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Determination of organic pollutants in meconium and its relationship with fetal growth. Case control study in Northwestern Spain

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Abstract

Objectives: Antenatal exposure to organic pollutants is a leading public health problem. Meconium is a unique matrix to perform prenatal studies because it enables us to retrospectively evaluate fetal exposure accumulated during the second and third trimester. The aim of the present study was to evaluate associations between organic pollutant levels in meconium and birth weight in NW Spain.

Methods: In this study, we quantify the concentrations of 50 organic pollutants together with the total values of the most important chemical groups in meconium using gas chromatography coupled to tandem mass spectrometry.

Results: Organochlorine pesticides, polychlorinated biphenyls and polybrominated diphenyl ethers were detected with the highest levels in meconium from small for gestational age newborns. It was estimated that several congeners were statistically significant ($p<0.05$). However, organophosphorus pesticides attained higher concentrations in newborns with an appropriate weight.

Conclusions: The occurrence of transplacental transfer can be confirmed. Prenatal exposure to organic pollutants was associated with a decrease in birth weight and, therefore, organic pollutants could have an impact on fetal growth. Nevertheless, these results need validation in larger sample sized studies.

Keywords: environmental pollutants; infant; meconium; prenatal exposure; prenatal exposure effects; small for gestational age.

Introduction

Fetal growth defect is defined by the impossibility to achieve intrauterine growth potential. This is usually due to placental insufficiency, normally of unknown origin. Interaction between environmental and genetic factors (fetal, placental or maternal) could be pointed out. For practical purposes, a child born "small for gestational age" (SGA) is defined as that newborn (NB) whose birth weight is less than the 10th percentile for gestational age and sex and according to data derived from an appropriate reference population [1].

This is the same definition as obstetricians apply to define a "small fetus for gestational age" [2]. SGA neonates are exposed to a higher risk of health problems not only during neonatal development but also during young adulthood, especially short stature, neurocognitive dysfunction, diabetes mellitus, hypertension and higher risk of cardiovascular disease [3].

Organic pollutants (OPs) are among possible etiological factors for SGA [4–7]. OPs are a set of lipophilic chemical products, most of them resistant to environmental breakdown that can bio-accumulate and biomagnify inside the food chain [8] or transfer to animals and humans. Some OPs may act as potent Endocrine Disrupting Chemicals defined as "a chemical agent or mixture of chemicals that interferes with any aspect of hormonal action" [9].

Some endocrine disruptors are highly lipophilic, such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), dioxins and organochlorinated pesticides (OCPs). These organic molecules are stored in adipose tissue and have very long half-lives ranging from

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months to several years. Different authors have demonstrated the ability of OPS to deposit in the placenta and pass to the fetus [4, 10, 11].

Meconium is the earliest fecal excretion of an NB. In 70% of cases meconium is expelled in the first 12 h of life. In term infants, 99% pass their first formed stool by 48 h [12, 13]. In situations of stress, some fetuses expel meconium into the amniotic fluid before birth but even in these NB most of the meconium remains in the bowel. Meconium is comprised of water, lipids, proteins, sterols and cholesterol precursors, free fatty acids and other products derived from swallowing amniotic fluid, epithelial cells, bile, lanugo and intestinal secretions [14–16].

OPs can deposit in the meconium by several routes [17]:
- Dissemination of chemicals transported by the blood.
- Swallowing of OPs excreted by the skin, kidneys and fetal defecation itself in the amniotic fluid.
- Excretion in the gastrointestinal tract by means of bile after liver metabolism or other intestinal secretions.

Different studies suggest that meconium is metabolically inert, such that once toxic substances attain this, they remain "fossilized" [10, 18]. Therefore, meconium is a unique matrix for the NB and enables a retrospective evaluation of fetal exposure to OPs during a broad window of detection in the second and third trimesters.

Meconium is a stable record of antenatal exposure and has been used previously to evaluate exposure to environmental toxic agents and maternal substance abuse [19, 20]. Their most important advantages [21, 22] are:
a) easily obtained as waste material;
b) generally large biological sample;
c) Meconium usually begins to form at the beginning of the second trimester.

To the best of our knowledge, this is the first study about the relationship between OPs and SGA using a non-invasive biological matrix as meconium.

Materials and methods

Study design

This is a case control study with prospective and retrospective data collection from the electronic Clinical History at the University Hospital of Ourense (Ourense – Latitude: 42°20′12″ N; Longitude: 7°51′50″ O-, Spain).

Fifty pregnant women with low obstetric risk were recruited between October and December 2017. They were given the opportunity to take part in the study by transferring their clinical data and donating the meconium expelled by their NB the first 48 h after birth to study environmental contaminants (Supplementary Table 1). They all signed the informed consent after receiving information. The initial cohort was split into two subgroups according to the NB's birth weight.

