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Distribution of fipronil in humans, and adverse health outcomes of *in utero* fipronil sulfone exposure in newborns

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ABSTRACT

Fipronil is a highly effective insecticide with extensive usages; however, its distribution and toxic/health effects in the human population after chronic exposure have not yet been clearly identified. Our objectives were to determine the levels of serum fipronil and fipronil sulfone, a primary fipronil metabolite, in a general and sensitive human population using a birth cohort of parent-infant triads in Korea. We further investigated whether *in utero* exposure to fipronil and fipronil sulfone can affect health outcomes in newborn infants.

Blood and umbilical cord blood from 169 participants, 59 mother-neonate pairs and 51 matching biological fathers, were collected; serum fipronil and fipronil sulfone (both blood and cord blood) and serum thyroid hormones (cord blood) were measured. Demographic, physiological, behavioral, clinical, and socioeconomic data for each participant were collected via a one-on-one interview and a questionnaire survey.

Fipronil sulfone was detected in the serum of mothers, fathers, and infantile cord blood, while fipronil itself was not. Maternal fipronil sulfone levels were correlated to those of matched biological fathers and newborn infants. Adjusted analyses identified significant associations between parental fipronil sulfone levels and household income. Infantile fipronil sulfone levels were significantly associated with both maternal and paternal levels as well as maternal pre-pregnant BMI. Furthermore, infantile fipronil sulfone levels were inversely associated with cord blood T3 and free T3 levels as well as 5-min Apgar scores of newborn infants.

Serum fipronil sulfone was detected in a specific population of mother-neonate pairs and their matched biological fathers in a manner suggestive of regular exposure to fipronil among urban residents. The findings also suggest that serum fipronil sulfone placentally transfers to the fetus and affects infantile adverse health outcomes. This is a first of its kind study; therefore, future studies are warranted.

1. Introduction

Fipronil is a widely used phenylpyrazole insecticide with systemic effects (Mohamed et al., 2004; Simon-Delso et al., 2015; Tingle et al., 2003). Fipronil acts as a noncompetitive blocker of gamma-aminobutyric acid (GABA)-gated chloride channels in the central nervous system, which ultimately leads to insect death (Caboni et al., 2003). Fipronil exerts selective toxicity toward insect GABA receptors and is therefore often used as an alternative to other pesticides in many settings, including agricultural, commercial, industrial, and residential applications (Hainzl et al., 1998; Narahashi et al., 2007, 2010). Further, there is no known pesticide resistance in target insects—a phenomenon that has been shown to occur for most other pesticides. This, in turn, has led to a rapid increase in the usage of fipronil, which accounts for approximately 10% of the global pesticide market (Simon-Delso et al., 2015). However, the toxic properties of fipronil persist relatively long in aerobic soils and in water, with a half-life ranging from 2 days to 7.3 months depending on substrate and conditions (Bobé et al., 1997; Gunasekara et al., 2007).

Following the wide and extensive usage of fipronil and because of

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Abbreviations

BMI	body mass index
CI	confidence interval
C-section	Caesarean section
CV	coefficients of variance
CYP450	cytochrome P450
DDT	dichlorodiphenyltrichloroethane
ECLIA	electrochemiluminescence immunoassay
EPA	Environmental Protection Agency
ESI	electro spray ionization
GABA	gamma-aminobutyric acid
GM	geometric mean
GSD	geometric standard deviation
KRW	Korean Won
LC-MS/MS	liquid chromatography electrospray ionization mass spectrometry
LD50	lethal dose, 50%
LOD	limits of detection
NICU	neonatal intensive care unit
OCP	organochlorine pesticides
PCBs	polychlorinated biphenyls
POPs	persistent organic pollutants
RSD	relative standard deviation
SD	standard deviation
SPE	solid phase extraction
T3	triiodothyronine;
T4	thyroxine;
TR	thyroid hormone receptor
TSH	thyroid-stimulating hormone
WHO	World Health Organization

its physicochemical properties, fipronil has become a widespread environmental contaminant (Stone et al., 2014). Although fipronil more selectively binds to insect GABA receptors than mammalian GABA receptors (Hainzl et al., 1998), its undesirable effects on non-target organisms have led to concern about the potential for adverse health effects in humans (Hayasaka et al., 2012a, 2012b; Liu et al., 2012). Several *in vitro* and animal studies have shown that fipronil can induce cytotoxicity or cell death in human cell lines (Vidau et al., 2009, 2011); further, disturbed behavior (Udo et al., 2014; Magalhães et al., 2015) and thyroid, endocrine, or reproductive dysfunction in rats has been observed (Herin et al., 2011; Leghait et al., 2009; Ohi et al., 2004; Tingle et al., 2003).

Human exposure (both environmental and occupational exposures) to fipronil can occur through ingestion, inhalation, and dermal contact (Chodorowski and Anand, 2004; Mohamed et al., 2004). Acute poisoning in response to fipronil exposure in humans reportedly causes headache, dizziness, sweating, nausea, vomiting, agitation, and seizures (Cravedi et al., 2013; Mohamed et al., 2004). Information on the effects of chronic exposure to fipronil in the human population is limited; however, the U.S. Environmental Protection Agency (EPA) has classified fipronil as a possible human carcinogen based on data showing an increase in thyroid follicular cell tumors in rats after long term exposure (Hurley, 1998). The World Health Organization (WHO) also classified fipronil as a Class II moderately hazardous pesticide, with an acute oral LD50 in rats (97 mg/kg) (World Health Organization et al., 2009). Consequently, the use of some fipronil-based products on domestic animals is not recommended (Tingle et al., 2003).

Meanwhile, in July/August 2017, fipronil-contaminated eggs were distributed to several European and Asian countries, including Germany, Switzerland, France, Hong Kong, and South Korea. Fipronil-contaminated eggs may have been consumed for a long time prior to the discovery, and exposure levels may have exceeded the threshold, posing a threat to human health. In addition, fipronil sulfone, a primary metabolite of fipronil, is reported to be more persistent in organisms than fipronil, with a relatively longer half-life (estimated half-life is 150–245 h in rodents) (Cravedi et al., 2013; Leghait et al., 2010; F. Mohamed et al., 2004); further, fipronil sulfone-induced toxicity is more severe in mammals (Das et al., 2006) or human cells than fipronil-induced toxicity (Romero et al., 2016). Another study by McMahen et al. (2015) suggested that serum fipronil sulfone levels are a useful biomarker for environmental fipronil exposure in rodents and humans. However, there is very limited human data available regarding the distribution of fipronil and fipronil sulfone in the healthy general population or its health effects after chronic exposure.

In this study, we aimed to determine the levels of serum fipronil and its major metabolite, fipronil sulfone, among a general and sensitive human population using a birth cohort of parent-infant triads in Korea. We further investigated whether fipronil and fipronil sulfone can be placentally transferred and affect health outcomes in newborn infants. To this end, we measured fipronil and fipronil sulfone levels in blood serum and cord blood serum, as well as thyroid hormone levels, including thyroid-stimulating hormone (TSH), triiodothyronine (T3), thyroxine (T4), free T3, and free T4 in cord blood serum. We further evaluated factors that may influence fipronil exposure among pregnant women, including physiological, behavioral, and socioeconomic characteristics.

2. Materials and methods

2.1. Study population

Sixty-five healthy pregnant woman-newborn infant-matching biological-father pairs were recruited before delivery from Inje University Ilsan Paik Hospital in South Korea from March 2013 to July 2015. Pregnant women receiving prenatal care and delivering their newborn infants at 31–41 weeks of gestation were included. Written informed consents were obtained from each patient using consent forms and a protocol approved by the Institutional Review Board of Ilsan Paik Hospital (IRB number: IB-1211-038).

2.2. Blood and data collection

Maternal and paternal blood samples were collected when the participating pregnant women visited the hospital for their delivery, and umbilical cord blood from newborn infants was collected during delivery. Blood samples were collected using serum separation tubes on site and stored at −80 °C until analysis. All samples and data were processed blindly.

A one-on-one interview and a questionnaire survey for demographic, physiological, behavioral, and socioeconomic data of each participant were competed at the time when blood samples were obtained. The questions included age, body mass index (BMI, kg/m^2), weight gain during the gestation (kilograms (kg)), waist circumference (centimeters (cm)), age of menarche, duration of menstrual cycle, history of dysmenorrhea and related surgeries, gravity, parity, cigarette smoking, alcohol consumption, exposure to second-hand smoke, physical activity, owing a pet or pets (i.e. dog or cat), and socioeconomic characteristics. Current or previous health status of study population and newborn infantile birth outcomes were also collected from the medical record. For the birth outcomes for newborn infants, gestational age (weeks), birth weight (g), birth length (cm), head circumference (cm), ponderal index (g/cm^3), cord pH, Apgar score, neonatal intensive care unit (NICU) admission status, significant morbidity (in case of that infant had at least one diagnosis from following; intraventricular

hemorrhage, respiratory distress syndrome, bronchopulmonary dysplasia, and necrotizing enterocolitis), and thyroid function were examined. Gestational age was assessed by the obstetrician based on last menstruation or early ultrasound estimates.

