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A human exposure based mixture of persistent organic pollutants affects the stress response in female mice and their offspring

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HIGHLIGHTS

- Increased basal cortisol in mothers exposed to POPs.
- A prolonged stress response in mothers exposed to POPs.
- An over-sensitised stress response following POP exposure in male offspring.
- No effect of POPs in female offspring.

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ABSTRACT

Persistent organic pollutants (POPs) are found in the food chain of both humans and animals and exert a wide spectrum of potentially adverse effects. The present experiment aimed to investigate whether a defined mixture of 29 POPs, based on the dietary intake of Scandinavians, could affect the stress response in female mice exposed through ingestion, and in their offspring. Female mice 129:C57BL/6F0 hybrids were exposed from weaning, throughout pregnancy, and up until necropsy, to either 5000 × or 100 000 × the estimated daily intake for Scandinavians. The offspring were fed a reference diet containing no POPs. Both the mothers and their offspring were tested for basal and stress responsive corticosterone levels, and in an open field test to measure locomotor activity and anxiety-like behaviours. We found mothers to have elevated basal corticosterone levels, as well as a prolonged stress response following POP exposure. In the offspring, there was no effect of POPs on the stress response in females, but the exposed males had an over-sensitised stress response. There was no effect on behaviour in either the mothers or the offspring. In conclusion, we found a human relevant POP mixture can lead to subtle dysregulation of the hypothalamus-pituitary-adrenal axis in mice. As HPA axis dysregulation is commonly associated with neurological disorders, further studies should explore the relevance of this outcome for humans.

1. Introduction

Persistent organic pollutants (POPs) are organic compounds that are resistant to degradation and so remain within the environment for extended periods of time. These compounds were produced for use in industry and agriculture, but were subsequently found to have wide-ranging toxic effects in both humans and wildlife (for a review, see Jones and de Voogt, 1999; Carpenter, 2006). This has led to many POPs being banned from production via the Stockholm Convention, but some are still in use due to a lack of alternative compounds or strict regulation. Due to their resistance to

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degradation, POPs have been transported around the globe, primarily via wind and water, and continue to persist within the environment (Law et al., 2014). Furthermore, POPs bioaccumulate within living organisms and biomagnify up the food chain, even in locations previously considered pristine (e.g. Oskam et al., 2004). As such, POPs continue to be a considerable human and environmental issue.

To date, around 30 POPs are listed in the Stockholm Convention, but these may have multiple congeners. For instance, there are 209 possible congeners of polychlorinated biphenyls (PCBs). Due to the substantial number of compounds and their ubiquitous nature within the environment, vast cocktails of POPs are typically found in both humans and animals at any given time (i.e. Costopoulou et al., 2006). As such, there is a need to understand how such a body burden can influence an individual's health. The traditional approach has been to study single compounds at relatively high doses, but subsequent research would indicate that toxicants can have biphasic response curves, and/or additive, synergistic, or antagonistic effects on biological endpoints (Altenburger et al., 2013). Therefore, effects can be difficult to predict from modelling the results of relatively high doses of individual compounds.

Some POPs are endocrine disruptors, compounds that can result in alterations in hormone synthesis or metabolism, or receptor target modulation via mimicking, antagonising, or altering endogenous hormone levels (reviewed in Frye et al., 2012). Due to the central role of the endocrine system in developing, organising, and maintaining the central nervous system, there is concern over the ability of POPs to impair early brain development. This is especially true within the concept of foetal programming, whereby rapidly developing biological systems appear to be particularly vulnerable to disorganising influences that may then have chronic implications (Gluckman and Hanson, 2004). For instance, POPs are known to cross the placenta (Needham et al., 2011), and have been associated with neural tube defects at birth (Ren et al., 2011), as well as neurodevelopmental outcomes in children such as poor attention and low IQ (for a review, see Berghuis et al., 2015).

Glucocorticoids are essential for maintaining a normal physiological response to stressful situations and are therefore an essential aspect of lifetime fitness. The hypothalamic-pituitary-adrenal (HPA) axis coordinates the response to stress via corticotropin-releasing factor (CRF), adrenocorticotropic hormone (ACTH), and cortisol (corticosterone in mice). These hormones play a key role in directing energy away from non-essential life processes towards immediate survival. During development, cortisol is also essential for normal foetal development (Liggins, 1994), including development of the brain (Harris and Seckl, 2011). For example, prenatal stress or glucocorticoid exposure can reprogram the HPA axis in offspring leading to long-term dysregulation and alterations in anxiety-like behaviour (Glover et al., 2010; Davis et al., 2011). Of concern, ecotoxicological studies have found POPs to be associated with alterations in cortisol dynamics in polar bears (Oskam et al., 2004), arctic birds (Verboven et al., 2010; Tartu et al., 2014), and fish (Hontela et al., 1992) whilst experimental studies have confirmed that POPs can lead to alterations in basal cortisol/corticosterone in rodents (Pereiro et al., 2014), goats (Zimmer et al., 2009), sheep foetuses (Zimmer et al., 2013), and fish (Jørgensen et al., 2002), as well as impairing the stress response (Jørgensen et al., 2002; Zimmer et al., 2009).

In the current study, we explore whether a human based POP mixture affects basal corticosterone and the stress response in female mice and their offspring. We hypothesise that those mice exposed to POPs will show HPA axis dysregulation. Therefore, we measured corticosterone levels in unstressed and stressed mice, and anxiety-like behaviour and locomotor activity in an open field test.

2. Materials and methods

The study was performed at the Section for Experimental Biomedicine at The Norwegian University of Life Sciences in Oslo, Norway. The unit is licensed by the Norwegian Animal Research Authority (NARA) and accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (www.aaalac.org). The study was approved by the unit's animal ethics committee (Institutional Animal Care and Use Committee/IACUC; FOTS: 5583) and NARA (2013/39783).

2.1. Chemicals and feed

A thorough description of the design and preparation of the POP mixture can be found in Berntsen et al. (2017). In brief, the mixture reflects the levels of POPs found in a Scandinavian food basket as the experiment was designed in order to be of interest to a human exposure. A list of the individual compounds and the concentrations of each within the feed can be found in Table 1. A literature review identified the most relevant POPs and the estimated daily intake (EDI) levels of these compounds for a human of 70 kg. Based on the human EDI, corresponding EDIs of the different compounds for a 25 g mouse were calculated (Table 1). Due to the possibility of background exposure via the mice feed and the higher drug metabolism of mice than humans (Walton et al., 2001), the feed concentration of the mixture was set to provide a mouse consuming 3 g feed/day a daily dose of 5000 × and 100 000 × the EDI for humans (see Berntsen et al., 2017 for further discussion). This resulted in between 24 and 25 of the 29 compounds being detected in the plasma of the mothers depending on the exposure group, and between 18 and 20 were found in the pups (Table 2). In brief, most compounds were found in higher levels in the mothers than the pups and a dose dependent increase (low vs high dose) was found for all compounds. Most (26/29, low and high dose group, respectively) of the compounds were detected in the brains of mothers, as well as the pups brain (20/29 and 22/29 in the low and high dose, respectively) (Berntsen et al. in prep). When comparing blood values between the experimental mice and the Scandinavian population, those mothers exposed to the high dose had values ranging from 100 up to 5000 × the levels of individuals compounds found in Scandinavians (Table 2). For the low exposed mothers, these values ranged from 8–500 × the average Scandinavian (Table 2). Therefore, the tissue concentrations were more similar to the human scenario than the EDI values, as expected based on the higher drug metabolism in mice.

