



Comparison of prenatal and postnatal exposure to neonicotinoids and their temporal trends in breast milk

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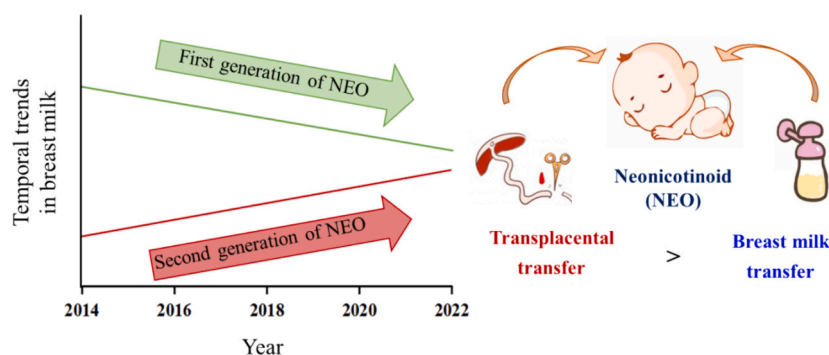
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HIGHLIGHTS

- Four p-NEOs and one m-NEO were widely detected in maternal-neonate samples.
- N-dm-ACE was the predominant NEO in all kinds of samples.
- N-dm-ACE (−30 %) and THM (30 %) had the highest annual changes in breast milk.
- The increasing trend of THM in level and contribution to EDI should be of concern.
- The neonatal prenatal period may be a more critical window of NEO exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

Although the potential effects of neonicotinoids (NEOs) in early life have received considerable attention, data on the exposure of mothers and infants to NEOs are scarce. In this study, four parent NEOs and one metabolite were widely detected in paired maternal serum (MS), umbilical cord serum (UCS) and breast milk (BM) samples, with median total NEO concentrations (Σ NEOs) of 113, 160 and 69 ng/L, respectively. Decreasing trends were observed for *N*-desmethyl-acetamiprid (30 %/year), acetamiprid (22 %/year) and Σ NEOs (15 %/year) in breast milk between 2014 and 2022, whereas increasing trends were seen for clothianidin (17 %/year) and thiamethoxam (30 %/year). *N*-desmethyl-acetamiprid was the predominant compound in all matrices. However, the contributions of *N*-desmethyl-acetamiprid (35 %) and thiamethoxam (36 %) in breast milk were similar in 2022. Moreover, thiamethoxam has become the predominant contributor to the estimated daily intake of Σ NEOs since

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2018, with the highest contribution of 71 % in 2022, suggesting the effects of NEOs continue to evolve and more attention should be paid to the new NEOs. Notably, the correlations and ratios of NEOs between paired UCS and MS were more significant and higher than those between paired BM and MS, respectively, indicating that NEO exposure was largely affected by the prenatal period.

1. Introduction

The introduction of neonicotinoids (NEOs) began in the early 1990s as replacements for conventional, long-established pesticide classes, such as chlorinated hydrocarbons, organophosphates, pyrethroids and carbamates (Jeschke and Nauen, 2008; Jeschke et al., 2011). The market share of NEOs grew to >25 % of total global pesticide sales by 2014 (Bass et al., 2015). As potent agonists of nicotinic acetylcholine receptors, they act selectively on insects' central nervous systems, causing paralysis and death (Jeschke and Nauen, 2008). However, many *in vitro* and *in vivo* experiments have shown that NEOs may have toxic effects in mammals, including hepatotoxicity (Arfat et al., 2014; Toor et al., 2013), neurotoxicity (Lonare et al., 2014; Ozdemir et al., 2014), reproductive toxicology and genetic toxicity (Kapoor et al., 2011; Galdikova et al., 2015; Kataria et al., 2016). Therefore, adverse effects of NEOs are of increasing public health concern.