2.3. Measurement of fipronil and fipronil sulfone

Serum fipronil and fipronil sulfone from a birth panel including pregnant woman-newborn infant-matching biological-father pairs were measured with liquid chromatography electrospray ionization mass spectrometry (LC-MS/MS) using Nexera X2 (Shimadzu, Kyoto, Japan) and API 4500 (Applied Biosystems, Foster, CA) as detailed in a previous publication (Bichon et al., 2008). In brief, fipronil and fipronil sulfone were extracted from serum using solid phase extraction (SPE) cartridges (Strata-X (30mg/1 cc, Phenomenex, UK). Serum samples (200 µL) were applied to the cartridges conditioned with 2 mL MeOH and 2 mL ultrapure water. The cartridges were washed with 2 mL ultrapure water as well as 2 mL 30% methanol. The analytes were then eluted with 3 mL methanol. The extract was dried using a vacuum evaporator and reconstituted in 200 µL MeOH, and 5 µL was injected into a LC-MS/MS system. The separation of fipronil sulfone was carried out using a YMC-Pack ODS-AQ (50 mm × 2.0 mm, 3 µm) column. The flow rate used was 500 µL min^{-1}, and MS/MS was performed in electro spray ionization (ESI) negative mode.

For each analysis, method and matrix blanks were evaluated for contamination or background levels of the compounds of interest. The limits of detection (LOD) were determined from guidelines established by the US FDA. The LOD for fipronil was 0.027 ng/mL and for fipronil sulfone was 0.087 ng/mL. For each instrumental run, reagent blanks and QC samples were included for quality control. The method validation test was performed with bovine serum spiked with 3 points of standard materials. The accuracies were between 80% and 120%, and precisions were lower than 15% relative standard deviation (RSD) for high, medium and low concentrations of spiked samples (n = 7). More details regarding determination of fipronil and fipronil sulfone are provided in Tables S1, S2, and S3.

2.4. Measurement of thyroid hormones in cord blood

Cord blood samples were kept at −80 °C until thyroid hormone analysis. The measurement of thyroid hormones, including TSH, total T3, total T4, free T3, and free T4 in cord serum samples was performed using the electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics GmbH, Mannheim, Germany). The interassay coefficients of variance (CV) for the thyroid assay did not exceed 10%, which met the laboratory's established requirements for precision. The LOD was 0.026 ng/dL for free T3, 0.023 ng/dL for free T4, 0.195 ng/mL for T3, 0.420 µg/dL for T4, and 0.005 uIU/mL for TSH. Samples were treated with monoclonal antibodies labeled with a ruthenium complex (T3-specific antibody, T4-specific antibody, TSH-specific antibody, free T3-specific antibody, free T4-specific antibody, respectively) and incubated. A sandwich-complex formed with target analytes, and chemiluminescent emission was measured.

2.5. Statistical analysis

Among the subjects whose blood/cord blood samples were collected, levels of serum fipronil and fipronil sulfone were available for 59 mothers, 59 paired neonates, and 51 matching fathers; thus, a total of 59 mother-neonate pairs and 51 matching biological fathers were finally chosen for this study. Mean ± standard deviation (SD), median (range), geometric mean (95% confidence interval (CI)), or number (frequency) was tabulated to describe demographic characteristics, fipronil and fipronil sulfone levels, cord blood thyroid profiles, or birth outcomes when appropriate. Serum fipronil sulfone levels were detected in all study participants; however, serum fipronil levels from the study participants (except one paternal serum sample) were not detected above the LOD. Thus, only serum fipronil sulfone levels were analyzed/considered in this study.

Serum fipronil sulfone levels from all participants were compared by maternal, paternal, or newborn infantile characteristics using a two-sample *t*-test or ANOVA test. The following covariates were introduced: age at delivery (< 30 or ≥ 30 years), maternal (pre-pregnancy) and paternal BMI (< 25 or ≥ 25 kg/m^2), parity (primipara or multipara), mode of delivery (vaginal delivery or caesarean section), education level (< University or ≥ University), previous and second-hand smoking status of mother (yes or no), paternal smoking (yes or no), household income (< 3,000,000 or ≥ 3,000,000 South Korean Won (KRW) per month; 1000 KRW is approximately equivalent to U$ 1.00), infantile gender (male or female), birthweight (< 2500 or ≥ 2500 g), gestational age (< 37 or ≥ 37 weeks), NICU admission (yes or no), ponderal index (< 2.32, 2.32–2.85, or > 2.85), and significant morbidity (yes or no). For household income, the total income from both paternal and/or maternal incomes was considered (if maternal income was available); thus, household income from 51 households was considered in this study.

The correlation between mother, father, and matched infant fipronil sulfone levels was tested using the Pearson correlation test. We performed linear regression analyses to explore whether maternal, paternal, or infantile fipronil sulfone levels were affected by maternal or paternal covariates, including age, BMI (pre-pregnant BMI for maternal level), parity (primipara or multipara), weight gain during pregnancy, smoking status (indirect smoking status for maternal levels; yes or no), education level (< University or ≥ University), and household income (< 3,000,000 or ≥ 3,000,000 KRW/month). To identify the factors related to fipronil sulfone levels in parental serum and infantile cord serum, multiple linear regression analyses were performed considering various covariates (stated above) based on our linear regression models and previous reported literature (Chen et al., 2012; Fei et al., 2007; Hamm et al., 2010; Maisonet et al., 2012).

Associations between fipronil sulfone levels in cord blood and newborn infantile birth outcomes were evaluated using linear regression and multiple linear regression models. Since infantile morbidity and gestational age significantly affected Apgar scores and thyroid hormone levels (i.e. T3, Free T3, and TSH) in cord blood, infantile morbidity and gestational age were also considered for the associations between fipronil sulfone levels in cord blood and newborn infantile birth outcomes; therefore, maternal age, maternal pre-pregnant BMI, parity, education level, smoking status (indirect smoking status for maternal level), household income, infantile sex, gestational age, birth weight, birth length, head circumference, birth morbidity, Apgar score, and ponderal index were considered for the multiple linear regression models. All statistical analyses were conducted using SPSS statistical software (version 21, Chicago, IL). *P* values less than 0.05 were considered statistically significant.

3. Results

There were a total of 169 participants in this study: fifty nine pregnant women-paired newborn infants, and 51 corresponding biological fathers. All pregnant women and biological fathers (husbands) were healthy urban residents, and only one household owned a pet (dog) at home during the pregnancy; thus, most were not directly nor occupationally exposed to any pesticides, including fipronil, during the pregnancy. The distribution of demographic, behavioral, and socioeconomic characteristics of the study population is shown in Table 1. Most of our participants (both pregnant women and biological fathers) were educated at least a university levels (> 80.4%) and have house income more than 3,000,000 KRW/month, which is the approximate average income among 20 to 30 year-old office workers in Korea (64.7%). The health outcomes of newborn infants, including physical, hormonal, and health screening data from are shown in Table 1. Mean

Table 1
Characteristics of the study population.

Variable	Mean ± SD (range)	Number (%)
All		169 (100)
Maternal characteristics (N = 59)		
Age at delivery (years)	32.08 ± 3.23 (26–41)	
Pre-pregnant BMI (kg/m^2)	21.92 ± 3.75 (16.61–32.19)	
Mode of delivery		
Vaginal delivery		37 (62.7)
C-section[a]		22 (37.3)
Parity		
Primipara		33 (55.9)
Multipara		26 (44.1)
Smoking status before pregnancy		
No		55 (93.2)
Yes		4 (6.8)
Smoking status during pregnancy		
No		59 (100)
Yes		0
Second hand smoking status		
No		39 (66.1)
Yes		20 (33.9)
Alcohol drinking status during pregnancy		
No		58 (98.3)
Yes		1 (1.7)
Education		
< University		9 (15.3)
≥ University		50 (84.7)
Paternal characteristics (N = 51)		
Age at delivery (years)	34.31 ± 4.43 (27–46)	
BMI (kg/m^2)	23.95 ± 3.09 (17.90–34.84)	
Smoking status during pregnancy		
No		14 (27.5)
Yes		37 (72.5)
Alcohol drinking status during pregnancy		
No		0
Yes		51 (100)
Education		
< University		10 (19.6)
≥ University		41 (80.4)
Household income (KRW/month)[b]		
< 3,000,000		18 (35.3)
≥ 3,000,000		33 (64.7)
Infants characteristics (N = 59)		
Sex		
Male		35 (59.3)
Female		24 (40.7)
Gestational age (weeks)	37.44 ± 2.59 (30.6–41.0)	
Birth weight (g)	2983.66 ± 547.09 (1710–3940)	
Head circumference (cm)	33.11 ± 1.92 (28.5–36.0)	
Birth length (cm)	48.57 ± 2.76 (41.5–53.5)	
Ponderal index (g/cm^3)[c]	2.51 ± 0.19 (2.16–2.97)	
Cord pH	7.30 ± 0.06 (7.2–7.5)	
Base deficit	−3.32 ± 2.69 (−13.4–4.2)	
Apgar score at 1 min	7.85 ± 1.19 (5–10)	
Apgar score at 5-min	9.07 ± 0.64 (7–10)	
Thyroid hormones		
T3 (ng/mL)[d]	0.59 ± 0.08 (0.41–0.80)	
T4 (ug/dL)[e]	8.06 ± 1.21 (5.55–10.69)	
Free T3 (ng/dL)[f]	0.13 ± 0.02 (0.08–0.19)	
Free T4 (ng/dL)[g]	1.25 ± 0.14 (0.99–1.58)	
TSH (uIU/mL)[h]	10.98 ± 6.70 (2.97–40.55)	