All polybrominated diphenyl ethers (PBDEs), PCBs and other organochlorines were originally purchased from Chiron As (Trondheim, Norway). All perfluorinated compounds and hexabromocyclododecane (HBCD) were obtained from Sigma-Aldrich (St. Louis, MO, USA), with the exception of perfluorohexane sulfonic acid (PFHxS) potassium salt which was from Santa Cruz (Dallas, US). All chemicals were dissolved in an appropriate solvent and added to corn oil (Jasmin, fully refined, Yonca Gida San A.S., Manisa, Turkey) intended for human consumption. All solvents were thoroughly evaporated under N_2-flow before the oil containing the POPs was sent to the feed company (TestDiets, St. Louis, MO) to be incorporated in the mouse feed. Four different diets were made, three exposure diets for pregnant mice, control (non-exposed), low dose (5000 × EDI) and high dose (100 000 × EDI), and a reference diet for males and pups after weaning. For the control feed, the corn oil included the solvents at identical levels to those found in the two exposure diets, whereas for the reference feed only untreated corn oil was used. In all diets, all soybean oil in the original feed recipe was exchanged with corn oil intended for human consumption, in order to reduce background POP exposure,

Table 1
A mixture of persistent organic pollutants (POPs) based on a literature review on estimated daily intake (EDI) values in the Scandinavian population (Berntsen et al., 2017). Average EDI values for a 70 kg human and corresponding values for a 25 g mouse are shown. EDI values for a 25 g mouse consuming 3 g of feed designed to provide daily doses of POPs corresponding to the low (5000× human EDI) and high (100,000× human EDI) doses are shown in grey, and are based on measured feed concentrations. The table is adapted from Berntsen et al. (2017).

Compound	Average EDI [a] 70 kg person ng/day	Daily intake human ng/kg/day	EDI [b] 25 g mouse pg/day	EDI [c] 25 g mouse 5000× ng/day	EDI [d] 25 g mouse 100,000× ng/day	Feed measured [e] 5000× ng/g feed	Feed measured [f] 100,000× ng/g feed	EDI [g] 25 g mouse 5000× ng/day	EDI [h] 25 g mouse 100,000× ng/day
Chlorinated									
PCB 28	10	0.14	3.5	18	350	3.1	46	9	138
PCB 52	23	0.33	8.3	41	825	15.0	182	45	546
PCB 101	39	0.56	14.0	70	1400	25.4	377	76	1131
PCB 118	68	0.97	24.3	121	2425	37.2	612	112	1836
PCB 138	97	1.38	34.5	173	3450	53.8	957	161	2871
PCB 153	97	1.38	34.5	173	3450	61.4	981	184	2943
PCB 180	26	0.37	9.3	46	925	17.4	263	52	789
∑ PCBs	360	5.13	128.4	642	12,825	213.3	3418	640	10,254
p,p'-DDE	201	2.87	71.8	359	7175	136.0	2390	408	7170
HCB	84	1.20	30.0	150	3000	37.4	588	112	1764
α-Chlordane	63	0.90	22.5	113	2250	45.0	723	135	2169
Oxychlordane	21	0.30	7.5	38	750	9.8	297	29	891
trans-Nonachlor	21	0.30	7.5	38	750	14.9	264	45	792
α-HCH	36	0.52	13.0	65	1300	21.2	421	64	1263
β-HCH	29	0.42	10.5	53	1050	22.3	398	67	1194
γ-HCH (Lindane)	40	0.57	14.3	71	1425	31.4	435	94	1305
Dieldrin	126	1.80	45.0	225	4500	70.4	1470	211	4410
∑ OCPs	621	8.88	222.1	1112	22,200	388.4	6986	1165	20,958
∑ PCBs + OCPs	981	14.01	350.5	1754	35,025	601.7	10,404	1805	31,212
Brominated									
PBDE 47	68	0.97	24.3	121	2425	39.7	642	119	1926
PBDE 99	13	0.19	4.8	24	475	8.6	126	26	378
PBDE 100	11	0.15	3.8	19	375	5.6	91	17	272
PBDE 153	2	0.03	0.8	4	75	1.5	22	5	67
PBDE 154	4	0.06	1.5	8	150	2.8	38	8	114
PBDE 209	105	1.50	37.5	188	3750	64.8	1141	194	3423
HBCD	21	0.30	7.5	38	750	9.9	203	30	609
∑ BFRs	224	3.2	80.2	402	8000	132.9	2263	399	6789
Perfluorinated									
PFHxS	1.2	0.017	0.4	2	43	1.7	42	5	125
PFOS	18	0.26	6.5	33	650	3.2	74	10	222
PFOA	31	0.44	11.0	55	1100	6.0	121	18	363
PFNA	9.5	0.14	3.5	18	350	2.1	42	6	127
PFDA	13	0.19	4.8	24	475	3.1	57	9	172
PFUnDA	6.7	0.096	2.4	12	240	1.6	28	5	84
∑ PFAAs	79.4	1.14	28.6	144	2858	17.7	364	53	1094

Abbreviations: PCBs (polychlorinated biphenyls); OCPs (organochlorine pesticides); BFRs (brominated flame retardants); PFAAs (perfluoroalkyl acids).
[a] Average EDI (Estimated daily intake) values of POPs for a 70 kg human – based on a literature review of Scandinavian EDI values (Berntsen et al., 2017).
[b] EDI values for a 25 g mouse corresponding to human EDI values.
[c] EDI values for a 25 g mouse corresponding to human EDI values * 5000
[d] EDI values for a 25 g mouse corresponding to human EDI values * 100,000.
[e] Measured concentrations of the various compounds in the 5000× feed.
[f] Measured concentrations of the various compounds in the 100,000× feed.
[g] EDI values for a 25 g mouse consuming 3 g of the 5000× feed/day – based on concentrations measured in the feed of the current project.
[h] EDI values for a 25 g mouse consuming 3 g of the 100,000× feed/day – based on concentrations measured in the feed of the current project.

Table 2
Plasma levels of persistent organic pollutants in ng/g wet weight (ng/g ww) measured in mothers and pups of the control, low, and high exposed groups (white columns). Measured levels expressed relative to average human blood levels (ng/g ww) in the Scandinavian population (Berntsen et al., 2017) are also included (grey columns). Adapted from Berntsen et al. (in prep).