Case group: Pregnant women who accepted to participate and whose NB presented a birth weight lower than the 10th percentile according to the tables of Spanish neonatal weights for gestational age and sex.

Control group: Pregnant women who accepted to participate and whose NB presented at birth a weight greater/equal to the 10th percentile.

Exclusion criteria: Pregnant women aged under 18, pregnant women with pregestational chronic pathology (diabetes mellitus, chronic hypertension, conjunctive tissue diseases, etc.), twin gestations, pregnant women with an intrauterine diagnosis of fetal pathology (excluding growth abnormalities) and/or vertically transmitted infections and patients who did not agree to take part in the study after reading the informed consent.

Pontevedra-Vigo-Ourense Research Ethics Committe approved the study with registration number 2014/410. The Declaration of Helsinki on biomedical research was applied at all times.

Collection of samples

Meconium samples were collected immediately after deposition into a diaper in the first 48 h after NB birth. The samples were also coded and kept refrigerated until arrival at the laboratory in the University of Vigo. Afterwards, the samples were separate from the diaper, placed into 60 mL amber glass bottles, and frozen at –20 °C until analysis.

Extraction and detection methodologies

Meconium samples were processed following analytical procedures optimized by Fernández-Cruz et al. [8]. Briefly, 0.50 g of the homogenised meconium samples were spiked with 0.50 µg/kg of surrogate standards of each target family of chemicals (PCBs: PCB 14, PCB 65 and PCB 166; PBDEs: PBDE 77; PAHs: Chrysene-D12; OCPs: αHCH-D6, HCB-13C6, γ-HCH-D6; DDTs: DDE-D8 and OPPs: Chlorpyrifos-D10) (Supplementary Table 1, 2). Afterwards, the meconium samples were pre-treated by pulverization with 2.4 g of diatomaceous earth and extracted with acetonitrile in a SPLE (Selective Pressurized Liquid Extraction) at 100 °C, 100 bar, three cycles and 5.0 min of cycle. Prior the extraction, the 40 mL PLE (Pressurized Liquid Extraction) cells with cellulose filters (BÜCHI, Switzerland) were filled from bottom to top with two glass fibre filters, 10 g neutral silica and the pre-treated meconium, and finally one glass fibre filter on top. The final extract was reduced until dryness under a gentle nitrogen (analytical grade C-45 nitrogen, Carburos Metálicos, Vigo, Spain) stream using a TurboVap (LifeScience, Hopkinton, MA, USA). The dried extract was redisolved in 500 uL of acetonitrile (CHROMASOLV for HPLC≥99.9%) before the clean-up step. For such purpose, dual-layer EZ-POP SPE cartridges (Supelco, Bellefonte, PA) were conditioned with 20 mL of acetone (CHROMASOLV for HPLC≥99.8%), then the extracts were added and finally 40 mL of acetonitrile were used as eluting solvent. The eluate extracts were reduced until dryness and redissolved again with 100 µL of acetone with APs containing 50 ng of the internal

standards (PCB 30 and DDT-D8 and) for GC-QqQ-MS/MS detection (gas chromatography-triple quadrupole tandem mass spectrometry) (Supplementary Table 3).

Statistical analyses

Descriptive statistics were performed with all variables included. A descriptive analysis of all variables included in the study was performed.

Quantitative variables were expressed as mean and standard deviation. Qualitative variables were reported with absolute and relative frequency (percentage). Mean values of variables of interest were compared according to the case/control group by means of the Student's *t*-test or Mann-Whitney test, as appropriate; this was after prior verification whether the variables followed a normal distribution (Kolgomorov-Smirnov test). Qualitative variables association was estimated with the Chi-squared test. Multivariate linear regression models were utilized in order to evaluate the correlation between OPs and the birth weight of the NB, adjusted for maternal age, maternal BMI, gestational week, tobacco, and the gender of the NB.

IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.was used for statistical data analysis. Statistical significance was considered $p<0.050$

Results

Twenty percent NB weighed less than the 10th percentile for gestational age and, sex and 80% NB were of normal weight and identified as a control group.

Table 1 shows the most important clinical features of the total cohort, as well as of the case and the control group. Neither group presented any significant differences in terms of age, BMI or gestational age. In both groups 80% were primiparous (p=0.658). A total of 35 and 40% of the case and control groups, respectively, were smokers (p=0.941).

Table 1: Details about the donors.