In recent years, NEOs and their metabolites have been unequivocally detected in various human samples. Urine is the most frequently used matrix for analyses of NEOs and their metabolites. Based on urine samples, human exposure to NEOs was shown to be linked to an increased prevalence of neurological symptoms, such as postural finger tremor, recent memory loss and fever (Marfo et al., 2015). In another study, calculation of the estimated daily intake (EDI) of NEOs from urine and indoor dust measurements suggested that young females were more exposed to NEOs than young males (Zhang et al., 2021). Ueyama et al. investigated changes in NEO levels in urine samples from Japanese women between 1994 and 2011, and found that detection rates of urinary NEOs increased significantly during the period, suggesting higher exposure to NEOs over time (Ueyama et al., 2015).

The effects of NEO exposure on vulnerable populations are of particular concern. Data has shown that pregnant women in Japan are likely to be exposed to NEOs in their daily lives (Anai et al., 2021). Moreover, multiple NEOs have been detected in cerebrospinal fluid, plasma and urine samples from children (Laubscher et al., 2022). Boekelheide et al. has suggested that early-life pollutant exposure could increase susceptibility to diseases over a person's lifetime (Boekelheide et al., 2012). According to two mother–fetus population studies (Carmichael et al., 2014; Yang et al., 2014), maternal exposure to NEOs may be associated with birth defects in infants. For example, Ichikawa et al. observed higher urinary *N*-desmethyl-acetamiprid (*N*-dm-ACE) detection rates and levels in early gestational age infants than in normal gestational age infants, but no correlation was found between *N*-dm-ACE levels and infant physique indexes (Ichikawa et al., 2019). Dórea suggested that exposure to neurotoxicants in breast milk could correlate to lasting influences of predelivery exposure (Dorea, 2021). Considering the potentially harmful effects of early-life exposure to NEOs, it is crucial to evaluate levels of NEO exposure in fetuses or neonates through placental transfer and breastfeeding. However, data characterizing NEO transfer across the placenta and excretion into breast milk are so far limited.

In the present study, concentrations of parent NEOs (p-NEOs) and their metabolites (m-NEOs) in paired maternal serum, umbilical cord serum and breast milk samples, as well as breast milk samples from 2014 to 2022, were determined. The aims of this study were to investigate exposure to NEOs (including p-NEOs and m-NEOs) in infants through the placenta and breast milk and analyze temporal trends in exposure to NEOs in infants from breastfeeding between 2014 and 2022 in China.

2. Materials and methods

2.1. Chemicals and reagents

Seven native analytical standards (dinotefuran [DIN], *N*-dm-ACE, acetamiprid [ACE], clothianidin [CLO], imidacloprid [IMI], thiamethoxam [THM] and thiacloprid [THD]) and six internal standards (DIN-*d*₃, ACE-*d*₃, CLO-*d*₃, IMI-*d*₄, THM-*d*₃ and THD-*d*₄) were purchased from Sigma-Aldrich (St. Louis, MO, USA) with purity >97 %. The remaining two metabolites of IMI, olefin-imidacloprid (Of-IMI) and 5-hydroxy-imidacloprid (5-OH-IMI), were obtained from First Standard (Tianjin, China) with purity >98 %. Because labeled standards of m-NEOs are not commercially available, ACE-*d*₃ and IMI-*d*₄ were used as internal standards for the analysis of *N*-dm-ACE and two IMI metabolites, respectively. β -glucuronidase/arylsulfatase (30 U/mL and 60 U/mL, respectively) was used as hydrolase and purchased from Roche Diagnostics GmbH (Mannheim, Germany). Formic acid (purity >98 %) was bought from Anpel Technologies (Shanghai, China), acetonitrile and methanol (LC-MS grade) were purchased from Merck (Darmstadt, Germany) and ultrapure water (18.2 M Ω cm) was prepared using a Milli-Q system (Millipore Corp., Bedford, USA).