Compound	Human ng/g ww	Control				Low				High			
		Mother ng/g ww	×Human levels	Pup ng/g ww	×Human levels	Mother ng/g ww	×Human levels	Pup ng/g ww	×Human levels	Mother ng/g ww	×Human levels	Pup ng/g ww	×Human levels
Chlorinated													
PCB 28	0.013	n.d.	N/A	n.d.	N/A	n.d.	N/A	n.d.	N/A	n.d.	N/A	n.d.	N/A
PCB 52	0.010	n.d.	N/A	n.d.	N/A	1.1	108	0.12	12	4.7	472	2.8	281
PCB 101	0.008	n.d.	N/A	n.d.	N/A	1.2	147	n.d.	N/A	2.4	295	n.d.	N/A
PCB 118	0.064	0.07	1.1	0.3	4.4	2.5	39	0.4	6	42	658	11	185
PCB 138	0.222	0.08	0.4	1.2	5.2	12	54	4.0	18	91	409	50	224
PCB 153	0.362	0.13	0.3	1.2	3.2	7.4	21	2.9	8	87	241	49	135
PCB 180	0.194	0.03	0.2	0.3	1.7	2.7	14	0.8	4	24	126	14	72
p,p'-DDE	0.502	n.d.	N/A	n.d.	N/A	5.3	11	0.3	0.6	51	102	1.7	3
HCB	0.117	0.09	0.8	0.3	2.3	2.9	25	0.8	6	41	347	9.5	81
α-Chlordane	0.011	n.d.	N/A	n.d.	N/A	n.d.	N/A	n.d.	N/A	n.d.	N/A	n.d.	N/A
Oxychlordane	0.022	n.d.	N/A	n.d.	N/A	1.6	72	1.3	58	22	978	14	621
trans-Nonachlor	0.041	n.d.	N/A	n.d.	N/A	1.1	26	0.4	9	9.8	240	6.2	152
α-HCH	0.006	n.d.	N/A	n.d.	N/A	0.2	35	n.d.	N/A	n.d.	N/A	n.d.	N/A
β-HCH	0.053	n.d.	N/A	n.d.	N/A	1.5	29	0.15	3	14	272	10	189
γ-HCH (Lindane)	0.006	n.d.	N/A	n.d.	N/A	n.d.	N/A	n.d.	N/A	n.d.	N/A	n.d.	N/A
Dieldrin	0.024	n.d.	N/A	n.d.	N/A	13	537	1.4	56	50	2100	17	701
Brominated													
BDE 47	0.009	n.d.	N/A	n.d.	N/A	1.2	137	n.d.	N/A	11	1196	n.d.	N/A
BDE 99	0.004	n.d.	N/A	n.d.	N/A	0.5	124	n.d.	N/A	3.8	952	n.d.	N/A
BDE 100	0.002	n.d.	N/A	n.d.	N/A	0.5	263	0.09	45	3.8	1903	0.6	305
BDE 153	0.010	n.d.	N/A	n.d.	N/A	0.18	18	n.d.	N/A	2.5	249	1.1	110
BDE 154	0.002	n.d.	N/A	n.d.	N/A	0.3	132	n.d.	N/A	0.9	460	2.0	976
BDE 209	0.011	n.d.	N/A	n.d.	N/A	4.9	444	n.d.	N/A	26	2374	n.d.	N/A
HBCD	0.025	n.d.	N/A	n.d.	N/A	n.d.	N/A	n.d.	N/A	n.d.	N/A	n.d.	N/A
Perfluorinated													
PFHxS	3.45	n.d.	N/A	6.4	1.9	161	47	16	5	3381	994	317	93
PFOS	29.43	0.89	0.03	16	0.5	223	8	29	1	3403	116	635	22
PFOA	4.52	0.53	0.12	12	2.5	345	76	26	6	6980	1543	598	132
PFNA	0.80	0.32	0.4	13	16	176	220	24	29	2531	3164	470	588
PFDA	0.50	0.25	0.5	14	29	246	497	28	56	2580	5212	503	1016
PFUnDA	0.56	0.16	0.3	3.2	5.6	63	113	6.2	11	724	1293	114	204

n.d. = Not detected.
N/A = Not applicable.

and to limit the amount of phytoestrogens, suspected to interfere with hormone homeostasis, and known to be present in soy-based food (Berntsen et al., 2017).

2.2. Animal model

In total, 10 male C57BL/6J mice and 20 female 129S1/SvImJ (Jackson Laboratory, Maine, USA) were used for breeding. The mice were newly sexually mature and were acclimated to the unit for 1 week before mating started. In total, 110 hybrid pups were born (129:C57BL/6F0, $n = 63$ males and 47 females). Of these, the 47 females were assigned to one of three groups, the control, a low dose (5000 × EDI POP mixture), or a high dose (100 000 × EDI POP mixture), and given their respective diet from weaning prior to their use as breeders of the F1 generation. In total, the F1 generation consisted of 320 pups (129:C57BL/6F1, $n = 163$ males and 157 females). At weaning, 18 males and 18 females from each respective group ($n = 36$/group) were assigned to the open field test. The remaining pups were assigned to a later project. Throughout, the assignment of animals to housing, exposure groups, and testing groups was done randomly, either by simple lottery or by computer random numbering.

2.3. Housing and husbandry

All animals were housed in open type III cages (Tecniplast, Buguggiate, Italy) in group housing. All cages contained standard aspen bedding (Scanbur BK, Nittedal, Norway) and cellulose nesting material. The animals had free access to their assigned feed. Tap water was available from standard drinking bottles (Tecniplast, Buguggiate, Italy). The animal room was on a 12:12 light–dark cycle, with a room temperature of 21 ± 2 °C with 20 air changes per hour and 45 ± 5% relative humidity. The cages, bedding, nesting material, and water bottles were changed once a week, when animals were not tested. At weaning, the F0 females were randomly assigned to one of three exposure groups: High ($n = 16$ females), low ($n = 16$ females), and control ($n = 15$ females). All females were given their assigned feed from weaning and throughout the project (approx. 6 months). The F0 generation females were housed in groups of four from weaning until sexual maturity (marked with simple ear punch holes from 1 to 4), then caged with the F0 generation males (non-brothers) in triplets (1 male and 2 females) for one week. One week before predicted birth, they were single housed until their pups were weaned. At weaning, the F1 pups were randomly assigned to a same sex and exposure group cage/housing group of two, where one was marked with a microtransponder. The F1 generation were fed the reference diet after weaning. Feed intake was recorded for one week prior to necropsy and not found to be affected by exposure (data not shown). Similarly, body weights were assessed at weaning and necropsy and not found to be significantly affected by exposure in either the mothers or pups. No mice died or showed clinical signs of disease during the experiment, but 7 males (3 from the control group and 4 from the high exposure) were euthanized due to wounds attained through fighting. These 7 euthanized males occurred at weaning and were replaced with naïve males from their respective groups (the

mothers had undergone exactly the same exposure protocol, but had been housed in a different room).

2.4. Open field test

All testing (open field and HPA axis responsivity test) was done by 3 researchers with FELASA C credentials. Each person had assigned specific responsibilities during the testing and this was kept constant throughout the testing period to ensure consistency and elimination of deviations between experiments.