		Age, years	BMI, kg/m^2	Amenorrhea at birth, weeks	Newborn weight, g
Controls	n	40	40	40	40
	Mean	32.89	26.07	39.59	3146.2
	DS	4.45	4.88	1.56	558.94
	Median	34	25.2	39.85	3,230
	Minimum	22	18	35.28	1900
	Maximum	42	36	41.85	4,340
Cases (SGA)	n	10	10	10	10
	Mean	30.1	22.31	38.84	2,564
	DS	6.22	2.39	1.53	368.91
	Median	30	22	39	2742.5
	Minimum	20	18.4	35.4	1940
	Maximum	40	26.2	40.7	2,980
	p-Value	0.116	0.09	0.07	<0.0001

Tables 2–4 show statistical values detected for OCPs, OPPs, PCBs and PBDEs in meconium both in case and control groups, as well as in the total sample. Significant differences between case and control groups have been included.

The analysis of results by chemical groups is as follows:

Organochlorinated pesticides (OCPs): Their presence was determined in 90% of meconium samples (Table 2).

Dieldrin and some 1,1-Bis-(4-chlorphenyl)-2,2,2-trichelorethan (DDT) isomers were not detected in any of the cohort samples. *Cis*-Chlordane was detected in higher concentrations in the SGA group than the control group with statistical significance (p=0.0040). *Trans*-Chlordane, ∑DDT and ∑OCPs values followed the same trend without statistical significance.

Organophosphorus pesticide (OPPs): The compounds evaluated are shown in Table 2. Detectable levels were determined in 86% of samples. All congeners from this group had concentrations that were detectable in meconium. Diazinon and ∑OPPs had higher concentrations in the control group than the SGA group with statistically significant difference (p=0.033 and p=0.029 respectively).

Polychlorinated biphenyls (PCBs): Levels detected of PCB congeners are shown in Table 3. As can be seen, all of the target congeners were found in 94% of meconium samples except PCB123 that was not detected in any sample.

Highest concentrations were determined for PCB 157 (p=0.042), PCB 209 (p=0.005) and PCB 189 (p=0.05) in the case group vs. the control group with statistically significant difference.

Polycyclic aromatic hydrocarbon (PAHs): PAH levels are shown in Table 4. They were only detected in 43% of the meconium samples and benzo[*a*]anthracene (BaA), chrysene (Chry) and benzo[*k*]fluoranthene (BkF) were no detected in any sample. The congeners with the highest contribution were pyrene (P), dibenzo[ah]anthracene (DBahA), indeno[1,2,3-cd]perileno (Ind123cdP) and fluoranthene (F). None of the target PAHs analyzed attained statistically significant differences among groups.

Polybrominated diphenyl ethers (PBDEs): Table 4 shows the target PBDEs.

The rate of detection in meconium was 48%. The average values of PBDE congeners studied were always higher in the case vs. the control group, except for PBDE 166 that was not identified in any meconium sample from the SGA group. Statistically significant differences were detected for PBDE 154 between groups (p=0.049).

Table 2: Statistical values for OCPs and OPPs obtained for the selected meconium samples grouped in controls and cases.

OPs, ng/g lw		α-HCH	HCB	β-HCH	Heptachlor	Ald	End	*t*-Chlord	*c*-Chlord	∑DDT	∑OCP	Diaz	Parat	Chlorpy	Fen	∑OPP
Controls	n	40	40	40	40	40	40	40	40	40	39	38	39	39	39	39
	Mean	0.1204	1.200	0.03691	0.06667	0.08789	0.03537	0.04978	0.00067	0.3671	1.608	0.21501	0.1362	0.1036	0.07126	0.5205
	D.S.	0.3456	1.670	0.1608	0.1468	0.3130	0.1173	0.1624	0.004246	1.0115	1.779	0.3138	0.1839	0.5531	0.1175	0.7030
	Median	0.00	0.6206	0.00	0.00	0.00	0.00	0.00	0.00	0.1036	1.119	0.08783	0.00	0.00	0.00	0.4019
	Minimum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Maximum	1.948	6.661	0.781	0.724	1.837	0.484	0.775	0.027	6.329	8.275	1.646	0.622	3.413	0.388	4.238
Cases	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Mean	0.00853	1.05082	0.00	0.06340	0.03439	0.02648	0.39395	0.25765	0.4072	2.24243	0.04529	0.13211	0.00	0.03624	0.21364
	D.S.	0.02697	1.069939	0.00	0.153043	0.074222	0.083737	0.886574	0.493653	0.53782	2.477625	0.096812	0.238929	0.00	0.06097	0.332964
	Median	0.00	0.83216	0.00	0.00	0.00	0.00	0.00	0.00	0.1799	1.21982	0.00	0.02122	0.00	0.00	0.11053
	Minimum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Maximum	0.085	2.550	0.00	0.475	0.206	0.265	2.823	1.428	1.803	7.086	0.297	0.766	0.00	0.159	1.102
p-Value		0.135	0.767	0.377	0.519	0.820	0.963	0.192	0.004	0.219	0.637	0.033	0.913	0.469	0.93	0.029
Total	n	50	50	50	50	50	50	50	50	50	49	48	49	49	49	49
	Mean	0.09803	1.170	0.02953	0.06602	0.07719	0.0336	0.1186	0.05207	0.3751	1.73724	0.1797	0.1354	0.08245	0.06412	0.4579
	D.S.	0.3118	1.5604	0.1443	0.1465	0.2819	0.1107	0.4298	0.2357	0.9315	1.929872	0.2901	0.1936	0.4939	0.1088	0.6539
	Median	0.00	0.6344	0.00	0.00	0.00	0.00	0.00	0.00	0.1298	1.11937	0.06389	0.00	0.00	0.00	0.3287
	Minimum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Maximum	1.948	6.661	0.781	0.724	1.837	0.484	2.823	1.428	6.329	8.275	1.646	0.766	3.413	0.388	4.238