2.2. Sample collection

Thirty-two paired maternal serum, umbilical cord serum and breast milk samples were collected from pregnant women who gave birth at the Nanfang Hospital of Southern Medical University in 2022. These pregnant women did not experience any pregnancy complications during pregnancy and delivered at 39–41 weeks of gestation, reducing the possibility of interference from other disease factors on the distribution of NEOs in the body. Other breast milk samples from 2014 to 2020 were obtained from the Guangzhou Donor Milk Bank of the Guangzhou Women and Children's Medical Center. Due to limited storage conditions, only five breast milk samples per year were included from 2014 to 2018, whereas 25 samples per year were included for 2019 and 2022. At the time of recruitment, participating pregnant and lactating women were informed of the goals and requirements of the study and asked to sign a consent form. A physical health report was obtained for each participant certifying that they were free of infectious and genetic diseases. A detailed questionnaire, which included general demographic parameters, was completed by each participant. Demographic information on the participants is listed in the Supporting Information, Table S1. The day before delivery, venous blood samples were drawn from the mother and collected in 5 mL vacutainer anticoagulant-free serum tubes. Immediately after childbirth, umbilical cord blood samples were taken from the umbilical cord vein and collected in 5 mL vacutainer anticoagulant-free serum tubes. The serum was isolated by centrifugation at 3000 rpm for 10 min within 3 h of collection and then transferred to a 2 mL Eppendorf tube. Breast milk samples were collected 3–4 weeks after delivery. All samples were stored in the cold kept at -18 °C during transportation and at -80 °C until analysis. Ethical approval for the study was granted by the Medical Ethics committee of Nanfang Hospital of Southern Medical University (NFEC-2017-055).

2.3. Sample preparation

An automated magnetic solid phase extraction (mSPE) system (Suzhou Agile Biotech Co., Ltd., Suzhou, China, Fig. S1) was used to

extract NEOs in serum and breast milk samples. Application of mSPE to extract trace organic compounds from human biomatrices has been described previously by Huang et al. (2024). Prior to analysis, 200 μL of breast milk or serum sample was spiked with 5 μL β -glucuronidase enzyme and hydrolyzed at 37 $^{\circ}\text{C}$ in an incubator shaking for 12 h. After diluted with 600 μL of ultrapure water containing 2 % formic acid, the mixture was then added to the hole positions of rows 3 and 9. Next, each sample was spiked with 100 pg of each labeled internal standard solution in MeOH. A magnetic HLB sorbent (30–50 μm , Agile Bio, Suzhou, China) was selected as the extraction and purification material due to the broad-spectrum application of HLB packing. The sorbent beads were activated and equilibrated with 800 μL of MeOH and 600 μL of ultrapure water for 2 min, respectively. Subsequently, the magnetic HLB sorbent was transferred into the sample hole position and allowed to extract the target compounds for 2 min, before being magnetically separated and washed with 600 μL of ultrapure water for 2 min. Target NEOs were eluted with 600 μL of acetonitrile for 2 min. The above steps were repeated automatically to ensure that the magnetic SPE materials were fully transferred. Afterwards, the eluate was magnetically separated from the sorbent and then evaporated using a vacuum centrifugal concentrator (25 $^{\circ}\text{C}$, 1400 r/min, Ji Aim, Beijing, China). The residues were reconstructed with 100 μL of ultrapure water containing 25 % acetonitrile for liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) analysis.

2.4. Instrument analysis

Separation of the nine target chemicals was achieved using an ExionLC™ system (the UHPLC system) with a Poroshell 120 EC-C18 column (100 mm \times 4.6 mm, 2.7 μm ; Agilent Technologies, Santa Clara, CA, USA). The injection volume was 10 μL and the flow rate of the mobile phase was set at 0.35 mL/min. Solvent A (0.01 % formic acid in water) and solvent B (acetonitrile) were used as the mobile phase. The gradient elution program began with 5 % solvent B held for 2 min, then increased to 99 % solvent B within 6 min and held for 2 min, finally returned to the initial mobile phase conditions and equilibrated for 4.5 min before the next run. An API 6500 triple quadrupole tandem mass spectrometry system (QTRAP LC-MS/MS System, Sciex, Washington DC, WA, USA) was operated in positive ion mode to identify and quantify the target chemicals. The source temperature was set at 500 $^{\circ}\text{C}$, and the ion spray voltage was set at 5500 V. Further details of the mass transitions and multiple reaction monitoring (MRM) parameters are provided in Table S2.