Both the F0 mothers and their offspring were tested. The open field test was performed during the animals' dark cycle, between 20:00 and 03:00. Four testing boxes (each 50 × 50 × 22 cm) (Noldus, Wageningen, the Netherlands) were used. Each box was divided into three zones, a center zone, corners, and a border zone. Four mice were tracked at the same time using EthoVision 9 (Noldus). The animal was placed inside a disposable non-transparent cardboard cylinder (guinea pig play tunnel from Scanbur BK, Nittedal, Norway) in the center of the arena and left there for 3 s. When released from the cylinder, each mouse was tracked for 15 min. Different endpoints were evaluated: the time spent within the different zones, the total distance moved, and velocity. Rearing (exploratory behaviour) and grooming were tracked manually. Following the behavioural test, the number of urine puddles and faecal pellets was recorded. The testing arena boxes were thoroughly washed with water and dried with paper towels between each animal. Male mice were tested before female mice, and all animals underwent testing only once. Background light intensity was around 1600 lx (TES Light Meter 1337, Presisjons Teknikk AS, Oslo, Norway) and background noise was stable at 32 dB (Castle GA 112 sound level meter, Presisjons Teknikk AS, Oslo).

2.5. HPA axis responsivity test

All F0 mothers and the offspring tracked in the open field test underwent the HPA axis responsivity test. The test was done inside the housing room to eliminate transport stress. The mouse was picked up, scanned for its micro-transponder, and then restrained inside a 50 ml Falcon tube for 15 min. Blood samples were taken from the tip of the tail at 4 different time points: 0, 15, 30, and 120 min. The mouse was returned to a home-cage after the 15 min time point and retrieved when needed for sampling. All samples were taken with a 20 μL Minivette POCT capillary collecting tube coated with EDTA (Sarstedt, Ski, Norway) and transferred to an eppendorf tube on ice. The blood samples were spun at 5000 rpm at 4 °C for 10 min to obtain the plasma, which was stored at −80 °C until further analyses. Corticosterone was measured in the plasma using an MP Biomedicals ImmuChem ™ Double Antibody Corticosterone ^{125} Ria Kit (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturers' instructions.

2.6. Statistical analyses

For mothers, we analysed differences in basal levels of corticosterone between levels of exposure using a linear regression model. Basal levels of corticosterone in pups were analysed against exposure levels, stratified by sex, using linear mixed effect models allowing for random intercepts for mother.

We also used linear regressions to model changes in the levels of corticosterone over time. Due to clear peaks in corticosterone levels at 30 min, we first analysed the change in corticosterone levels between 0 to 30 min and 30–120 min against exposure levels. These analyses were carried out in mothers and pups separately. For mothers, the level of exposure and time were the explanatory variables, while for pups we also studied the effects of sex along with exposure. To analyse longitudinal trends in corticosterone levels across exposure, we used longitudinal mixed effect models with a piecewise linear structure of the population average corticosterone levels. The piecewise linear model was preferred due to increasing corticosterone levels from 0 to 30 min and decreasing levels from 30 to 120 min. Repeated measurements of corticosterone within each mouse implies a possibility of within individual variability, which is modelled using an autoregressive correlation structure of the order one over time. In all raw profiles, we observed a variation in the starting levels of corticosterone. Further, in the case of pups, we observed a greater inter-individual variability in female mice. To account for these observations, we included a random intercept (for both mother and pup models) and a random slope for sex (in the pups model) in the mixed effect longitudinal models. As a sensitivity analysis, we repeated the longitudinal modelling stratified by sex in the pups, including exposure as the variable of interest and a random intercept for each mouse.

We also analysed the data from the open field experiments using univariate and multivariate linear regression models with sex, exposure, and the interaction between sex and exposure as the

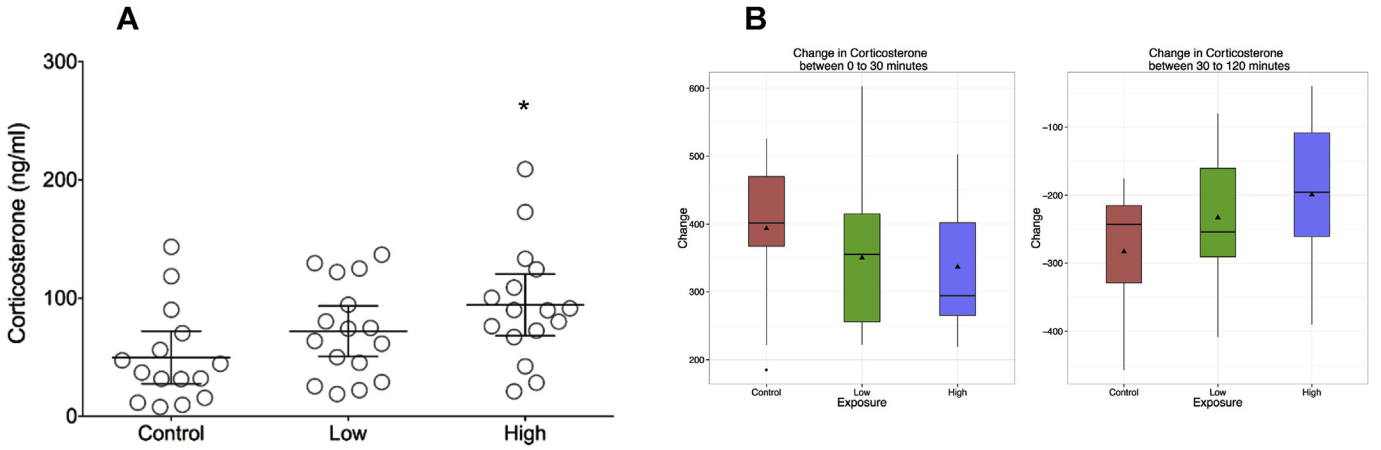

Fig. 1. Plasma corticosterone in mice mothers exposed to a mixture of persistent organic pollutants. (A) Basal corticosterone and (B) corticosterone dynamics following stress. In (A), data includes mean ± 95% CI and an asterisk represents a significant exposure effect compared to controls (Linear model, $p = .007$). In (B), each box represents the first, second, and third quartile, the upper whisker is the third quartile plus 1.5 × the interquartile range (IQR) and the lower whisker is the first quartile minus 1.5 × IQR. There was a significant effect of the high exposure between 30 and 120 min compared to controls ($p = <0.05$, see Table 1).

Table 3
Results of modelling maternal corticosterone changes over time (0–30 and 30–120 min) following exposure to two doses (high and low) of a mixture of persistent organic pollutants using linear regression models.

	Dependent variable:	
	Change in Corticosterone (0–30) (1)	Change in Corticosterone (30–120) (2)
Low Exposure	−43.775	50.437
	(36.884)	(34.744)
High Exposure	−57.018	84.077**
	(36.884)	(34.744)
Constant	393.547***	−283.381***
	(26.081)	(24.568)
Observations	45	45
R2	0.059	0.124
Adjusted R2	0.014	0.082
Residual Std. Error (df = 42)	101.010	95.152
F Statistic (df = 2; 42)	1.309	2.967*

Note: *p < .1; **p < .05; ***p < .01.