[a] C-section, Caesarean section.
[b] Thousand South Korean Won (KRW) is approximately equivalent to U$ 1.00.
[c] Ponderal index, birth weight (gram) divided by third power of body length (centimeter), then multiplied by 100.
[d] T3, Triiodothyronine.
[e] T4, Thyroxine.
[f] Free T3, Free triiodothyronine.
[g] FreeT4, Free Thyroxine.
[h] TSH, Thyroid-stimulating hormone.

Apgar scores in the newborn infants at 1 and 5 min were 7.85 ± 1.19 and 9.07 ± 0.64, respectively, indicating overall good health at birth. Infant serum thyroid hormones, including T3, free T3, T4, free T4, and TSH were within normal range, which are defined in other publications (Kapelari et al., 2008; Oddie et al., 1979).

Levels of fipronil and fipronil sulfone among the study population are shown in Table 2. Fipronil sulfone was detected in the serum of mothers, fathers, and infantile cord blood, while fipronil itself was not (except one paternal serum sample). Fipronil sulfone levels were highest in the paternal samples (geometric mean = 1.163 ± 0.797 ng/mL, range, 0.130–3.570 ng/mL) and were significantly higher than those in the maternal (0.744 ± 0.426 ng/mL, range, 0.0790–2.910 ng/mL) and infant samples (0.525 ± 0.240 ng/mL, range, 0.159–1.750 ng/mL), as measured from cord blood.

The associations between parental fipronil sulfone levels and parental characteristics are shown in Table 3 as well as Table S4. The level of fipronil sulfone in the multiparous women was significantly higher than that in primipara women. Household income was a borderline significant variable for both maternal and paternal fipronil sulfone levels, while paternal fipronil sulfone levels were not significantly different by any characteristics. However, both maternal and paternal fipronil sulfone levels were significantly associated with household income ($\beta = 0.354$, 95% CI = 0.030, 0.678; and $\beta = 0.493$, 95% CI = 0.030, 0.970, respectively) after adjustments for age, BMI (pre-pregnant BMI for maternal level), weight gain during pregnancy, parity, smoking status (indirect smoking for maternal level), education level, and household income (Table 3).

Further univariate analysis examining differences in associations between infantile fipronil sulfone levels and maternal, paternal, and infant characteristics revealed household income as a significant variable (distinguished between < 3,000,000 or ≥ 3,000,000 KRW/month) (Table S5). This finding is consistent with both maternal and paternal analysis trends towards significance ($p < 0.1$) detected in comparisons of parental fipronil sulfone levels to household income level (Table S4).

As shown in Fig. 1, maternal fipronil sulfone levels were strongly correlated with paternal and infantile levels (Fig. 1A and B, respectively), and paternal fipronil sulfone levels were also significantly correlated with infantile levels (Fig. 1C). Concordantly, Table 4 shows that infantile cord blood fipronil sulfone levels were significantly associated with both maternal and paternal levels ($\beta = 0.306$, 95% CI = 0.131, 0.480; and $\beta = 0.183$, 95% CI = 0.069, 0.298, respectively) after adjusting for maternal age, maternal pre-pregnant BMI, maternal weight gain during pregnancy, parity, maternal indirect smoking status, paternal age, paternal BMI, paternal smoking status, parental education levels, and household income. These results indicate that maternal fipronil sulfone can be placentally transferred to the fetus and subsequently to newborn infants. In addition, adjusted analysis revealed that maternal pre-pregnant BMI was inversely associated with infantile fipronil sulfone levels ($\beta = -0.019$, 95% CI = −0.039, −0.001).

Interestingly, negative associations between birth outcomes and cord blood serum fipronil sulfone were detected before and after making model adjustments as shown in Table 5. In univariate linear regression analysis, cord blood T3 levels were significantly associated with infant fipronil sulfone levels (β = −0.066, 95% CI = −0.130, −0.001). Further analyses showed significant associations between both cord blood T3 ($\beta = -0.105$, 95% CI = −0.190, −0.020) and free T3 ($\beta = -0.021$, 95% CI = −0.040, −0.002), which were significantly associated with infantile fipronil sulfone level after adjusting for maternal age, maternal pre-pregnant BMI, parity, maternal indirect smoking status, paternal smoking status, parental education levels, household income, infant sex, gestational age, birth weight, birth length, head circumference, birth morbidity, Apgar scores, and Ponderal index. Multiple regression models after adjustments also revealed a significant negative association between infantile fipronil sulfone levels and 5-min Apgar scores ($\beta = -0.477$, 95% CI = −0.902, −0.051).

Table 2
Distribution of fipronil and fipronil sulfone in study population.

	Maternal serum (N = 59)		Paternal serum (N = 51)		Cord blood serum (N = 59)	
	Fipronil	Fipronil sulfone	Fipronil	Fipronil sulfone	Fipronil	Fipronil sulfone
GM[a] ± GSD[b] (ng/mL)		0.744 ± 0.426		1.163 ± 0.797*		0.525 ± 0.240
LOD [c]	0.078	0.036	0.078	0.036	0.078	0.036
Number > LOD (%)	0	59 (100)	1 (2.78)	51 (100)	0	59 (100)
Minimum		0.079	0.12	0.130		0.159
Median		0.753	0.12	1.310		0.506
Maximum		2.910	0.12	3.570		1.750

* Significantly different from maternal and newborn infant levels ($p < 0.05$).
[a] GM, geometric mean.
[b] GSD, geometric standard deviation.
[c] LOD, limit of detection.

Table 3
Association between serum fipronil sulfone concentration and characteristics of study population.

Variable	Beta coefficient (95% CI[a])	
	Crude	Adjusted[b]
Maternal serum fipronil sulfone		
Age	0.029 (−0.011, 0.068)	−0.026 (−0.086, 0.033)
Pre-pregnant BMI	0.006 (−0.029, 0.040)	0.006 (−0.038, 0.050)
Weight gain during pregnancy	−0.013 (−0.036, 0.009)	−0.133 (−0.037, 0.019)
Parity	**0.262 (0.013, 0.510)***	0.303 (−0.066, 0.673)
Indirect smoking status	0.045 (−0.224, 0.316)	0.024 (−0.291, 0.339)
Education	0.115 (−0.240, 0.469)	0.195 (−0.302, 0.692)
Household Income	0.237 (−0.046, 0.520)#	**0.354 (0.030, 0.678)***
Paternal serum fipronil sulfone		
Age	0.008 (−0.038, 0.055)	−0.014 (−0.066, 0.038)
BMI	−0.020 (−0.066, 0.025)	0.019 (−0.054, 0.093)
Smoking status	0.075 (−0.392–0.542)	−0.030 (−0.494, 0.436)
Education	−0.352 (−0.867, 0.164)	−0.437 (−1.103, 0.229)
Household income	0.374 (−0.049, 0.797)#	**0.493 (0.017, 0.970)***

*$p < 0.05$; # borderline significance ($p < 0.1$).
[a] CI, confidence interval.
[b] Model was adjusted for age, pre-pregnant BMI, weight gain during pregnancy, parity, indirect smoking status, education level, and household income for the maternal fipronil sulfone level. Model was adjusted for age, BMI, smoking status, education level, and household income for the paternal fipronil sulfone level.

4. Discussion

Serum fipronil and fipronil sulfone levels were examined to determine their distribution in a general and sensitive human population of parent-infant triads. Our study demonstrates that urban residents are likely to be exposed to fipronil, and fipronil sulfone can be placentally transferred, thereby affecting health outcomes in newborn infants. To our knowledge, this is the first study to report distribution of serum fipronil and fipronil sulfone levels and the consequent outcomes using a birth cohort.