2.5. Estimation of daily NEO intake and risk assessment

To evaluate potential risks posed to infants, a relative potency factor (RPF) approach was applied to integrate the cumulative exposure of NEOs and their metabolites present in the same breast milk sample into an imidacloprid-equivalent total NEO value (IMI_{eq} , ng/L) using Eq. (1). For N-dm-ACE, 5-OH-IMI and Of-IMI, the same RPF value of their corresponding parent NEOs was used in the IMI_{eq} calculation (Mahai et al., 2022; USEPA, 2010).

$$\text{IMI}_{\text{eq}} = \sum \text{RPF}_k \times C_k$$

$$= 2.9 \times C_{\text{DIN}} + 5.8 \times C_{\text{CLO}} + 14.2 \times C_{\text{THD}} + 1.0 \times C_{\text{IMI}} + 9.5 \times C_{\text{THM}} + 0.8 \times C_{\text{ACE}} + 0.8 \times C_{\text{N-dm-ACE}} + 1.0 \times C_{\text{5-OH-IMI}} + 1.0 \times C_{\text{Of-IMI}} \quad (1)$$

wherein RPF_k represents the relative potency factor of corresponding NEOs and C_k (ng L^{-1}) represents the concentration of individual NEOs.

Subsequently, EDI was calculated from Eq. (2):

$$\text{EDI} = \frac{\text{IMI}_{\text{eq}} \times \text{CV}}{\text{bw}} \quad (2)$$

where CV represents the breast milk consumption volume, assumed to be 0.75 L day^{-1} , bw represents the body weight, assumed to be 6 kg, and EDI is expressed in ng kg^{-1} of bw day^{-1} (Chen et al., 2020). Finally, the risk index (RI) (Chen et al., 2020), which can be used to assess the risk level of non-cancer-related health effects, was calculated from Eq. (3):

$$\text{RI} = \frac{\text{IMI}_{\text{eq}}}{\text{cRfD}_{\text{IMI}}} \quad (3)$$

where cRfD_{IMI} represents the chronic reference dose of IMI expressed in 0.057 ng kg^{-1} of bw day^{-1} , which was appropriate for infants who are more susceptible to external stimuli (Chen et al., 2020). An RI value of <1.0 indicated that EDI of the sum of all NEOs was likely not associated with chronic adverse effects.

2.6. Quality assurance and quality control (QA/QC)

Instrumental QC was checked by regular injection of solvent blanks and standard solutions for every batch of ten field samples. The instrumental QC values were 100 ± 20 % of the nominal values, indicating low batch-analysis variability. For method QC, a set of QC samples was evaluated using procedural blanks and spiked matrices. The recoveries of NEOs in spiked matrix samples (including blank bovine milk and fetal bovine serum samples) were 74.6–117 %. The limit of detection (LOD) of target compounds in breast milk and serum ranged from 0.6 to 10.0 ng/L and 0.6–20.0 ng/L, respectively, whereas the limit of quantitation (LOQ) ranged from 2.0 to 30.0 ng/L and 2.0–60.0 ng/L, respectively. The calibration curves were linear in concentration range of 0.01–50.0 ng/mL, with correlation coefficients (R^2) >0.996 for all target analytes. The concentrations of the analytes in the field samples were not recovery corrected. None of the analytes were detected in the procedural blanks.