Table 4
Estimated parameters from the longitudinal mixed effects model for the mother and pups exposed to two doses (high and low) of a mixture of persistent organic pollutants. Sex (male vs female) and time (0–30 vs 30–120 min) were also within the model.

	Mother levels	Pup levels
Low Exposure	10.96	−31.54
	(17.57)	(19.34)
High Exposure	30.59	−14.95
	(17.57)	(17.92)
Time	12.00***	9.55***
	(0.68)	(0.29)
(Time - 30)+	−15.22***	−12.03***
	(0.81)	(0.35)
Male		−118.80***
		(14.36)
Low Exposed Male		45.69*
		(22.28)
High Exposed Male		47.64*
		(20.75)
Intercept	109.18***	129.87***
	(16.56)	(13.43)
AIC	2145.67	4863.83
BIC	2170.99	4928.48
Log Likelihood	−1064.84	−2415.92
Num. obs.	180	428
Num. groups	45	107

***p < .001, **p < .01, *p < .05.

explanatory variables. Frequency at particular locations (borders, centres, and corners) and cumulative duration at those locations were treated as bivariate outcomes in each multivariate regression. In addition, frequency of grooming and cumulative duration of grooming was also treated as a bivariate outcome. Univariate regressions were carried out with two responses, i) the ratio of time spent moving against not moving, and ii) the ratio of time spent grooming against rearing. Multivariate and univariate ANOVA was used to determine the significance of the factors and a *p*-value of <0.05 was treated as a significant finding. All statistical analyses were carried out in R statistical software (Version 3.2.1., R Development Core Team, http://www.r-project.org) and the R scripts can be found in the supplementary material.

3. Results

3.1. Corticosterone

High exposed mothers had significantly higher basal corticosterone levels than controls, whereas the low exposure showed only a trend for higher values than controls (Fig. 1A). When modelling changes in corticosterone following stress, we found a significant effect (Table 3) of the high exposure between 30 and 120 min, as the controls showed a greater level of change during this period compared to the high exposed group, with the low exposed mothers having an intermediate value (Fig. 1B). When modelling the absolute values across the whole experiment, there was no effect of any exposure (Table 4).

There was no effect of exposure on basal cortisol levels in either male or female pups (Fig. 2A). When modelling changes in corticosterone following stress, there was a significant interaction between sex and exposure. Here, high exposed males showed a significantly greater increase in corticosterone between 0 and 30 min, but also a greater decrease between 30 and 120 min, than controls (Table 5), whereas the opposite non-significant pattern was observed in females (Fig. 2B). When modelling the absolute values of corticosterone across the whole experiment, there was a clear dose response in males with both the low and high exposed groups having significantly greater values than controls, but no such trends were observed in females (Table).

3.2. Behaviour

There was no effect of exposure on any behavioural endpoint (see supplementary Figs. 1-14). There was a significant effect of sex on the bivariate outcome comprised of frequency of grooming and cumulative duration of grooming (Pillai's multivariate F-statistic, p value = .007). Both frequency of grooming and cumulative time spent in grooming were higher in female mice. In addition, there was also a significant sex effect on the ratio of the duration of grooming against rearing, with male mice spending 1.79 (95% CI = 1.06, 3.06, p = .03) times more on grooming than rearing compared to female mice. The results are summarised in supplementary Tables 1-3.

4. Discussion

The stress response in mice exposed to a human based mixture of POPs was assessed in mothers and their offspring. We found evidence that POP exposure can influence corticosterone dynamics in mothers and their offspring. However, the effects on the HPA axis were sex specific and were not evident in measures of anxiety-like behaviour. These results have important implications on neurological development in both humans and wildlife.

We found that exposed mothers had elevated basal levels and a prolonged period of corticosterone elevation following stress compared to controls. This conforms to both ecotoxicological and laboratory studies that have found associations between stress hormones and POPs across taxa (i.e. Gendron et al., 1997; Jørgensen et al., 2002; Oskam et al., 2004; Franceschini et al., 2008; Pereiro et al., 2014). However, the direction of change is not consistent between studies. For example, basal cortisol was elevated in Arctic char (Jørgensen et al., 2002) and guinea pigs (Kato et al., 1981), but decreased in polar bears (Oskam et al., 2004) and rats (Pereiro et al., 2014). Similarly, POPs have been found to either blunt (Hontela et al., 1992; Jørgensen et al., 2002; Verboven et al., 2010) or oversensitise (Zimmer et al., 2009) the stress response. These inconsistencies may be explained by the POPs studied. For example, *in vitro*, PCBs 118 and 126 were found to induce cortisol production in human H295R adrenal cells, but not PCB 153 (Kraugerud et al., 2010) or perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), or perfluorononanoic acid (PFNA) (Kraugerud et al., 2011). Similarly, a study in wild polar bears found cortisol to be

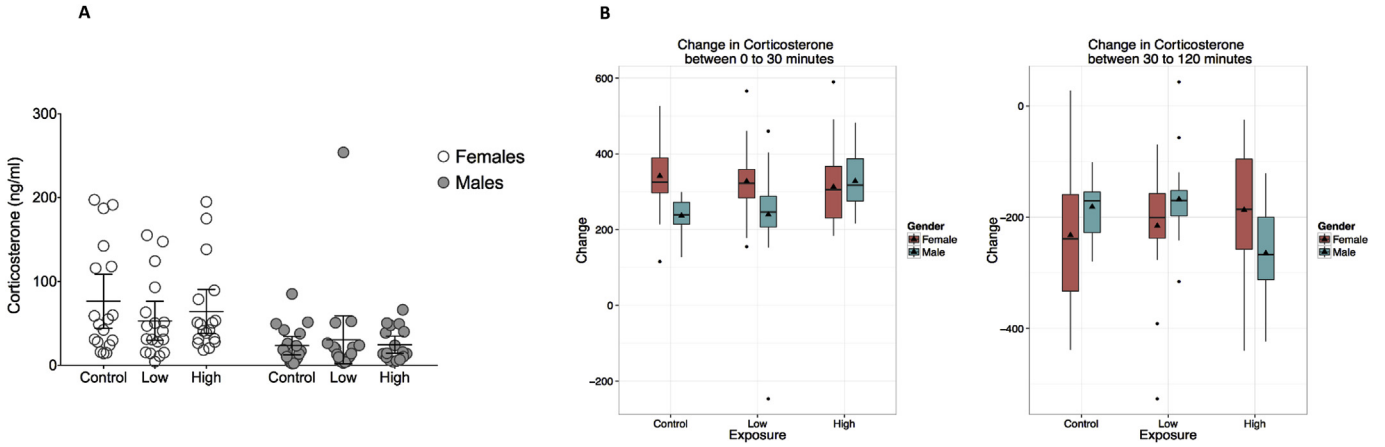

Fig. 2. Plasma corticosterone in pups exposed to a mixture of persistent organic pollutants via their mothers. (A) Basal corticosterone and (B) corticosterone dynamics following stress. In (A), data are means ± 95% CI. In (B), each box represents the first, second, and third quartile, the upper whisker is the third quartile plus 1.5 × the interquartile range (IQR) and the lower whisker is the first quartile minus 1.5 × IQR. There was a significant interaction between exposure and gender between 0-30 and 30–120 min ($p = <0.05$, see Table 3).