When fipronil is absorbed in mammals following exposure, it is rapidly metabolized through three major mechanisms: (1) an oxidization step, leading to fipronil sulfone formation via cytochrome P450 (CYP450); (2) a reduction step, leading to fipronil sulfide; and (3) a hydrolysis step, leading to fipronil amide (Brennan et al., 2009a, 2009b; Caboni et al., 2003; Mohamed et al., 2004). No appreciable gender differences have been identified for the absorption, metabolism, distribution, or excretion of fipronil after exposure in rodents (FAO, 1998).

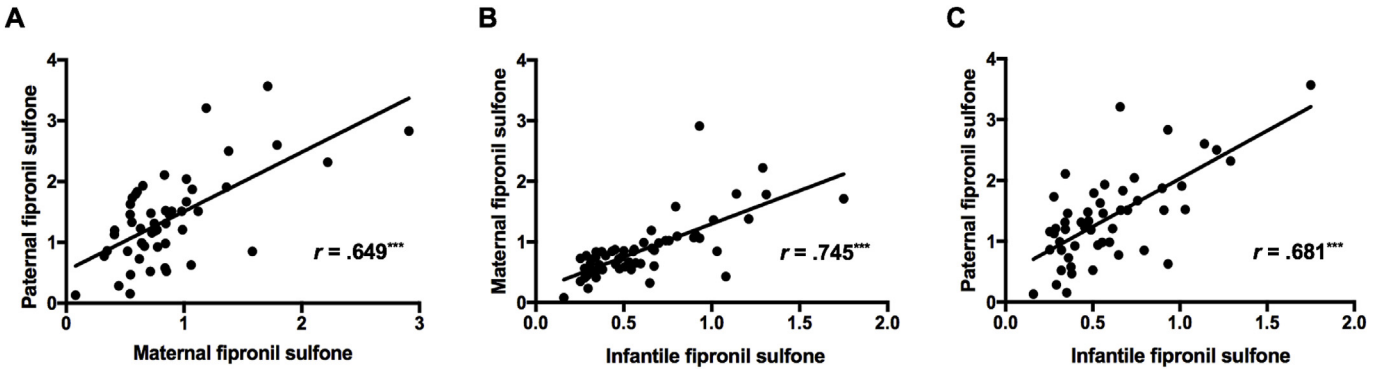

Fig. 1. Scatter plots between variables. Fipronil sulfone concentrations from (A) paternal and maternal samples, (B) maternal and infantile samples, and (C) paternal and infantile samples are shown. r = Pearson's correlation coefficient. *** = $p < 0.001$.

Table 4
Association between cord blood serum fipronil sulfone concentration and characteristics of study population.

Variable	Beta coefficient (95% CI[a])	
	Crude	Adjusted[b]
Maternal serum fipronil sulfone level	**0.486 (0.365, 0.607)** *	**0.306 (0.131, 0.480)** *
Maternal age	0.015 (−0.012, 0.041)	0.015 (−0.018, 0.047)
Maternal pre-pregnant BMI	−0.007 (−0.030, 0.016)	**−0.019 (-0.039, -0.001)***
Maternal weight gain during pregnancy	−0.008 (−0.022, 0.007)	−0.003 (−0.014, 0.008)
Parity	0.130 (-0.038, 0.297)	−0.090 (−0.259, 0.078)
Maternal indirect smoking status	−0.007 (−0.187, 0.173)	−0.074 (−0.208, 0.060)
Maternal education	0.108 (−0.127, 0.343)	0.078 (−0.158, 0.313)
Paternal serum fipronil sulfone level	**0.295 (0.204, 0.386)** *	**0.183 (0.069–0.298)** *
Paternal age	0.010 (−0.010, 0.029)	0.004 (−0.023, 0.031)
Paternal BMI	−0.004 (−0.034, 0.025)	−0.002 (−0.023, 0.018)
Paternal smoking status	0.044 (−0.158, 0.246)	−0.0001 (−0.132, 0.132)
Paternal education	−0.001 (−0.228, 0.226)	0.090 (−0.112, 0.293)
Household income	**0.197 (0.016, 0.377)** *	0.030 (−0.124, 0.174)

*$p < 0.05$; # borderline significance ($p < 0.1$).
[a] CI: confidence interval.
[b] Model adjusted for parental age, parental BMI (pre-pregnant BMI for maternal level), parity, maternal weight gain during pregnancy, smoking status (indirect smoking status for maternal level), parental education levels, and household income.

A pharmacokinetic study showed that once absorbed, fipronil is highly converted to fipronil sulfone, with a biotransformation rate of approximately 75% in rats (Roques et al., 2012). Among fipronil metabolites, fipronil sulfone is the predominant form in humans and rats (Tang et al., 2004; McMahen et al., 2015); thus, only fipronil sulfone levels were measured in this study. Several studies using rodent models have shown that fipronil sulfone is more persistent in organisms, with an estimated half-life of 150–245 h; it is slowly eliminated, attributable to being highly distributed to adipose tissue and moderately distributed to adrenal glands, thyroid, pancreas, skin, liver, kidneys, muscle, ovaries, and uterus. Further, fipronil sulfone is widely found in foodstuffs (e.g. milk and eggs) (FAO, 1998).

Fipronil was not detected in mother, father, or infantile cord blood serum, except one paternal serum sample; however, fipronil sulfone in serum or cord blood was detected in all study participants. This suggests that our study participants were regularly and chronically exposed to fipronil, which is readily metabolized to fipronil sulfone. Fipronil sulfone is more persistent and is eliminated more slowly than fipronil, as shown in rodents. A case report of acute fipronil exposure after self-poisoning in 7 patients who consumed 1600–3740 ng/mL fipronil also reported that plasma fipronil concentrations fall rapidly and disappear from the blood over the first 15–20 h, and fipronil metabolites (i.e. fipronil sulfone) concentrations plateau over time (Mohamed et al., 2004). In addition, McMahen et al. (2015) measured and reported serum fipronil and fipronil sulfone levels in 96 individuals with no known fipronil exposure, and only serum fipronil sulfone was detected in 25% of their samples. Furthermore, the detected fipronil sulfone levels (0.1–4 ng/mL) were in a similar range as our results. A study by

Table 5
Association between cord blood serum fipronil sulfone concentration and birth outcomes.

Birth outcomes	Beta coefficient (95% CI[a])		
	Crude	Adjusted (Model I)[b]	Adjusted (Model II)[c]
T3[d]	**−0.066 (-0.130, -0.001)***	**−0.104 (-0.177, -0.029)** *	**−0.105 (-0.190, -0.020)** *
T4[e]	−0.297 (−1.287, 0.693)	−0.902 (−1.927, 0.122)	−0.677 (−1.790, 0.435)
FreeT3[f]	−0.013 (−0.029, −0.003)	**−0.021 (-0.037, -0.004)** *	**−0.021 (-0.040, -0.002)** *
FreeT4[g]	−0.042 (−0.154, 0.071)	−0.039 (−0.162, 0.083)	−0.033 (−0.163, 0.096)
TSH[h]	0.290 (−5.191, 5.771)	0.984 (−5.412, 7.380)	0.537 (−6.745, 7.818)
Gestational age (weeks)	−0.054 (−2.171, 2.063)	−0.343 (−1.940, 1.254)	0.109 (−1.604, 1.822)
Birth weight (g)	19.873 (−437.97, 477.720)	−14.368 (−65.946, 37.211)	5.965 (−44.652, 56.583)
Head circumference (cm)	−0.062 (−1.634, 1.510)	−0.089 (−1.176, 0.997)	−0.183 (−1.312, 0.946)
Birth length (cm)	0.050 (−2.209, 2.309)	0.092 (−0.204, 0.388)	−0.018 (−0.310, 0.275)
Ponderal index (g/cm^3)	0.023 (−0.129, 0.175)	0.014 (−0.029, 0.056)	−0.004 (−0.045, 0.038)
Cord pH	−0.028 (−0.077, 0.021)	−0.010 (−0.068, 0.048)	−0.023 (−0.089, 0.042)
Base deficit	−0.418 (−2.689, 1.853)	1.029 (−1.697, 3.756)	1.397 (−1.785, 4.579)
Apgar score at 1 min	−0.050 (−1.020, 0.920)	−0.217 (−1.132, 0.697)	0.375 (−0.430, 1.180)
Apgar score at 5-min	−0.314 (−0.830, 0.203)	**−0.538 (-1.061, - 0.015)** *	**−0.477 (-0.902, -0.051)** *

*$p < 0.05$; # borderline significance ($p < 0.1$).
[a] CI, confidence interval.
[b] Model I was adjusted for maternal age, maternal pre-pregnant BMI, parity, smoking status (indirect smoking status for maternal level), parental education levels, household income, infant sex, gestational age, birth weight, birth length, head circumference, and Ponderal index.
[c] Model II was adjusted for maternal age, maternal pre-pregnant BMI, parity, smoking status (indirect smoking status for maternal level), parental education levels, household income, infant sex, gestational age, birth weight, birth length, head circumference, Ponderal index, birth morbidity, Apgar score at 1 min, and Apgar score at 5 min.
[d] T3, Triiodothyronine.
[e] T4, Thyroxine.
[f] Free T3, Free triiodothyronine.
[g] FreeT4, Free Thyroxine.
[h] TSH, Thyroid-stimulating hormone.