2.7. Statistical analysis

Statistical analysis was performed with SPSS (Version 13.0). If the concentration of an analyte was below LOD, it was set to LOD divided by the square root of 2. If the concentration was above LOD but below LOQ, it was replaced by 1/2 LOQ when the detection frequency (DF) was higher than 50 %; and 1/4 LOQ was used when the DF was lower than 50 %. Statistical differences in concentrations between different matrices were assessed by the Kruskal Wallis H-test. Due to the abnormal distribution of NEO concentrations according to the Kolmogorov-Smirnov test, Spearman's correlation coefficients were used to analyze correlations of NEOs between matrices. Temporal trends in NEOs in breast milk samples were explored by linear regression (the concentrations of target compounds were ln-transformed to achieve normality). A p -value <0.05 was assumed to indicate statistical significance.

3. Results and discussion

Six p-NEOs (ACE, CLO, IMI, THM, THD and DIN) and three m-NEOs (N-dm-ACE, 5-OH-IMI and Of-IMI) were analyzed in all samples. However, the concentrations of THD, DIN, Of-IMI and 5-OH-IMI were $< \text{LOD}$

in all samples. Therefore, only results for the remaining five compounds (ACE, CLO, IMI, THM and N-dm-ACE) are presented and discussed

Table 1

Concentrations of NEOs in paired maternal serum samples (ng/L), umbilical cord serum samples (ng/L) and breast milk samples (ng/L).

	Maternal serum (n = 32)				Umbilical cord serum (n = 32)				Breast milk (n = 32)			
	Range	Mean	Median	DF ^a	Range	Mean	Median	DF	Range	Mean	Median	DF
N-dm-ACE	11.0–391	88.1	47.9	100 %	11.9–520	98.5	60.7	100 %	4.23–573	48.7	23.3	100 %
ACE	2.50–15.4	4.62	4.55	100 %	2.50–12.9	4.00	2.50	100 %	< LOD–33.9	2.23	< LOD	38 %
CLO	< LOD–79.9	18.1	15.9	75 %	< LOD–144	30.9	28.4	81 %	< LOD–109	24.1	13.2	75 %
IMI	< LOD–87.6	9.94	5.04	66 %	< LOD–88.8	13.3	6.16	78 %	< LOD–93.3	14.5	6.94	84 %
THM	< LOD–111	22.4	10.4	56 %	< LOD–186	37.6	26.7	63 %	< LOD–268	50.3	21.5	56 %
ΣNEOs	27.2–527	143	113	100 %	43.2–649	184	160	100 %	12.9–868	140	69.0	100 %

^a Compounds with concentrations > LOD were considered as detected.

below.

3.1. Concentrations and profiles of NEOs in paired maternal serum, cord serum and breast milk samples

The concentrations and DFs of the target compounds in paired maternal serum, umbilical cord serum and breast milk samples collected in 2022 are presented in Table 1. N-dm-ACE was the most frequently observed compound (100 %) in the three matrices. Although ACE was detected in all the maternal serum and umbilical cord serum samples, 47 % and 66 % of them, respectively, had concentrations < LOQ and the DF of ACE was only 38 % in breast milk samples. These trace concentrations and low DF of ACE in human body may be possibly due to the rapid elimination of ACE in the human body. A previous human *in vivo* experiment suggested that ACE could be rapidly metabolized to N-dm-ACE after oral administration, with a half-life of 1.65 days for urinary excretion (Harada et al., 2016). The DFs of the other compounds ranged from 56 % to 84 %. The ubiquitous detection of p-NEOs and N-dm-ACE in umbilical cord serum and breast milk samples indicated that the neonates had been subjected to prenatal and postnatal exposure to NEOs.

The total concentration of NEOs (ΣNEOs, including p-NEOs and m-NEOs) in umbilical cord serum samples ranged from 43.2 to 649 ng/L with a median of 160 ng/L, significantly higher than the equivalent values in maternal serum samples (range: 27.2–527 ng/L, median: 113 ng/L) and breast milk samples (range: 12.9–868 ng/L, median: 69.0 ng/mL) ($p < 0.05$) (Table 1). The composition profiles of NEOs were similar in maternal serum and umbilical cord serum samples but different in breast milk samples (Fig. 1). N-dm-ACE was the predominant target compound in the maternal serum and umbilical cord serum samples,