Table 5
Results of modelling corticosterone changes using linear regression models in pups exposed to two doses (high and low) of a mixture of persistent organic pollutants. Sex (male vs female) and time (0–30 and 30–120 min) were also included within the model.

	Dependent variable:	
	Change in Corticosterone (0–30) (1)	Change in Corticosterone (30–120) (2)
Low Exposure	−14.224	16.800
	(33.429)	(32.381)
High Exposure	−29.099	45.426
	(32.986)	(31.952)
Male	−104.687***	50.961
	(33.429)	(32.381)
Low Exposure and Male	17.037	−3.066
	(47.276)	(45.794)
High Exposure and Male	120.051**	−128.582***
	(47.702)	(46.206)
Intercept	341.493***	−232.442***
	(23.638)	(22.897)
Observations	107	107
R2	0.162	0.106
Adjusted R2	0.120	0.062
Residual Std. Error (df = 101)	100.287	97.143
F Statistic (df = 5; 101)	3.902***	2.399**

Note: *p < .1; **p < .05; ***p < .01.

negatively associated with PBDE 99 and 153, as well as PCB 170, 180, 190 and 201, but positively associated with PCB 66/95, α-HCH, dieldrin, PBDE 47, and para, para'-dichlorodiphenyldichloroethane (*p,p'*- DDD) (Bechshøft et al., 2012). Environmental factors may also influence the response to POPs, as Jørgensen et al. (2002) found that the PCB mixture Aroclor 1254 suppressed basal cortisol in food-deprived fish, but elevated cortisol in fed fish. Therefore, it appears the effect of POPs on the HPA axis is dependent on the mixture composition, most likely because different POPs will have different mechanistic pathways, and environmental factors.

Although they did not receive POP containing food, sex specific effects on the stress response were found in the offspring. This demonstrates the risk to offspring of POP exposure via mothers. Although POPs were found in brain tissue from both mothers and pups (Berntsen et al. in prep), we cannot conclude whether the effects in the offspring were due to direct effects of the POPs transferred via the mother or due to secondary physiological effects of exposure in the mothers. For example, POP exposure can have a wide-ranging effect on many physiological parameters, including the thyroid axis (Gilbert et al., 2012), sex steroids (Lilienthal et al., 2006), and metabolism (Swedenborg et al., 2009) that are important for neurological development in offspring. These systems were not investigated in the current study, but in the mothers, we did see alterations in corticosterone dynamics. Here, cortisol is known to regulate neurodevelopment and alterations in stress hormones during pregnancy can have long lasting effects on the levels of anxiety (Davis and Sandman, 2010) and lead to dysregulation of the HPA axis in children (Davis et al., 2011). The current sex effect on the stress response is not unexpected, as comparable results on the stress response following POP exposure have been found in other animal models (Zimmer et al., 2009; Verboven et al., 2010). Why male pups were found to have an over-sensitised stress response compared to females is currently unknown. However, previous work has demonstrated that gonadal hormones influence development of the HPA axis (reviewed in Bale and Epperson, 2015), POPs are known to interfere with testosterone production in male mice (Kaya et al., 2002), and we found effects of our POP mixture on sperm quality in a sub sample of the pups from the current experiment (Khezri et al., 2017a).

Alterations in stress levels are commonly linked with changes in behaviour, such as anxiety (Cohan et al., 2006). However, in the current study we observed no effect of POP exposure on behavioural endpoints of anxiety in mothers or offspring in the open field test. For the mothers, in which we observed alterations in basal corticosterone, this may appear contradictory to the expected. However, Cohan et al. (2006) reported that rats showing higher levels of anxiety-like behaviour did not have higher basal corticosterone compared to comparative groups. Therefore, increased basal cortisol may not necessarily be associated with increased anxiety-like behaviour. However, in the future it may be beneficial to include behavioural tests that involve the activation of the stress response to gain a more detailed insight into the link between the observed changes in the HPA axis following POP exposure and potential modification of behaviour.

High exposed mothers were found to have elevated basal corticosterone and a prolonged elevation of corticosterone following stress. This could be due to one of several possibilities. For instance, the burden of chemicals may have continuously stimulated a stress response, the feedback mechanism to reduce elevated corticosterone may have been impaired, or maybe the mothers were less capable of metabolising corticosterone. Here, we note

that in a separate experiment on CD1 mothers and their pups, exposed to the same concentrations of the POP mixture used in the current study, there were transient effects on the liver, including an early increase in relative liver size, alterations in liver morphology (centralobular hypertrophy), and the induction of detoxification enzyme activity (CYP1A1, CYP1A, CYP3A, CYP2B, CYP2E1, CYP2A) in the offspring, which disappeared over time (unpublished data). This suggests the doses used impacted on liver morphology and physiology, but were not pathological in the offspring, although the mothers remained untested. Liver endpoints were not assessed in the current study, but would be of interest with regards to the ability of the liver to metabolise corticosterone.

We find evidence of HPA dysregulation in both the mothers and their male offspring exposed to the high dose, and male pups exposed to the low dose, of our POP mixture. The implication of this finding is unclear, but such dysregulation has previously been associated with neurological disorders. For example, in laboratory studies rats with a blunted stress response were found to show more extreme responses to a stressor, similar to post traumatic stress disorder (Cohan et al., 2006). Furthermore, elevated levels of glucocorticoids have negative effects on the cognitive abilities of animals, causing neuronal cell death and reducing neurogenesis (Sapolsky et al., 2000). In humans, elevated or decreased basal cortisol levels have been associated with post-traumatic stress disorder (Yuhuda et al., 1990; Bremner et al., 1997) and dissociative disorders (Simeon et al., 2001), whereas a hypersensitive stress response has been associated with panic disorder (Abelson et al., 2006), and both an elevated and blunted cortisol response has been associated with depression (Burke et al., 2005; Van den Bergh et al., 2008). Therefore, further work should assess whether exposure to our POP mixture can lead to other neurological disorders in mice and other vertebrate models.

We used a POP mixture based on human dietary intake levels in a mouse model. Based on tissue analysis, although mice were fed 5000 × and 100 000 × the estimated daily intake of humans, the levels within the plasma, adipose tissue, and brain were in some instances comparable to the levels of POPs found in humans and wildlife (Berntsen et al. in prep). For example, compounds detected in the plasma of the low dose group of pups ranged from 0,6 (para, para'-dichlorodiphenyldichloroethylene [*p,p'*-DDE]) to 58 (perfluorodecanoic acid [PFDA]) times the levels in human blood (Berntsen et al. in prep). Similarly, concentrations of organochlorines measured in autopsy tissue from Greenland (Dewailly et al., 1999) were in between the pup and the mother brain levels of the low dose group of the present study when lipid adjusted levels were compared. Therefore, the effects on corticosterone HPA dysregulation observed in the male pups exposed to the low dose in the current study is a concern for human safety. As such, further work is required to understand which compounds are acting upon the HPA axis, and whether the effects of these compounds are mediated by the presence of other compounds similar to that seen in other species (i.e. Oskam et al., 2004; Bechshøft et al., 2012). Of note, we recently tested the effect of a second POP mixture on larval zebrafish behaviour that contained the exact same 29 compounds as used in the current study, but the concentrations were based on human blood levels rather than the estimated dietary intake (see Berntsen et al., 2017). Nevertheless, we found this alternative POP mixture could influence anxiety-like behaviour in larval zebrafish (Khezri et al., 2017b). This observed effect was found to be mimicked by the perfluorinated fraction of the mixture, and of these six compounds, only PFOS could mimic the effect. Therefore, it would be interesting to see whether PFOS itself can mimic the effects on HPA regulation we see in the present study when using the total mixture. Especially as PFOS was the dominating perfluorinated congener measured in brain tissue from the current experiment (Berntsen et al. in prep).