Herin et al. (2011) also reported serum fipronil and fipronil sulfone levels among 159 workers from a factory manufacturing fipronil-containing veterinary drugs. Among these 159 workers, serum fipronil sulfone was detected in 155 samples, with levels ranging from 0.37 to 42.45 ng/mL, which are higher than those measured in our general population. In the same study of 159 workers, serum fipronil levels were detectable in only 33 workers (20.1%), attributable to its short half-life in serum; the detected serum fipronil concentrations probably corresponded to the previous 24 h of occupational exposure.

We observed a strong correlation for fipronil sulfone levels between family members (i.e. maternal levels vs. paternal or infantile levels; paternal levels vs. infantile levels), suggesting that mother and biologically matched father (husband) likely share similar exposure routes, including lifestyle and diet. Furthermore, our multiple regression models showed that both maternal and paternal fipronil sulfone levels were significantly associated with household income after adjustments. It is generally known that a lower income increases the risk of exposure to environmental hazards, including pesticides and air pollutants (Evans and Kantrowitz, 2002; Ma et al., 2002; Mohd Shariff et al., 2015). There are few studies regarding fipronil exposure among humans, especially urban residents; thus, it is hard to directly compare the association between household income and fipronil sulfone levels reported here with the results of other studies. Exposure to fipronil or fipronil sulfone likely occurred via dietary intake, dermal contact, or household dust; for those with indoor pets, exposure may be attributable to the use of veterinary medicine (Chun and Kang, 2003; Cochran et al., 2015: Mahler et al., 2009). Households with higher incomes are more likely to own pets (i.e. dogs) (Saunders et al., 2017), providing a higher chance for exposure to fipronil from dogs treated for fleas and ticks (Jennings et al., 2002). However, only one family reported owning a dog in our study; thus, contact with pets would not be the main route of fipronil exposure, and dietary intake is likely an important factor in our study. Furthermore, Higher income households tend to spend more money per person per month on food eaten both at home and when dining out than lower income households (French et al., 2010); thus, they may be more prone to eating meats (i.e. chicken), eggs, or grains contaminated with fipronil.

Interestingly, our results show that infantile fipronil sulfone levels were inversely associated with maternal pre-pregnant BMI after adjusting for confounders, while maternal fipronil sulfone levels were not associated with pre-pregnant BMI. Again, there are no known studies available to compare with this inverse association between maternal pre-pregnant BMI and infantile fipronil sulfone levels. However, fipronil sulfone is lipophilic and therefore preferably stored in adipose tissue and slowly eliminated from the fat. Consequently, lower serum fipronil sulfone levels may be measured in persons with a higher BMI. While not discussed here, several studies of persistent organic pollutants (POPs), a group of lipophilic chemicals that includes organochlorine pesticides (OCP) and polychlorinated biphenyls (PCBs), reported a similar inverse relationship (Agudo et al., 2009; Dirinck et al., 2011; Wolff et al., 2005). During the early pregnancy (before actual weight gain), pregnant women with a lower BMI might have higher serum fipronil sulfone levels; consequently, this may affect infantile exposure levels throughout the pregnancy period and be involved in fetal programing (Kind et al., 2006). Furthermore, the lack of an association between maternal fipronil sulfone levels and pre-pregnant BMI is likely attributable to the increased weight gain observed during the later stages of pregnancy, which might affect serum fipronil sulfone levels. However, additional studies using a larger sample size or animal models are needed to confirm this.

Fipronil has selective toxicity toward insects rather than mammals (Hainzl et al., 1998); however, it is becoming increasingly evident that fipronil causes neurotoxic, hepatotoxic, nephrotoxic, and cytotoxic effects in humans and animals, as well as reproductive and endocrine toxicities (Das et al., 2006; de Oliveira et al., 2012; Khan et al., 2015; Ohi et al., 2004; Romero et al., 2016; Roques et al., 2013). In recent years, oxidative stress has been suggested to play a critical role in fipronil-induced toxicities by altering the antioxidant defense system, thereby damaging cellular macromolecules such as lipids, proteins, and DNA (Badgujar et al., 2016; Clasen et al., 2012; Khan et al., 2015; Slotkin and Seidler, 2005; Wang et al., 2016). Mechanisms underlying the toxicity associated with fipronil metabolites, including fipronil sulfone, are relatively unknown; however, fipronil sulfone has been suggested to be the cause of fipronil-induced toxicity rather than fipronil itself (Wang et al., 2016). Fipronil sulfone reportedly has a 6-fold greater binding affinity for GABA receptors in mice and humans (Hainzl et al., 1998) and higher toxicity in mammals than does fipronil (Das et al., 2006; Romero et al., 2016).

The adverse effects of pesticides on thyroid dysfunction and thyroid cancer have been investigated (Goldner et al., 2010; Maervoet et al., 2007; Meeker et al., 2007; Sørmo et al., 2005; Scollon et al., 2004; Tingle et al., 2003; Zhang et al., 2010). Several animal and human studies revealed that pesticides, including dichlorodiphenyltrichloroethane (DDT), thiocarbamate, insecticides, and fungicides can act as thyroid disruptors by decreasing T4, freeT4, T3, and free T3 levels as well as increasing TSH levels (Brucker-Davis, 1998; Farokhi and Taravati, 2014; Garry, 2005; Toft et al., 2006). Fipronil has also been reported to cause thyroid disruption in several studies (Grimalt et al., 1994; Herin et al., 2011; Leghait et al., 2009; Roques et al., 2012). Fipronil disrupts GABAergic signaling activity (Cole et al., 1993), and the GABA system closely interacts with thyroid hormones (Wiens and Trudeau, 2006). An *in vitro* study by Lu et al. (2015) showed that fipronil sulfone has endocrine disrupting potential via thyroid hormone receptor (TR)β antagonistic activity. Further, significant disorganization in the thyroidean tissue and follicular alterations were observed in fipronil-exposed mice (Ferreira et al., 2012), and fipronil treatment increased the plasma concentration of TSH, decreased the concentration of T4, and increased the prevalence of thyroid gland tumors in rats (FAO, 1998). In a human study, fipronil sulfone concentrations were negatively correlated with serum TSH concentrations in fipronil production facility workers occupationally exposed to fipronil, suggesting that fipronil has a central inhibitory effect on TSH secretion in humans (Herin et al., 2011).

Our adjusted analyses identified significant inverse associations between infantile fipronil sulfone levels and cord blood T3 and free T3 levels as well as 5-min Apgar scores in newborn infants. These results provide the first evidence that *in utero* fipronil sulfone exposure adversely affects thyroid hormone levels and subsequent developmental defects. Fetal thyroid hormone levels at birth are associated with fetal growth, since thyroid hormones play a crucial role in fetal development as well as neurodevelopment and metabolism (Alvarez-Pedrerol et al., 2009; Ausó et al., 2004; Bernal, 2007; Cam et al., 2018; Cuevas et al., 2005; Lavado-Autric et al., 2003). Furthermore, thyroid status and timing during development has a significant impact on behavior, locomotor ability, speech, and cognition (Anderson, 2008; Bernal, 2007; Costeira et al., 2011; Zoeller and Rovet, 2004). Human fetuses are particularly sensitive to pesticide exposure, attributable to rapidly developing brains and underdeveloped detoxification systems (Eskenazi et al., 2008). Several previous studies have shown that *in utero* exposure to pesticides, including organophosphate pesticides, can adversely affect fetal development (Barr et al., 2010; Perera et al., 2003).

In this study, 5-min Apgar scores were inversely associated with infantile fipronil sulfone levels, suggesting *in utero* exposure to fipronil sulfone may induce subsequent health risk in infants. Lower 5-min Apgar scores are considered a marker for developmental vulnerability in 5-year-old children (Razaz et al., 2016), including an increased risk of developing several diseases such as premature retinopathy (Marinov et al., 2017), childhood cancer (Li et al., 2012), and psychosis (Kotlicka-Antczak et al., 2014). Although our mean 5-min Apgar scores (9.07 ± 0.64) were relatively high, additional studies with larger sample sizes are needed to confirm subsequent health effects in infants born with lower Apgar scores and higher fipronil sulfone levels.