OCP, organochlorinated pesticides; OPP, organophosphorus pesticide; α-HCH, α-hexachlorocyclohexane; HCH, hexachlorocyclohexane; β-HCH, β –hexachlorocyclohexane; Heptachl; Ald, aldrin; End, endrin; *t*-Chlord, *trans*-Chlordane; *c*-Chlord, *cis*-Chlordane; ∑DDT, ∑1,1-bis-(4-chlorphenyl)-2,2,2-trichelorethan; Diaz, diazinon; Parat, paranthion; Chlorpy, chlorpyrifos; Fen, fenthion.

Table 3: Statistical values for PCBs obtained for the selected meconium samples grouped in controls and cases.

PCBs, ng/g lw		PCB11	PCB209	PCB28	PCB52	PCB101	PCB118	PCB138	PCB153	PCB180	PCB77	PCB118	PCB126	PCB 156	PCB 157	PCB81	PCB105	PCB114	PCB167	PCB 169	PCB 189	∑PCB
Control	n	37	38	38	38	38	39	38	35	38	39	39	39	39	39	39	39	39	39	39	39	40
	Mean	1.394	0.03316	0.5199	0.08999	0.01466	0.00639	0.1014	0.02977	0.1998	0.00437	0.00639	0.00130	0.01343	0.03831	0.00979	0.1171	0.00640	0.01510	0.01765	0.02737	1.163
	D.S.	0.7403	0.07622	0.4909	0.1371	0.06487	0.02307	0.2699	0.1207	0.3159	0.01893	0.02307	0.006763	0.04209	0.09420	0.06117	0.3499	0.03996	0.04648	0.05202	0.07397	1.324
	Median	1.433	0.0000	0.4109	0.03549	0.0000	0.0000	0.0000	0.0000	0.01228	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.6732
	Min.	0.071	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Max.	3.251	0.279	2.004	0.646	0.3910	0.1030	1.265	0.6040	1.289	0.0980	0.1030	0.041	0.211	0.466	0.382	2.133	0.250	0.229	0.242	0.344	6.122
Cases	n	10	10	10	10	10	10	10	9	10	10	10	10	10	10	10	10	10	10	10	10	10
	Mean	1.155	0.1384	0.4264	0.03955	0.03001	0.03284	0.0000	0.0000	0.1953	0.0000	0.03284	0.0000	0.02029	0.08638	0.0000	0.1349	0.0000	0.04602	0.05236	0.06815	1.165
	D.S.	1.029	0.2013	0.3661	0.06476	0.09491	0.09681	0.0000	0.0000	0.1474	0.0000	0.09681	0.0000	0.06415	0.1395	0.0000	0.1576	0.0000	0.1141	0.1243	0.1479	1.121
	Median	1.224	0.1245	0.4010	0.0000	0.0000	0.0000	0.0000	0.0000	0.2356	0.0000	0.0000	0.0000	0.0000	0.03541	0.0000	0.07408	0.0000	0.0000	0.0000	0.00269	0.8900
	Min.	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.132
	Max.	3.342	0.671	1.091	0.1610	0.3000	0.3080	0.0000	0.0000	0.3560	0.0000	0.308	0.0000	0.203	0.4460	0.0000	0.449	0.0000	0.358	0.389	0.470	3.845
p-value		0.287	**0.0050**	0.7030	0.2160	0.842	0.2570	0.1180	0.369	0.2330	0.3710	0.257	0.4690	0.862	**0.042**	0.613	0.169	0.613	0.860	0.279	**0.050**	0.544
Total	n	47	48	48	48	48	49	48	44	48	49	49	49	49	49	49	49	49	49	49	49	50
	Mean	1.343	0.05508	0.5005	0.07948	0.0179	0.01178	0.08029	0.02368	0.1989	0.00348	0.01178	0.00104	0.01483	0.04812	0.00780	0.1207	0.00509	0.02141	0.02473	0.03569	1.163
	D.S.	0.8037	0.119	0.4657	0.1266	0.07126	0.04790	0.2431	0.1079	0.2876	0.01693	0.04790	0.006041	0.04671	0.1052	0.05457	0.3188	0.03565	0.06565	0.07239	0.09331	1.28
	Median	1.318	0.0000	0.4109	0.00166	0.0000	0.0000	0.0000	0.0000	0.04689	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.7031
	Min.	0.0000	0.0000	0.000	0.000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Max.	3.342	0.671	2.004	0.646	0.391	0.3080	1.265	0.6040	1.289	0.09800	0.3080	0.0410	0.2110	0.4660	0.3820	2.133	0.250	0.358	0.389	0.470	6.122