accounting for 62 % and 53 % of the ΣNEOs concentration, respectively, followed by THM (17 % and 20 %, respectively) and CLO (13 % and 17 %, respectively). In contrast, N-dm-ACE and THM were the dominant compounds in breast milk samples, accounting for 35 % and 36 % of the ΣNEOs concentration, respectively. N-dm-ACE concentrations in breast milk samples ranged from 4.23 to 573 ng/L with a median of 23.3 ng/L, significantly lower than the equivalent values in maternal serum samples (range: 11.0–391 ng/L, median: 47.9 ng/L) and umbilical cord serum samples (range: 11.9–520 ng/L, median: 60.7 ng/L) ($p < 0.01$). However, there was no significant difference in the concentration of N-dm-ACE between maternal serum and umbilical cord serum samples. This may be attributed to differences in the microenvironment of different matrices, as there are more proteins in the blood that have a strong affinity for N-dm-ACE (Han et al., 2003). THM concentrations in breast milk samples ranged from < LOD–268 ng/L with a median of 21.5 ng/L, which was higher than the equivalent values in maternal serum samples (range: 11.0–391 ng/L, median: 10.4 ng/L) ($p > 0.05$) but lower than that in umbilical cord serum samples (range: < LOD–186 ng/L, median: 26.7 ng/L) ($p > 0.05$). This suggests a difference in the distribution of N-dm-ACE and THM in serum and breast milk. Moreover, the concentrations of other compounds were not significantly different between the three matrices, with the exception that the concentrations of CLO in umbilical cord serum samples (range: < LOD to 144 ng/L, median: 28.4 ng/L) were significantly higher than those in maternal serum samples (range: < LOD to 79.9 ng/mL, median: 15.9 ng/mL) ($p < 0.01$).

Owing to the limited availability of biomonitoring data on NEOs in maternal serum, umbilical cord serum and breast milk samples, comparison to residue profiles in the same matrices was difficult. In a

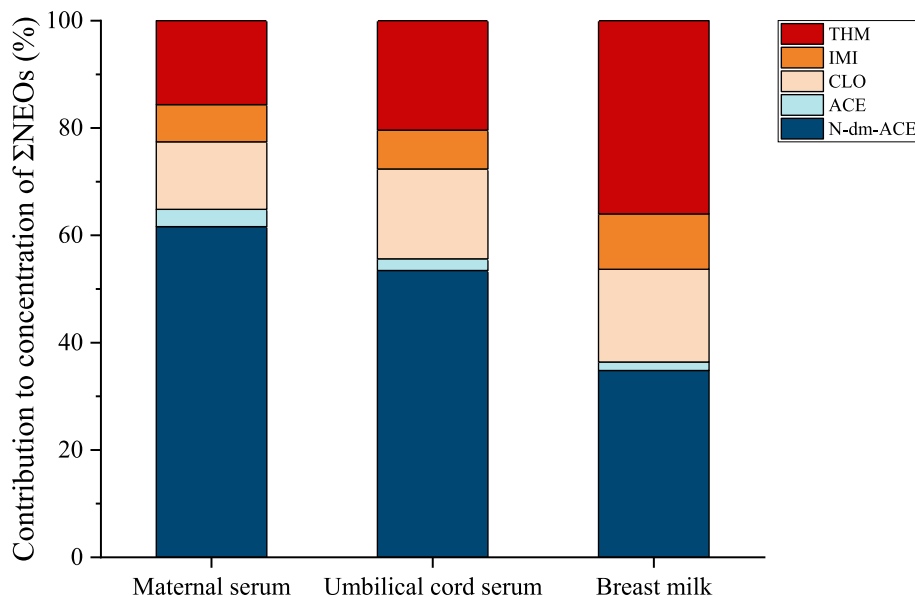


Fig. 1. Composition profiles of NEOs in paired maternal serum, umbilical cord serum and breast milk samples collected in 2022.