In conclusion, a human based POP mixture dysregulated the HPA axis in female mice and their male offspring. As HPA dysregulation is associated with neurological disease in humans, further work should be carried out to determine which compounds within the POP mixture are acting upon the HPA axis and the risk to humans.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2018.01.085.

References

Abelson, J.L., Khan, S., Liberzon, I., Young, E.A., 2006. HPA axis activity in patients with panic disorder: review and synthesis of four studies. Depress. Anxiety 24, 66–76.

Altenburger, R., Backhaus, T., Boedeker, W., Faust, M., Scholze, M., 2013. Simplifying complexity: mixture toxicity assessment in the last 20 years. Environ. Toxicol. Chem. 32, 1685–1687.

Bale, T.L., Epperson, C.N., 2015. Sex differences and stress across the lifespan. Nat. Neurosci. 18, 1413–1420.

Bechshøft, T.Ø., Sonne, C., Dietz, R., Born, E.W., Muir, D.C.G., Letcher, R.J., Novak, M.A., Henchey, E., Meyer, J.S., Jenssen, B.M., Villanger, G.D., 2012. Associations between complex OHC mixtures and thyroid and cortisol hormone levels in East Greenland polar bears. Environ. Res. 116, 26–35.

Berghuis, S.A., Bos, A.F., Sauer, P.J.J., Roze, E., 2015. Developmental neurotoxicity of persistent organic pollutants: an update on childhood outcome. Arch. Toxicol. 89, 687–709.

Berntsen, H.F., Berg, V., Lyche, J.L., Ropstad, E., Zimmer, K.E., in prep. The design of an environmentally relevant mixture of POPs for use in *in vivo* studies: concentrations in female mice and their offspring.

Berntsen, H.F., Berg, V., Thomsen, C., Ropstad, E., Zimmer, K.E., 2017. The design of an environmentally relevant mixture of persistent organic pollutants for use in *in vivo* and *in vitro* studies. J. Toxicol. Environ. Health A. https://doi.org/10.1080/15287394.2017.1354439.

Bremner, J.D., Licinio, J., Darnell, A., Krystal, J.H., Owens, M.J., Southwick, S.M., Nemeroff, C.B., Charney, D.S., 1997. Elevated CSF corticotropin-releasing factor concentrations in posttraumatic stress disorder. Am. J. Psychohist. 154, 624–629.

Burke, H.M., Davis, M.C., Otte, C., Mohr, D.C., 2005. Depression and cortisol responses to psychological stress: a meta-analysis. Psychoneuroendocrinology 30, 846–856.

Carpenter, D.O., 2006. Polychlorinated biphenyls (PCBs): routes of exposure and effects on human health. Rev. Environ. Health 21, 1–23.

Cohan, H., Zohar, J., Gidron, Y., Matar, M.A., Belkind, D., Loewenthal, U., Kozlovsky, N., Kaplan, Z., 2006. Blunted HPA axis response to stress influences susceptibility to posttraumatic stress response in rats. Biol. Psychiatr. 59, 1208–1218.

Costopoulou, D., Vassiliadou, I., Papadopoulos, A., Makropoulos, V., Leondiadis, L., 2006. Levels of dioxins, furans and PCBs in human serum and milk of people living in Greece. Chemosphere 65, 1462–1469.

Davis, E.P., Sandman, C.A., 2010. The timing of prenatal exposure to maternal cortisol and psychosocial stress is associated with human infant cognitive development. Child Dev. 81, 131–148.

Davis, E.P., Waffarn, F., Sandman, C.A., 2011. Prenatal treatment with glucocorticoids sensitizes the HPA axis response to stress among full-term infants. Dev. Psychobiol. 53, 175–183.

Dewailly, E., Mulvad, G., Pedersen, H.S., Ayotte, P., Demers, A., Weber, J.P., Hansen, J.C., 1999. Concentration of organochlorines in human brain, liver, and adipose tissue autopsy samples from Greenland. Environ. Health Perspect. 107, 823–828.

Franceschini, M.D., Custer, C.M., Custer, T.W., Reed, J.M., Romero, M., 2008. Corticosterone stress response in tree swallows nesting near polychlorinated biphenyl- and dioxin-contaminated rivers. Environ. Toxicol. Chem. 27, 2326–2331.

Frye, C., Bo, E., Calamandrei, G., Calzà, L., Dessì-Fulgheri, F., Fernàndez, M., Fusani, L.,