PCB, polychlorinated biphenyls.

Table 4: Statistical values for PAHs and PBDEs obtained for the selected meconium samples grouped in controls and cases.

OPs, ng/g lw		F	P	B(b)F	B(a)P	Ind123cP	DB(ah)A	B(ghi)P	∑PAHs	PBDE 28	PBDE 47	PBDE 99	PBDE 100	PBDE 153	PBDE154	∑PBDE
Control	n	40	40	40	40	40	40	40	40	39	39	39	39	39	39	39
	Mean	0.1036	0.1868	0.00297	0.12200	0.4205	0.7339	0.09784	1.668	0.002225	0.02022	0.02291	0.02189	0.02404	0.04165	0.2176
	D.S.	0.6555	0.9264	0.01879	0.4639	1.019	1.319	0.31295	2.268	0.07930	0.07305	0.06769	0.04553	0.07077	0.1205	0.5522
	Median	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1776	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Minimum	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Maximum	4.145	5.600	0.1190	0.0000	4.303	4.002	1.684	7.795	0.333	0.427	0.264	0.191	0.339	0.552	2,582
Cases	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Mean	0.0000	0.0000	0.0000	0.0000	0.62015	0.9089	0.2719	1.801	0.009026	0.1895	0.08702	0.09300	0.09266	0.07964	0.6321
	D.S.	0.0000	0.0000	0.0000	0.0000	1.058	1.709	0.4788	2.703	0.2086	0.3829	0.2045	0.1365	0.1662	0.1075	1.025
	Median	0.0000	0.0000	0.0000	0.0000	0.0000	0.03273	0.0000	0.7415	0.0000	0.0000	0.0000	0.0000	0.0000	0.03555	0.2652
	Minimum	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	0.0000	0.0000	0.0000
	Maximum	0.0000	0.0000	0.0000	0.0000	2.611	5.259	1.329	7.630	0.637	1.091	0.651	0.400	0.514	0.332	3.224
p-Value		–	–	–	–	0.706	0.489	0.29	0.878	0.107	0.260	0.247	0.152	0.096	**0.049**	0.187
Total	n	50	50	50	50	50	50	50	50	49	49	49	49	49	49	49
	Mean	0.08291	0.1495	0.00238	0.0976	0.4604	0.7689	0.1327	1.694	0.03613	0.05477	0.03599	0.03640	0.03805	0.04940	0.3022
	D.S.	0.5863	0.8299	0.01681	0.4168	1.019	1.388	0.3536	2.332	0.1179	0.1909	0.1102	0.07729	0.09963	0.1179	0.6835
	Median	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2170	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.00423
	Minimum	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Maximum	4.145	5.600	0.119	2.378	4.303	5.259	1.684	7.795	0.637	1.091	0.651	0.400	514	0.552	3.224

PBDE, polybrominated diphenyl ethers; PAH, polycyclic aromatic hydrocarbon; F, fluoranthene; P, pyrene; B(b)F, benzo[b]fluoranthene; B(a)P, benzo(a)pyrene; Ind123cP, indeno(123-cd)pyrene; DB(ah)A, benzo[a,h]anthracene; B(ghi)P, benzo[ghi]perylene.

Discussion

OP detection rate in the selected meconium samples ranged between 43 and 90%, with an average rate of 75%. This suggests generalized fetal exposure to the target OPs during intra-uterine life in the city of Ourense located in NW Spain, with low level of industrialization and marked by a predominance of service sector.

Several scientific studies have been published in the medical literature over the last years that examine the fetal exposure to OPs, mainly to persistent OPs (POPs) and using other biological samples such as umbilical cord blood, urine and neonatal blood. Nevertheless, these invasive biological samples are difficult to collect, implicate ethical issues and some of them are only available at the precise time of birth. Moreover, they only show a temporary record of exposure to OPs and cannot reflect the mother and the child long-term exposure. The use of meconium as non-invasive samples, offer any advantages in sampling, handling, and ethical issues, while ensuring reliability and similar sensitivity.