nationwide biomonitoring study conducted in China (Chen et al., 2020), N-dm-ACE was also found to be the most frequently detected NEO (100 %) and the predominant NEO in breast milk samples, with a mean concentration three times higher (161 ng/L) than that reported in this study (48.7 ng/L). However, the contribution of other compounds (in descending order IMI > THM > ACE > CLO) was different from that reported in this study. This difference may have been due to variations in the geographical distribution of NEOs or differences in the timescale of breast milk collection (Chen et al., 2020; Zhu et al., 2006). Additionally, N-dm-ACE was found to be the most abundant NEO in serum samples from a Chinese elderly population, accounting for 33 %–44 % of the Σ NEOs concentration, consistent with the results of the current study (Zhang et al., 2022b). However, the DFs of six p-NEOs and three m-NEOs in the elderly population serum samples were >88 % and their concentrations were 1–2 orders of magnitude higher than those in maternal serum and umbilical cord serum samples in the current study. Conversely, in a study of general residents in Wuxi City, Eastern China, the DFs of NEOs in serum samples (< 30 %) were much lower than those in this study (Chen et al., 2021). N-dm-ACE was also shown to be the predominant NEO in urine samples from Chinese children and the general population (Wang et al., 2020a,b), and cerebrospinal fluid of patients aged 1 month to 89 years old (Laubscher et al., 2022; Li et al., 2022b). As the parent compound of N-dm-ACE, ACE has been shown to have hepatotoxic (Karaca et al., 2019), reproductive toxic and endocrine-disrupting potential in several *in vivo* studies (Arican et al., 2020; Halawa et al., 2021). A prevalence case-control study demonstrated that human urinary N-dm-ACE was associated with some typical neurological symptoms (Marfo et al., 2015). Therefore, N-dm-ACE monitoring should receive more attention at an early stage of life through maternal serum, cord serum and breast milk biomonitoring. A recent report found that IMI was the most abundant NEO in paired maternal serum and umbilical cord serum samples; this is in contrast with the current study, which showed 2–3 orders of magnitude lower median concentrations of IMI in maternal serum and umbilical cord serum (Zhang et al., 2022a). This may be attributed to differences in the composition of pesticides used at different times. Unfortunately, only limited data are available on the annual use of different pesticides in China. Additionally, the variation in results between studies may be due to the different equipment and analytical methods used. Although several laboratories have provided analytical data for NEOs in the human body, efforts to evaluate data quality and inter-laboratory data comparability are warranted.

3.2. Temporal trends of NEOs in breast milk

Concentrations and DFs of NEOs in breast milk samples from 2014 to 2022 are summarized in Table S3. N-dm-ACE was the most frequently detected compound (100 %) each year, followed by IMI and ACE, with fluctuations in DF between 60 % and 100 % for IMI, and 38 % and 100 % for ACE over time. DFs of CLO were lower than 40 % before 2018. Moreover, the concentrations of THM were < LOD in breast milk samples until 2018. The increasing variety of these compounds detected in

Table 2
Analysis of temporal trends by linear regression.

Compound	All breast milk samples			
	β	95 CI (%)	<i>p</i>	<i>R</i> ²
N-dm-ACE	−30	−38, −21	<0.01	0.31
ACE	−22	−34, −10	<0.01	0.12
CLO	17	5.0, 29	<0.01	0.07
IMI	−6.4	−19, 6.5	0.33	0.01
THM	30	17, 44	<0.01	0.15
Σ NEOs	−15	−23, −7.8	<0.01	0.14
EDI	3.5	−5.4, 12	0.44	0.01

Annual change (β , % per year) in concentrations (ln-transformed) of NEOs in breast milk samples from 2014 to 2022.

breast milk over time raises concerns about the risk of exposure to multiple NEOs through breast milk feeding for infants.