Our study has a few limitations. First, our sample size (59 mother-neonate pairs and 51 matching biological fathers) was relatively small. In addition to the small sample size, we lacked dietary data, although we assume fipronil exposure among our study participants was mainly attributable to dietary intake; therefore, these factors should be considered when interpreting these results and merits caution. Second, this study is a cross-sectional study based on analyses of blood samples (for fipronil and fipronil sulfone levels as well as thyroid hormone levels in cord blood) and demographic, physiological, behavioral, socio-economic, and clinical data collected simultaneously at birth; thus, determinations of causal association are limited. Furthermore, lack of information regarding serum lipid levels is another limitation, attributable to the lipophilic properties of fipronil (O'Brien et al., 2017); thus, consideration of serum lipids is needed when describing fipronil concentration and interpreting associations.

Despite these limitations, our study provides the distribution of fipronil sulfone in a general and sensitive population. Furthermore, our study is the first to evaluate adverse birth outcomes in newborns in relation to maternal and infantile fipronil sulfone exposure via transplacental transmission. However, these findings should be confirmed by a more appropriate longitudinal design with larger sample size, as well as more information regarding possible routes of fipronil exposure (i.e. diet or house dust).

5. Conclusions

Serum fipronil sulfone was detected in a specific population of mother-neonate pairs and their matched biological fathers in a manner suggestive of regular exposure to fipronil among general urban residents. Furthermore, this study is the first to show serum fipronil sulfone placentally transfers to the fetus and adversely affects infantile health outcomes, including measurements of thyroid function and 5-min Apgar scores.

Declarations of interest

The authors declare they have no actual or potential competing financial interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2019.01.009.

References

Agudo, A., Goñi, F., Etxeandia, A., Vives, A., Millán, E., López Amiano, P., Ardanaz, E., Barricarte, A., Chirlaque, M.D., Dorronsoro, M., Jakszyn, P., Larrañaga, N., Martínez, C., Navarro, C., Rodríguez, L., Sánchez, M.J., Tormo, M.J., González, C.A., 2009. Polychlorinated biphenyls in Spanish adults: determinants of serum concentrations. Environ. Res. 109, 620–628.

Alvarez-Pedrerol, M., Guxens, M., Mendez, M., Canet, Y., Martorell, R., Espada, M., Plana, E., Rebagliato, M., Sunyer, J., 2009. Iodine levels and thyroid hormones in healthy pregnant women and birth weight of their offspring. Eur. J. Endocrinol. 160, 423–429.

Anderson, G.W., 2008. Thyroid hormone and cerebellar development. Cerebellum 7, 60–74.

Ausó, E., Lavado-Autric, R., Cuevas, E., Del Rey, F.E., Morreale De Escobar, G., Berbel, P., 2004. A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticogenesis alters neuronal migration. Endocrinology 145, 4037–4047.

Badgujar, P.C., Chandratre, G.A., Pawar, N.N., Telang, A.G., Kurade, N.P., 2016. Fipronil induced oxidative stress involves alterations in sod1 and catalase gene expression in male mice liver: Protection by vitamins E and C. Environ. Toxicol. 31, 1147–1158.

Barr, D.B., Ananth, C.V., Yan, X., Lashley, S., Smulian, J.C., Ledoux, T.A., Hore, P., Robson, M.G., 2010. Pesticide concentrations in maternal and umbilical cord sera and their relation to birth outcomes in a population of pregnant women and newborns in New Jersey. Sci. Total Environ. 408 (4), 790–795.

Bernal, J., 2007. Thyroid hormone receptors in brain development and function. Nat. Clin. Pract. Endocrinol. Metabol. 3, 249–259.

Bichon, E., Richard, C.A., Le Bizec, B., 2008. Development and validation of a method for fipronil residue determination in ovine plasma using 96-well plate solid-phase extraction and gas chromatography-tandem mass spectrometry. J. Chromatogr., A 1201, 91–99.

Bobé, A., Coste, C.M., Cooper, J.F., 1997. Factors influencing the adsorption of fipronil on soils. J. Agric. Food Chem. 45, 4861–4865.

Brennan, A.A., Harwood, A.D., You, J., Landrum, P.F., Lydy, M.J., 2009a. Degradation of fipronil in anaerobic sediments and the effect on porewater concentrations. Chemosphere 77, 22–28.

Brennan, A.A., You, J., Lydy, M.J., 2009b. Comparison of cleanup methods for fipronil and its degradation products in sediment extracts. Talanta 78, 1408–1413.

Brucker-Davis, F., 1998. Effects of environmental synthetic chemicals on thyroid function. Thyroid 8, 827–856.

Caboni, P., Sammelson, R.E., Casida, J.E., 2003. Phenylpyrazole insecticide photochemistry, metabolism, and GABAergic action: ethiprole compared with fipronil. J. Agric. Food Chem. 51, 7055–7061.

Cam, M., Durieu, E., Bodin, M., Manousopoulou, A., Koslowski, S., Vasylieva, N., Barnych, B., Hammock, B.D., Bohl, B., Koch, P., Omori, C., Yamamoto, K., Hata, S., Suzuki, T., Karg, F., Gizzi, P., Haber, V.E., Bencetic Mihaljevic, V., Tavcar, B., Porteliu, s E., Pannee, J., Blennow, K., Zetterberg, H., Garbis, S.D., Auvray, P., Gerber, H., Fraering, J., Fraering, P.C., Meijer, L., 2018. Induction of amyloid-β42 production by fipronil and other pyrazple insecticides. J. Alzheimers Dis. 62 (4), 1663–1681.

Chen, M.H., Ha, E.H., Wen, T.W., Su, Y.N., Lien, G.W., Chen, C.Y., Chen, P.C., Hsieh, W.S., 2012. Perfluorinated compounds in umbilical cord blood and adverse birth outcomes. PLoS One 7, e42474.

Chodorowski, Z., Anand, J.S., 2004. Accidental dermal and inhalation exposure with fipronil–a case report. J. Toxicol. Clin. Toxicol. 42 (2), 189–190.

Chun, O.K., Kang, H.G., 2003. Estimation of risks of pesticide exposure, by food intake, to Koreans. Food Chem. Toxicol. 41 (8), 1063–1076.

Clasen, B., Loro, V.L., Cattaneo, R., Moraes, B., Lópes, T., de Avila, L.A., Zanella, R., Reimche, G.B., Baldisserotto, B., 2012. Effects of the commercial formulation containing fipronil on the non-target organism cyprinus carpio: implications for rice–fish cultivation. Ecotoxicol. Environ. Saf. 77, 45–51.

Cochran, R.C., Yu, L., Krieger, R.I., Ross, J.H., 2015. Postapplication fipronil exposure following use on pets. J. Toxicol. Environ. Health A. 78 (19), 1217–1226.

Cole, L.M., Nicholson, R.A., Casida, J.E., 1993. Action of phenylpyrazole insecticides at the GABA-gated chloride channel. Pestic. Biochem. Physiol. 46, 47–54.

Costeira, M.J., Oliveira, P., Santos, N.C., Ares, S., Saenz-Rico, B., de Escobar, G.M., Palha, J.A., 2011. Psychomotor development of children from an iodine-deficient region. J. Pediatr. 159, 447–453.

Cravedi, J., Delous, G., Zalko, D., Viguie, C., Debrauwer, L., 2013. Disposition of fipronil in rats. Chemosphere 93, 2276–2283.

Cuevas, E., Ausó, E., Telefont, M., Morreale de Escobar, G., Sotelo, C., Berbel, P., 2005. Transient maternal hypothyroxinemia at onset of corticogenesis alters tangential migration of medial ganglionic eminence-derived neurons. Eur. J. Neurosci. 22, 541–551.

Das, P.C., Cao, Y., Cherrington, N., Hodgson, E., Rose, R.L., 2006. Fipronil induces cyp isoforms and cytotoxicity in human hepatocytes. Chem. Biol. Interact. 164, 200–214.

de Oliveira, P.R., Bechara, G.H., Denardi, S.E., Oliveira, R.J., Mathias, M.I., 2012. Genotoxic and mutagenic effects of fipronil on mice. Exp. Toxicol. Pathol. 64, 569–573.

Dirinck, E., Jorens, P.G., Covaci, A., Geens, T., Roosens, L., Neels, H., Mertens, I., Van Gaal, L., 2011. Obesity and persistent organic pollutants: possible obesogenic effect of organochlorine pesticides and polychlorinated biphenyls. Obesity 19, 709–714.

Eskenazi, B., Rosas, L.G., Marks, A.R., Bradman, A., Harley, K., Holland, N., Johnson, C., Fenster, L., Barr, D.B., 2008. Pesticide toxicity and the developing brain. Basic Clin. Pharmacol. Toxicol. 102 (2), 228–236.