OP levels detected in the present study were relatively low in comparison with those reported in previous studies [23, 24]. However, they were determined in most of the selected meconium samples that showed a profile of the exposition of OPs during intra-uterine life. Despite their presence in such biological samples their production and use of most of them has been banned for more than 30 years. OPs still remain in the environment or in the mother's body and can cross the placenta [23] and remain in the fetus.

Organochlorinated pesticides: *Cis*-chlordane levels were higher in meconium of SGA group than in meconium of the control group with statistically significant difference. Literature about antenatal exposure to chlordane and its effects on the fetus is scarce. Tan et al. [25], with 41 NB umbilical cord blood samples from Singapore reported that chlordane concentration is inversely related to the neonate's weight, cranial circumference and height. Similar results, but without attaining statistical significance, have been published by other groups [24].

Despite not attaining statistical significance in the comparison of total DDT, we observed how these compounds present higher levels in the SGA group. These contaminants reached the environment after their production and massive use as a pesticide in the 1950s. Their use was prohibited in the USA and definitively in Spain in the 1970s and 1990s, respectively. However, they continue to be used in endemic malaria areas and are distributed into the environment from old or current waste.

These lipophilic OPs are resistant to breakdown and tend to bio-accumulate and therefore, they are commonly found at low levels in human adipose tissue [26, 27]. The Stockholm Convention recognizes POPs as "Highly toxic, stable and persistent. It takes decades for them to break down. They evaporate and travel long distances through air and water and build up in human adipose tissue and wildlife species" [28].

Different authors have reported associations between DDT levels and SGA concentrations [29–33]. Guo et al. [29] reported DDT levels in maternal and umbilical cord blood in 81 mother-child pairs. They used multiple linear regression analysis to find correlations between both biological matrices. They found that for every 1.0 ng/g of DDT in umbilical cord serum neonatal weight decreased 0.10 g.

DDT is classified as a neuroendocrine disruptor. The theoretical basis for its association with SGA focuses on its similarity to thyroid hormones. It is plausible that this may interfere with the hypothalamus-pituitary-thyroid axis with the consequent development of maternal hypothyroidism [33]. Thyroid hormones play an important role in bone growth by stimulating ossification center. Moreover, these hormones improve glycogenolysis and inhibit glycogen synthesis, whereby they increase use of glucose in the peripheral tissues by stimulating fetal growth [34–36].

Kim et al. [37] after analyzing thyroid hormone levels in umbilical cord blood observed inverse relationships between DDT concentrations with thyroxine (T4) and triiodothyronine (T3), which suggesting fetal hypothyroidism. In their meta-analysis, Gheidarloo et al. [38] specifically emphasized the disruptive effect of DDT and their interaction effects on neonatal or fetal thyroid function. However, they reported that the exact mechanism of action was not clearly determined. In animal experiments, it was observed lower thyroid hormone levels during DDT exposure, mainly after inhibition of the thyroid stimulating hormone (TSH) receptor in the peripheral gland [39]. Similar results were also obtained *in vitro* [40].

Furthermore, a low enzyme activity in the fetus should be pointed out, such as cytochrome P450. Therefore, OP biotransformation and detoxification processes could be limited during the immediate fetal and neonatal period and their anti-thyroid effect would be more noticeable [41].

Other authors [42] have published that DDT and its metabolites are capable of negatively modulating the insulin-like growth factor (IGF) system, especially in women. Insuline-like growth factors 1 and 2 (IGF1, IGF2) are expressed in the placenta and are known to regulate fetal growth [43]. Whilst it has been demonstrated that maternal IGF1 stimulates fetal growth by increasing nutrient transfer to the fetus [44], fetal IGF1 stimulates its growth by boosting anabolic events and DNA synthesis [45–47]. DDT

might alter fetal growth by acting on the IGF system on a maternal, placental and/or fetal level.

Nevertheless, we continue to detect conflicting data in the literature and after large series studies some authors do not reveal an association between DDT and SGA [6, 48]. It is possible that this inconsistency is due to the use of populations with different degrees of exposure to OPs, different definitions of growth abnormalities or possible confounding factors not being discarded. However, it may also be due to the lack of homogeneity in the matrices studied: progestational maternal serum, maternal serum at different stages of pregnancy, umbilical cord blood, placenta, etc.