Kah, O., Kajta, M., Le Page, Y., Patisaul, H.B., Venerosi, A., Wojtowicj, A.K., Panzica, G.C., 2012. Endocrine disruptors: a review of some sources, effects, and mechanisms of actions on behaviour and neuroendocrine systems. J. Neuroendocrinol. 24, 144–159.
Gendron, A.D., Bishop, C.A., Fortin, R., Hontela, A., 1997. In vivo testing of the functional integrity of the corticosterone-producing axis in mudpuppy (Amphibia) exposed to chlorinated hydrocarbons in the wild. Environ. Toxicol. Chem. 16, 1694–1706.
Gilbert, M.E., Rovet, J., Chen, Z., Koibuchi, N., 2012. Developmental thyroid hormone disruption: prevalence, environmental contaminants and neurodevelopmental consequences. Neurotoxicology 33, 842–852.
Glover, V., O'Connor, T.G., O'Donnell, K., 2010. Prenatal stress and the programming of the HPA axis. Neurosci. Biobehav. Rev. 35, 17–22.
Gluckman, P.D., Hanson, M.A., 2004. Living with the past: evolution, development, and patterns of disease. Science 305, 1733–1736.
Harris, A., Seckl, J., 2011. Glucocorticoids, prenatal stress and the programming of disease. Horm. Behav. 59, 279–289.
Hontela, A., Rasmussen, J.B., Audet, C., Chevalier, G., 1992. Impaired cortisol stress response in fish from environments polluted by PAHs, PCBs, and mercury. Arch. Environ. Contam. Toxicol 22, 278–283.
Jones, K.C., de Voogt, P., 1999. Persistent organic pollutants (POPs): State of the science, vol. 100, pp. 209–221.
Jørgensen, E.H., Vijayan, M.M., Aluru, N., Maule, A.G., 2002. Fasting modifies Aroclor 1254 impact on plasma cortisol, glucose and lactate responses to a handling disturbance in Arctic charr. Comp. Biochem. Physiol. C 132, 235–245.
Kato, N., Kawai, K., Yoshida, A., 1981. Effect of dietary level of ascorbic acid on the growth, hepatic lipid peroxidation, and serum lipids in Guinea pigs fed polychlorinated biphenyls. J. Nutr. 111, 1727–1733.
Kaya, H., Hany, J., Fastabend, A., Roth-Härer, A., Winneke, G., Lilienthal, H., 2002. Effects of maternal exposure to a reconstituted mixture of polychlorinated biphenyls on sex-dependent behaviors and steroid hormone concentrations in rats: dose-response relationship. Toxicol. Appl. Pharmacol. 178, 71–81.
Khezri, A., Fraser, T.W.K., Nourizadeh-Lillabadi, R., Kamstra, J.H., Berg, V., Zimmer, K.E., Ropstad, E., 2017b. A mixture of persistent organic pollutants and perfluorooctanesulfonic acid induces similar behavioural responses, but different gene expression profiles in zebrafish larvae. Int. J. Mol. Sci. 18, 291.
Khezri, A., Lindeman, B., Krogenæs, A.K., Berntsen, H.F., Zimmer, K.E., Ropstad, E., 2017a. Maternal exposure to a mixture of persistent organic pollutants (POPs) affects testis histology, epididymal sperm count and induces sperm DNA fragmentation in mice. Toxicol. Appl. Pharmacol. 329, 301–308.
Kraugerud, M., Zimmer, K.E., Dahl, E., Berg, V., Olsaker, I., Farstad, W., Ropstad, E., Verhaegen, S., 2010. Three structurally different polychlorinated biphenyl congeners (Pcb 118, 153, and 126) affect hormone production and gene expression in the human H295R in vitro model. J. Toxicol. Environ. Health Part A 73, 1122–1132.
Kraugerud, M., Zimmer, Z.E., Ropstad, E., Verhaegen, S., 2011. Perfluorinated compounds differentially affect steroidogenesis and viability in the human adrenocortical carcinoma (H295R) *in vitro* cell assay. Toxicol. Lett. 205, 62–68.
Law, R.J., Covaci, A., Harrad, S., Herzke, D., Abdallah, M.A.E., Fernie, K., Toms, L.M.L., Takigami, H., 2014. Levels and trends of PBDEs and HBCDs in the global environment: status at the end of 2012. Environ. Int. 65, 147–158.
Liggins, G.C., 1994. The role of cortisol in preparing the fetus for birth. Reprod. Fertil. Dev. 6, 141–150.
Lilienthal, H., Hack, A., Roth-Härer, A., Grande, S.W., Talsness, C.E., 2006. Effects of developmental exposure to 2,2′,4,4′,5-pentabromodiphenyl ether (PBDE-99) on sex steroids, sexual development, and sexually dimorphic behavior in rats. Environ. Health Perspect. 114, 194–201.
Needham, L.L., Grandjean, P., Heinzow, B., Jørgensen, P.J., Nielsen, F., Patterson, D.G., Sjödin, A., Turner, W.E., Weihe, P., 2011. Partition of environmental chemicals between maternal and fetal blood and tissues. Environ. Sci. Technol. 45, 1121–1126.
Oskam, I., Ropstad, E., Lie, E., Derocher, A., Wiig, Ø., Dahl, E., Larsen, S., Skaare, J.U., 2004. Organochlorines affect the steroid hormone cortisol in free-ranging polar bears (*Ursus maritimus*) at Svalbard. Norway. J. Toxicol. Env. Health Part A 67, 959–977.
Pereiro, N., Moyano, R., Blanco, A., Lafuente, A., 2014. Regulation of corticosterone secretion is modified by PFOS exposure at different levels of the hypothalamic–pituitary–adrenal axis in adult male rats. Toxicol. Lett. 230, 252–262.
Ren, A., Qiu, X., Jin, L., Ma, J., Li, Z., Zhang, L., Zhu, H., Finnell, R.H., Zhu, T., 2011. Association of selected persistent organic pollutants in the placenta with the risk of neural tube defects. Proc. Natl. Acad. Sci. U.S.A 108, 12770–12775.
Sapolsky, R.M., Uno, H., Rebert, C.S., Finch, C.E., 2000. Hippocampal damage associated with prolonged glucocorticoid exposure in primates. J. Neurosci. 10, 2897–2902.
Simeon, D., Guralnik, O., Knutelska, M., Hollander, E., Schmeidler, J., 2001. Hypothalamic-pituitary-adrenal axis dysregulation in depersonalization disorder. Neuropsychopharmacology 25, 793–795.
Swedenborg, E., Rüegg, J., Mäkelä, S., Pongratz, I., 2009. Endocrine disruptive chemicals: mechanisms of action and involvement in metabolic disorders. J. Mol. Endocrinol. 43, 1–10.
Tartu, S., Angelier, F., Herzke, D., Moe, B., Bech, C., Gabrielsen, G.W., Bustnes, J.O., Chastel, O., 2014. The stress of being contaminated? Adrenocortical function and reproduction in relation to persistent organic pollutants in female black legged kittiwakes. Sci. Total Environ. 476–477, 553–560.
Van den Bergh, B.R.H., Calster, B.V., Smits, T., Huffel, S.V., Lagae, L., 2008. Antenatal maternal anxiety is related to HPA-axis dysregulation and self-reported depressive symptoms in adolescence: a prospective study on the fetal origins of depressed mood. Neuropsychopharmacology 33, 536–545.
Verboven, N., Verreault, J., Letcher, R.J., Gabrielsen, G.W., Evans, N.P., 2010. Adrenocortical function of Arctic-breeding glaucous gulls in relation to persistent organic pollutants. Gen. Comp. Endocrinol. 166, 25–32.
Walton, K., Dorne, J.L., Renwick, A.G., 2001. Uncertainty factors for chemical risk assessment: interspecies differences in the in vivo pharmacokinetics and metabolism of human CYP1A2 substrates. Food Chem. Toxicol. 39, 667–680.
Yuhuda, R., Southwick, S.M., Nussbaum, G.B.S., Wahby, V., Giller, E.L., Mason, J.W., 1990. Low urinary cortisol excretion in patients with posttraumatic stress disorder. J. Nerv. Ment. Dis. 178, 145–149.
Zimmer, K.E., Gutleb, A.C., Lyche, J.L., Dahl, E., Oskam, I.C., Krogenæs, A., Skaare, J.U., Ropstad, E., 2009. Altered stress-induced cortisol levels in goats exposed to polychlorinated biphenyls (PCB 126 and PCB 153) during fetal and postnatal development. J. Toxicol. Environ. Health Part A 72, 164–172.
Zimmer, K.E., Kraugerud, M., Aleksandersen, M., Gutleb, A.C., Østby, G.C., Dahl, E., Berg, V., Skaare, J.U., Olsaker, I., Ropstad, E., 2013. Fetal adrenal development: comparing effects of combined exposures to PCB 118 and PCB 153 in a sheep model. Environ. Toxicol. 28, 164–177.