Evans, G.W., Kantrowitz, E., 2002. Socioeconomic status and health: the potential role of environmental risk exposure. Annu. Rev. Public Health 23, 303–331.

FAO, 1998. Pesticide Residues in Food-1997. Report of the Joint Meeting of the Fao Panel of Experts on Pesticide Residues in Food and the Environment and the Who Core Assessment Group on Pesticide Residues. FAO Plant Production and Protection Paper 145, Lyons, france 22 sept-1oct 1997.

Farokhi, F., Taravati, A., 2014. Pesticide exposure and thyroid function in adult male sprayers. Int. J. Med. Invest. 3, 127–132.

Fei, C., McLaughlin, J.K., Tarone, R.E., Olsen, J., 2007. Perfluorinated chemicals and fetal growth: a study within the Danish national birth cohort. Environ. Health Perspect. 115, 1677–1682.

Ferreira, M., De Oliveira, P.R., Denardi, S.E., Bechara, G.H., Mathias, M.I., 2012. Fipronil (active ingredient of acaricide Frontline®) acting on the mice thyroid. Microsc. Res. Tech. 75 (3), 265–270.

French, S.A., Wall, M., Mitchell, N.R., 2010. Household income differences in food sources and food items purchased. Int. J. Behav. Nutr. Phys. Activ. 7, 77.

Garry, V.F., 2005. Biomarkers of thyroid function, genotoxicity and agricultural fungicide use. J. Biochem. Mol. Toxicol. 19, 175.

Goldner, W.S., Sandler, D.P., Yu, F., Hoppin, J.A., Kamel, F., Levan, T.D., 2010. Pesticide use and thyroid disease among women in the agricultural health study. Am. J. Epidemiol. 171, 455–464.

Grimalt, J.O., Sunyer, J., Moreno, V., Amaral, O.C., Sala, M., Rosell, A., Anto, J.M., Albaiges, J., 1994. Risk excess of soft-tissue sarcoma and thyroid cancer in a community exposed to airborne organochlorinated compound mixtures with a high hexachlorobenzene content. Int. J. Canc. 56, 200–203.

Gunasekara, A.S., Truong, T., Goh, K.S., Spurlock, F., Tjeerdema, R.S., 2007. Environmental fate and toxicology of fipronil. J. Pestic. Sci. 32, 189–199.

Hainzl, D., Cole, L.M., Casida, J.E., 1998. Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct. Chem. Res.

