle for 48 h at a rate of 20 μl/min (Peristaltic "minipuls 3" pump, Gilson, Villiers-Le-Bel, France). CSF was collected in a series of 3 ml test tubes that were pooled to reach a volume of about 50 to 60 ml and were then stored at $-20$ °C until their contents were assayed for PCBs and THs. Similarly, 1 ml aliquots of each of the 35 samples from the second sampling session were pooled for further PCBs and THs analysis. Due to CSF collection problem mainly associated with completely or partially choked cannula in some animals, CSF was collected from all PCB153-treated animals and five control ewes in the LD Exp., while in the SD Exp. CSF was collected from all PCB153-treated animals and six control ewes.

### 2.5. Extraction and measurement of PCB congeners from blood plasma and CSF

Determination of PCBs was performed in an accredited laboratory (Laboratory for Trace Organic Analyses, Krakow University of Technology, Poland) using isotope-dilution high-resolution chromatography/tandem mass spectrometry (ID-HRGC/MS-MS) on a Thermo Scientific GCQ-1100/Trace2000 system adjusted to double fragmentation mode equipped with Xcalibur data acquisition and analysis software. Briefly, blood plasma (20 ml) and CSF (30 ml) were freeze-dried and spiked with clean-up standards using 1 ml of $^{13}$C-PCBs/g of the sample with a standard mixture containing 10 ng/ml of $^{13}$C-PCBs, with the exception of $^{13}$C-PCB153 where the concentration was 20 ng/ml, and extracted with toluene for 16 h in a 20 ml Soxhlet apparatus. The extract was cleaned up according to the previously published procedure (Surma-Zadora and Grochowalski, 2008). The cleaned-up extract was evaporated to dryness and reconstituted in 50 μl of nonane containing 100 pg/ml of $^{13}$C-1,2,3,4-tetrachlorodibenzodioxin ($^{13}$C-1,2,3,4-TCDD) as a precision and recovery standard. Separation of PCB congeners was performed on a 30 m × 0.25 mm i.d. DB5MS capillary column of 25 μm film and DB17 30 m × 0.25 mm i.d. A 2 μl-volume sample was injected into the SSL injector at 260 °C. The GC oven temperature was maintained at 100 °C for 1 min and was then raised to 180 °C at 20 °C/min, then by 2 °C/min to 260 °C, and then by 20 °C/min to a final temperature of 300 °C. The temperature was maintained at 300 °C for 5 min. The method is highly specific and does not give interfering signals between PCB congeners. Recovery for analysis ranges from 65 to 120%.