Exposure to OPPs may occur because of occupational use or proximity to agricultural areas. However, most populations are exposed to OPPs through diet [49, 50]. There is a strong biological plausibility that associates the effect of OPPs with fetal growth and development by means of interfering adenylyl cyclase activity. Cyclase activity is essential to cellular differentiation [51], thyroid hormone function alteration in mother or fetus [52] or deregulation of nutrient transport through the placenta [53].

Most of the reported studies have been focused on the determination of OPP metabolites in maternal urine and found an inverse relationship between these levels and neonatal weight [54–56]. However, other authors such as Ferguson et al. [57] observed an association of OPP values with delayed intra-uterine growth estimated by ultrasound. However, they did not detect an association between birth weight and OPP levels.

We have detected that diazinon and ∑OPP concentrations were higher in the control group than the SGA group, which conflicts with prior literature. It is not possible to compare the results of prior reports and our findings due to various methodological differences. Firstly, OPPs metabolize quickly in the human body and concentrations of their metabolite in urine (dialkyl phosphates) are moderately stable during pregnancy [50]. An isolated sample only reflects a precise moment from the pregnancy. Furthermore, OPPs are metabolized by means of paraoxanase enzymes (PON1 paroxonase) and, polymorphisms from just one nucleotide in genotypes of these enzymes can modify relationships between exposure to OPPs and perinatal outcomes [58–60]. The reported results about associations between prenatal OPP exposure and birth weight are ambiguous due to the ability of pregnant women to detoxify OPPs by means of the paraoxonase enzyme.

However, we have analyzed OPPs (not their metabolites) in meconium samples, which offers a profile of fetal exposure during the final trimester of gestation.

Finally, Koutroulakis et al. [61] quantified dialkyl phosphate values (OPP metabolite) in 514 samples of amniotic fluid liquid extracted by amniocentesis between week 18 and 20. They reported that macrosomic infants presented the highest dialkyl phosphate levels ($p=0.043$) with positive linear between the target OPP metabolite and birth weight percentile ($p=0.016$) and they obtained a positive linear association with birth weight percentile ($b=4.43$, $p=0.016$). These data are in accordance with our results.

None of the PAH studied in our analysis revealed statistically significant differences. However, the values of some congeners determined in SGA meconium were higher. We only found one study performed by the present authors [8], which analyzed meconium PAH concentrations. However, we found studies in the medical literature with PAH analyses in the placenta and umbilical cord blood [62, 63] which reveal their materno-fetal transfer.

PAHs are generated primarily during the incomplete combustion of organic materials (e.g. coal, oil, petrol, and wood). Outdoor PAH levels come from industrial combustion, forest fires, automobile exhaust gases [64] whereas heating, home cooking or tobacco exposure are the main sources of indoor PAHs [65].

PAHs can be classified into low and high molecular weight, according to the number of aromatic rings. The aqueous solubility decreases as molecular weight and molecular size increases, with the consequent increase in lipophilic nature. Our analysis of PAHs isolated more commonly and with higher levels corresponded to those of high molecular weight, just as reported by other authors [62].

In the absence of studies performed on PAHs in meconium, and even fewer studies that relate PAHs in meconium to SGA, we proceeded to review other matrices. NB weight and length have been related with PAH levels and DNA-PAHs adducts (chemical compounds formed by the union of PAH to DNA) detected in the food chain, as well as in outdoor and indoor places [66–69].

Drwal et al. [70] in a recent review argued that PAH levels can have a significant impact on fetal growth by means of two mechanisms. In the first mechanism, PAH interrupt endovascular proliferation of the early trophoblast and its ability to infiltrate the myometrium, which entails an altered vascular structure in the placenta, a reduced area of the fetoplacental vascular surface and altered apoptosis in fetal endothelial and syncytiotrophoblast cells; that is they will alter placental angiogenesis. The second mechanism proposed would be PHAs fetotoxicity.

We believe that in our study, statistical significance has not been attained because of the reduced number of samples studied.

A literature review showed that it is not possible to find studies that evaluate PCB levels in meconium and their relationship to fetal growth abnormalities. As commented before, in the present study PCBs were detected in 94% of meconium samples and PCB 157, 189 and 209 showed higher concentrations in the case group than in the control ones with statistical significance.

SGA related to PCBs was reported for the first time in humans since the Yusho (Japan) incident in 1968, where thousands of pregnant women were intoxicated with rice oil for cooking contaminated with PCBs [71]. Since then, multiple studies have found the same association: the higher the concentration of PCB, the higher the risk of SGA, after analyzing maternal blood at different stages of gestation [13, 72–77] or umbilical cord blood [13, 78, 79]. However, other authors have not managed to reveal this association [80–82].