Toxicol. 11, 1529–1535.
Hamm, M.P., Cherry, N.M., Chan, E., Martin, J.W., Burstyn, I., 2010. Maternal exposure to perfluorinated acids and fetal growth. J. Expo. Sci. Environ. Epidemiol. 20, 589–597.
Hayasaka, D., Korenaga, T., Suzuki, K., Sánchez-Bayo, F., Goka, K., 2012a. Differences in susceptibility of five cladoceran species to two systemic insecticides, imidacloprid and fipronil. Ecotoxicology 21, 421–427.
Hayasaka, D., Korenaga, T., Suzuki, K., Saito, F., Sánchez-Bayo, F., Goka, K., 2012b. Cumulative ecological impacts of two successive annual treatments of imidacloprid and fipronil on aquatic communities of paddy mesocosms. Ecotoxicol. Environ. Saf. 80, 355–362.
Herin, F., Boutet-Robinet, E., Levant, A., Dulaurent, S., Manika, M., Galatry-Bouju, F., Caron, P., Soulat, J.M., 2011. Thyroid function tests in persons with occupational exposure to fipronil. Thyroid 21, 701–706.
Hurley, P.M., 1998. Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents. Environ. Health Perspect. 106 (8), 437–445.
Jennings, K.A., Canerdy, T.D., Keller, R.J., Atieh, B.H., Doss, R.B., Gupta, R.C., 2002. Human exposure to fipronil from dogs treated with frontline. Vet. Hum. Toxicol. 44, 301–303.
Kapelari, K., Kirchlechner, C., Hogler, W., Schweitzer, K., Virgolini, I., Moncayo, R., 2008. Pediatric reference intervals for thyroid hormone levels from birth to adulthood: a retrospective study. BMC Endocr. Disord. 8, 15.
Khan, S., Jan, M.H., Kumar, D., Telang, A.G., 2015. Firpronil induced spermotoxicity is associated with oxidative stress, DNA damage and apoptosis in male rats. Pestic. Biochem. Physiol. 124, 8–14.
Kind, K.L., Moore, V.M., Davies, M.J., 2006. Diet around conception and during pregnancy–effects on fetal and neonatal outcomes. Reprod. Biomed. Online 12, 532–541.
Kotlicka-Antczak, M., Pawełczyk, A., Rabe-Jabłońska, J., Smigielski, J., Pawełczyk, T., 2014. Obstetrical complications and Apgar score in subjects at risk of psychosis. J. Psychiatr. Res. 48 (1), 79–85.
Lavado-Autric, R., Ausó, E., García-Velasco, J.V., Arufe Mdel, C., Escobar del Rey, F., Berbel, P., Morreale de Escobar, G., 2003. Early maternal hypothyroxinemia alters histogenesis and cerebral cortex cytoarchitecture of the progeny. J. Clin. Invest. 111, 1073–1082.
Leghait, J., Gayrard, V., Picard-Hagen, N., Camp, M., Perdu, E., Toutain, P.L., Viguié, C., 2009. Fipronil-induced disruption of thyroid function in rats is mediated by increased total and free thyroxine clearances concomitantly to increased activity of hepatic enzymes. Toxicology 255, 38–44.
Leghait, J., Gayrard, V., Toutain, P.L., Picard-Hagen, N., Viguié, C., 2010. Is the mechanisms of fipronil-induced thyroid disruption specific of the rat: Re-evaluation of fipronil thyroid toxicity in sheep? Toxicol. Lett. 194, 51–57.
Li, J., Cnattingus, S., Gissler, M., Vestergaard, M., Obel, C., Ahrensberg, J., Olsen, J., 2012. The 5-minute Apgar score as a predictor of childhood cancer: a population-based cohort study in five million children. BMJ Open 2 (4), e001095 pii.
Liu, T., Wang, P., Lu, Y., Zhou, G., Diao, J., Zhou, Z., 2012. Enantioselective bioaccumulation of soil-associated fipronil enantiomers in tubifex tubifex. J. Hazard Mater. 219–220, 50–56.
Lu, M., Du, J., Zhou, P., Chen, H., Lu, C., Zhang, Q., 2015. Endocrine disrupting potential of fipronil and its metabolite in reporter gene assays. Chemosphere 120, 246–251.
Ma, X., Buffler, P.A., Gunier, R.B., Dahl, G., Smith, M.T., Reinier, K., Reynolds, P., 2002. Critical windows of exposure to household pesticides and risk of childhood leukemia. Environ. Health Perspect. 110, 955–960.
Maervoet, J., Vermeir, G., Covaci, A., Van Larebeke, N., Koppen, G., Schoeters, G., Nelen, V., Baeyens, W., Schepens, P., Viaene, M.K., 2007. Association of thyroid hormone concentrations with levels of organochlorine compounds in cord blood of neonates. Environ. Health Perspect. 115 (12), 1780–1786.
Magalhães, J.Z., Udo, M.S., Sánchez-Sarmiento, A.M., Carvalho, M.P., Bernardi, M.M., Spinosa, H.S., 2015. Prenatal exposure to fipronil disturbs maternal aggressive behavior in rats. Neurotoxicol. Teratol. 52 (Pt A), 11–16.
Mahler, B.J., Van Metre, P.C., Wilson, J.T., Musgrove, M., Zaugg, S.D., Burkhardt, M.R., 2009. Fipronil and its degradates in indoor and outdoor dust. Environ. Sci. Technol. 43 (15), 5665–5670.
Maisonet, M., Terrell, M.L., McGeehin, M.A., Christensen, K.Y., Holmes, A., Calafat, A.M., Marcus, M., 2012. Maternal concentrations of polyfluoroalkyl compounds during pregnancy and fetal and postnatal growth in british girls. Environ. Health Perspect. 120 (10), 1432–1437.
Marinov, V.G., Koleva-Georgieva, D.N., Sivkova, N.P., Krasteva, M.B., 2017. The 5-minute Apgar score as a prognostic factor for development and progression of retinopathy of prematurity. Folia Med. (Plovdiv) 59 (1), 78–83.
McMahen, R.L., Strynar, M.J., Dagnino, S., Herr, D.W., Moser, V.C., Garantziotis, S., Andersen, E.M., Freeborn, D.L., McMillan, L., Lindstrom, A.B., 2015. Identification of fipronil metabolites by time-of-flight mass spectrometry for application in a human exposure study. Envrion. Int. 78, 16–23.
Meeker, J.D., Altshul, L., Hauser, R., 2007. Serum pcbs, p, p′-dde and hcb predict thyroid hormone levels in men. Environ. Res. 104 (2), 296–304.
Mohamed, F., Senarathna, L., Percy, A., Abeyewardene, M., Eaglesham, G., Cheng, R., Azher, S., Hittarage, A., Dissanayake, W., Sheriff, M.H., Davies, W., Buckley, N.A., Eddleston, M., 2004. Acute human self-poisoning with the n-phenylpyrazole insecticide fipronil—a gabaa-gated chloride channel blocker. J. Toxicol. Clin. Toxicol. 42, 955–963.
Mohd Shariff, Z., Lin, K.G., Sariman, S., Lee, H.S., Siew, C.Y., Mohd Yusof, B.N., Mun, C.Y., Mohamad, M., 2015. The relationship between household income and dietary intakes of 1-10 year old urban malaysian. Nutr. Res. Pract. 9, 278–287.
Narahashi, T., Zhao, X., Ikeda, T., Nagata, K., Yeh, J.Z., 2007. Differential actions of insecticides on target sites: basis for selective toxicity. Hum. Exp. Toxicol. 26, 361–366.
Narahashi, T., Zhao, X., Ikeda, T., Salgado, V.L., Yeh, J.Z., 2010. Glutamate-activated chloride channels: unique fipronil targets present in insects but not in mammals. Pestic. Biochem. Physiol. 97, 149–152.
O'Brien, K.M., Upson, K., Buckley, J.P., 2017. Lipid and creatinine adjustment to evaluate health effects of environmental exposures. Curr. Environ. Health Rep. 4 (1), 44–50.
Oddie, T.H., Bernard, B., Klein, A.H., Fisher, D.A., 1979. Comparison of T4, T3, rT3 and TSH concentrations in cord blood and serum of infants up to 3 months of age. Early Hum. Dev. 3 (3), 239–244.
Ohi, M., Dalsenter, P.R., Andrade, A.J., Nascimento, A.J., 2004. Reproductive adverse effects of fipronil in wistar rats. Toxicol. Lett. 146, 121–127.
Perera, F.P., Rauh, V., Tsai, W.Y., Kinney, P., Camann, D., Barr, D., Bernert, T., Garfinkel, R., Tu, Y.H., Diaz, D., Dietrich, J., Whyatt, R.M., 2003. Effects of transplacental exposure to environmental pollutants on birth outcomes in a multiethnic population. Environ. Health Perspect. 111 (2), 201–205.
Razaz, N., Boyce, W.T., Brownell, M., Jutte, D., Tremlett, H., Marrie, R.A., Joseph, K.S., 2016. Five-minute Apgar score as a marker for developmental vulnerability at 5 years of age. Arch. Dis. Child. Fetal Neonatal Ed. 101 (2), F114–F120.
Romero, A., Ramos, E., Ares, I., Castellano, V., Martínez, M., Martínez-Larrañaga, M.R., Anadón, A., Martínez, M.A., 2016. Fipronil sulfone induced higher cytotoxicity than fipronil in sh-sy5y cells: Protection by antioxidants. Toxicol. Lett. 252, 42–49.
Roques, B.B., Lacroix, M.Z., Puel, S., Gayrard, V., Picard-Hagen, N., Jouanin, I., Perdu, E., Martin, P.G., Viguié, C., 2012. CYP450-dependent biotransformation of the insecticide fipronil into fipronil sulfone can mediate fipronil-induced thyroid disruption in rats. Toxicol. Sci. 127, 29–41.
Roques, B.B., Leghait, J., Lacroix, M.Z., Lasserre, F., Pineau, T., Viguié, C., Martin, P.G., 2013. The nuclear receptors pregnane x receptor and constitutive androstane receptor contribute to the impact of fipronil on hepatic gene expression linked to thyroid hormone metabolism. Biochem. Pharmacol. 86, 997–1039.
Saunders, J., Parast, L., Babey, S.H., Miles, J.V., 2017. Exploring the differences between pet and non-pet owners: implications for human-animal interaction research and policy. PLoS One 12, e0179494.
Scollon, E.J., Carr, J.A., Cobb, G.P., 2004. The effect of flight, fasting and p, p′-DDT on thyroid hormones and corticosterone in Gambel's white-crowned sparrow, Zonotrichia leucophrys gambelli. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 137, 179–189.
Simon-Delso, N., Amaral-Rogers, V., Belzunces, L.P., Bonmatin, J.M., Chagnon, M., Downs, C., Furlan, L., Gibbons, D.W., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C.H., Liess, M., Long, E., McField, M., Mineau, P., Mitchell, E.A., Morrissey, C.A., Noome, D.A., Pisa, L., Settele, J., Stark, J.D., Tapparo, A., Van Dyck, H., Van Praagh, J., Van der Sluijs, J.P., Whitehorn, P.R., Wiemers, M., 2015. Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. Environ. Sci. Pollut. Res. Int. 22, 5–34.
Slotkin, T.A., Seidler, F.J., 2005. The alterations in cns serotonergic mechanisms caused by neonatal chlorpyrifos exposure are permanent. Brain Res. Dev. Res. 158, 115–119.
Sørmo, E.G., Jüssi, I., Jüssi, M., Braathen, M., Skaare, J.U., Jenssen, B.M., 2005. Thyroid hormone status in baltic and atlantic gray seal (halichoerus grypus) pups in relation to polychlorinated biphenyls and organochlorine pesticides. Environ. Toxicol. Chem. 24, 610–616.
Stone, W.W., Gilliom, R.J., Ryberg, K.R., 2014. Pesticides in U.S. streams and rivers: occurrence and trends durng 1992-2011. Envrion. Sci. Technol. 48 (19), 11025–11030.
Tang, J., Amin Usmani, K., Hodgson, E., Rose, R.L., 2004. In vitro metabolism of fipronil by human and rat cytochrome p450 and its interactions with testosterone and diazepam. Chem. Biol. Interact. 147, 319–329.
Tingle, C.C., Rother, J.A., Dewhurst, C.F., Lauer, S., King, W.J., 2003. Fipronil: environmental fate, ecotoxicology, and human health concerns. Rev. Environ. Contam. Toxicol. 176, 1–66.
Toft, G., Flyvbjerg, A., Bonde, J.P., 2006. Thyroid function in Danish greenhouse workers. Environ. Health 5, 32.
Udo, M.S., Sandini, T.M., Reis, T.M., Bernardi, M.M., Spinosa, H.S., 2014. Prenatal exposure to a low fipronil dose disturbs maternal behavior and reflex development in rat. Neurotoxicol. Teratol. 45, 27–33.
Vidau, C., Brunet, J.L., Badiou, A., Belzunces, L.P., 2009. Phenylpyrazole insecticides induce cytotoxicity by altering mechanisms involved in cellular energy supply in the human epithelial cell model caco-2. Toxicol. Vitro 23, 589–597.
Vidau, C., González-Polo, R.A., Niso-Santano, M., Gómez-Sánchez, R., Bravo-San Pedro, J.M., Pizarro-Estrella, E., Blasco, R., Brunet, J.L., Belzunces, L.P., Fuentes, J.M., 2011. Fipronil is a powerful uncoupler of oxidative phosphorylation that triggers apoptosis in human neuronal cell line shsy5y. Neurotoxicology 32, 935–943.
Wang, X., Martínez, M.A., Wu, Q., Ares, I., Martínez-Larrañaga, M.R., Anadón, A., Yuan, Z., 2016. Fipronil insecticide toxicology: oxidative stress and metabolism. Crit. Rev. Toxicol. 46, 876–899.
Wiens, S.C., Trudeau, V.L., 2006. Thyroid hormone and gamma-aminobutyric acid (GABA) interactions in neuroendocrine system. Comp. Biochem. Physiol. Mol. Integr. Physiol. 144, 332–344.
Wolff, M.S., Britton, J.A., Teitelbaum, S.L., Eng, S., Deych, E., Ireland, K., Liu, Z., Neugut, A.I., Santella, R.M., Gammon, M.D., 2005. Improving organochlorine biomarker models for cancer research. Cancer Epidemiol. Biomark. Prev. 14, 2224–2236.
World Health Organization, Research, SPf, Diseases, TiT, Diseases WHODoCoNT, Epidemic WHO, Alert, P., 2009. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control. WHO, Geneva.
Zhang, J., Jiang, Y., Zhou, J., Wu, B., Liang, Y., Peng, Z., Fang, D., Liu, B., Huang, H., He, C., Wang, C., Lu, F., 2010. Elevated body burdens of pbdes, dioxins, and pcbs on thyroid hormone homeostasis at an electronic waste recycling site in China. Environ. Sci. Technol. 44, 3956–3962.
Zoeller, R., Rovet, J., 2004. Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings. J. Neuroendocrinol. 16, 809–818.