We should highlight that studies that analyzed PCB levels in umbilical cord blood as a biological matrix manage to demonstrate a statistically significant association between PCBs and SGA. That is, analysis of a biological sample of the fetal compartment (cord blood, meconium, and amniotic fluid) is more sensitive in comparison to maternal blood or breast milk. Furthermore, some OPs, including PCBs, do not show a statistically significant correlation between maternal and fetal serum concentrations [4].

A meta-analysis that attempts to elucidate the relationship between PCBs and SGA was recently published in 2019 [83]. Its results revealed a statistically significant correlation between the reduction in birth weight and exposure to PCBs during pregnancy (β=−0.586 g; 95% CI:−0.629–0.543). They also shown an inverse correlation between birth weight and exposure to PCB using umbilical cord serum as a matrix (β=−0.833 g, 95%CI:−1.695–0.029) or maternal serum (β=−0.504 g, 95% CI:−0.785–0.223).

The biological mechanism whereby PCBs interfere with fetal growth is still not well established. Some mechanisms have been involved. Some PCBs and their metabolites have been identified as endocrine disruptors capable of modulating the thyroid hormonal system, such that they can interfere with and reduce circulating levels of maternal thyroid hormones [33, 84–86]. It has been revealed that even the subclinical type of maternal hypothyroidism may be a risk factor for SGA [87].

Maervoet el al. [88] reported negative statistically significant correlation between PCB levels and thyroid hormones (T3, T4) in umbilical cord blood. However, they did not detect an association with TSH values.

The other mechanism involved in the relationship between PCBs and SGA is their ability to alter the configuration of the placenta. Tsuji et al. [89] found that PCB trace levels in maternal blood of non-selected pregnant women (n=22) are negatively associated with the estimated volume of syncytiotrophoblast. Moreover, they observed a statistically significant positive association between exposure to PCBs and PIGF (placental growth factor) levels. However, they did not detect a relationship with sFlt-1 (soluble Fms-like tyrosine kinase-1) concentration. Therefore, PCB exposure could also affect to the vascular remodeling and to the nutrient transport.

Just as occurred with previous OPs, we have not found any studies that evaluate levels of PBDEs in meconium and their relationship to fetal growth abnormalities. Moreover, when comparing our results to other authors we find that studies performed with maternal samples are not valid to compare our findings, given that various authors [90–92] suggest that PBDE values in umbilical cord blood are higher and not correlated to values detected in maternal blood. This demonstrates that the placenta is not an effective barrier for transport of PBDEs. Therefore, we can suppose that something similar would occur in meconium samples.

Some authors account for the relationship between PBDEs and SGA because of alteration to these OPs in the thyroid system. Lin et al. [93], specifically report an inverse relationship between PBDE 153, PBDE 154 and PBDE 184 with thyroid hormones in umbilical cord blood. We have been able to determine higher and statistically significant concentrations of PBDE 154 in SGA.

To explain the relationship PBDEs and SGA is the recent proposal by Zhao et al. [94]. This working group justifies that the negative impact of PBDEs (at trace levels) on fetal growth could be accounted for by aberrant methylation of placenta DNA, specifically HSD11B2 (hypermethylation) or IGF2 (hypomethylation), which would hypothetically lead towards epigenetic changes in these two genes.

After a literature review, we can state that there is a sufficient body of scientific evidence that associates some OPs with fetal growth abnormalities.

We must bear in mind that the initial stages of life are especially vulnerable to exposure to environmental chemical

agents. The developing fetus is especially vulnerable to adverse toxicological actions due to their high levels of cell proliferation, reduced capacity to detoxify OPs, reduced capacity for an immune response and their physiological immaturity.

However, the problem of OPs goes beyond intra-uterine life and several authors have detected relationships between antenatal exposure and pathology during childhood and adult life. PCBs and PBDEs have been associated with adverse effects on neurological performance and cognitive development during childhood and adolescence [95–97]. Dichlorodiphenyldichloroethylene has been associated with child obesity [98] and endocrine abnormalities such as diabetes and hypothyroidism, among others.

Conclusions

To the best of our knowledge, this is the first study about the relationship between OPs and SGA using a non-invasive biological matrix as meconium. Meconium as a biological matrix will enable us to analyze a lengthy period of exposure to OPs during fetal life. OPs deposited in meconium build up and increase in concentration, which makes their detection more likely. Trace values in a region that is not very contaminated reveal a relationship between some OPs and fetal growth. The exact mechanism of action whereby OPs can have an impact on fetal growth is unknown. Study of antenatal exposure to OPs may contribute to shedding light on etiology and notifying strategies to prevent many adult diseases, especially diseases with a growing incidence such as obesity or diabetes.

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Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: The study was approved by Pontevedra-Vigo-Ourense Research Ethics Committee with registry code 2014/410. The Declaration of Helsinki on biomedical research was applied at all times.

Data availability: The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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