Although concentrations of the target compounds showed irregular variations over time (Table S3), linear regression was applied to evaluate the temporal trends after ln-transformation of the concentrations (Table 2). All the observed trends were highly significant ($p < 0.01$), except for IMI ($p = 0.33$). Decreasing trends were observed for N-dm-ACE, ACE and Σ NEOs between 2014 and 2022, with annual changes in concentration of 30 %, 22 % and 15 %, respectively. In contrast, increasing trends were seen for CLO and THM, with annual changes in concentration of 17 % and 30 %, respectively. This shows that N-dm-ACE and THM had the highest annual changes in concentration but in opposite directions.

As presented in Fig. 2, N-dm-ACE was the dominant NEO in breast milk samples. It accounted for 68–93 % of the Σ NEOs concentration between 2014 and 2018, with one to two orders of magnitude higher concentrations compared to the other detected compounds. The highest concentration of N-dm-ACE in breast milk samples was observed in 2014, with a mean value of 525 ± 679 ng/L. By 2022, the contribution of N-dm-ACE had decreased to 35 %, with the concentration decreasing to 48.7 ± 97.8 ng/L. Although the exact time when ACE started to be used in China is unclear, the overwhelming dominance of its metabolite in breast milk samples indicates widespread exposure of Chinese infants over the past decade. Furthermore, NEOs in breast milk became more diverse after 2018. THM appeared as a new contributor of NEOs in breast milk samples from 2018, with a mean value of 36.1 ± 29.6 ng/L. The contributions of THM increased from 7.2 % in 2019 to 36 % in 2022, with concentrations increasing from 21.7 ± 39.2 ng/L in 2019 to 49.7 ± 69.5 ng/L in 2022. Notably, the contributions and concentrations of N-dm-ACE and THM were comparable in 2022. This may reflect the fact that older NEOs were gradually replaced by newer ones during this period, and 2017 was the turning point for this replacement. More researches on the temporal Trends of NEOs in human body as well as in the environment matrices are need to verified the replacement of the use of the old NEOs to the new ones.

As shown in Fig. S2, the EDI of Σ NEOs was also calculated for exclusively breastfed infant younger than 6 months. No significant temporal trend ($p = 0.44$) was observed in the EDI of Σ NEOs through breastfeeding (Table 2), but the contribution of individual NEOs changed dramatically (Fig. S3). N-dm-ACE was the largest contributor, accounting for 88 %, 71 %, 67 % and 59 % of the EDI of Σ NEOs in 2014, 2015, 2016 and 2017, respectively. However, the contribution of N-dm-ACE to the EDI of Σ NEOs decreased from 34 % in 2019 to 5.9 % in 2022, whereas the contribution of THM increased from 39 % in 2019 to 71 % in 2022. Therefore, THM has replaced N-dm-ACE as the predominant contributor to the EDI of Σ NEOs since 2018. CLO also contributed >20 % of the EDI of Σ NEOs between 2018 and 2022. Although a decreasing trend was found in the concentrations of Σ NEOs between 2019 and 2022 (Table S2), the EDI of Σ NEOs between 2019 and 2022 increased (Fig. S3) because breast milk samples between 2019 and 2022 contained a higher proportion of THM and CLO with high RPFs. Furthermore, the trend in RI (used to estimate the risk of non-cancer-related health effects) was consistent with that of EDI (Fig. S2). The RI value was <1.0 for each year, suggesting that the EDI of NEOs was not associated with chronic adverse effects. Even so, the increasing contribution of THM to the EDI (or RI) of Σ NEOs is of concern.

THM and CLO are second-generation NEO insecticides widely used against a multitude of insect pests (Li et al., 2022a). They have been commonly found in food and environment matrices (Basley and Goulson, 2018; Li et al., 2018; Lu et al., 2018). THM (51 %) was shown to have a higher DF in fruits and vegetables than ACE (43 %) and CLO (19 %) in a China study (Lu et al., 2018). Moreover, CLO is a metabolite of THM and the metabolism of THM starts in the liver, which could cause hepatotoxicity and hepatocarcinogenic effects in mammals (Green et al., 2005). Metabolites of THM have even been found in the brains of mice administered 20 mg/kg of THM (Ford and Casida, 2006). Rodrigues

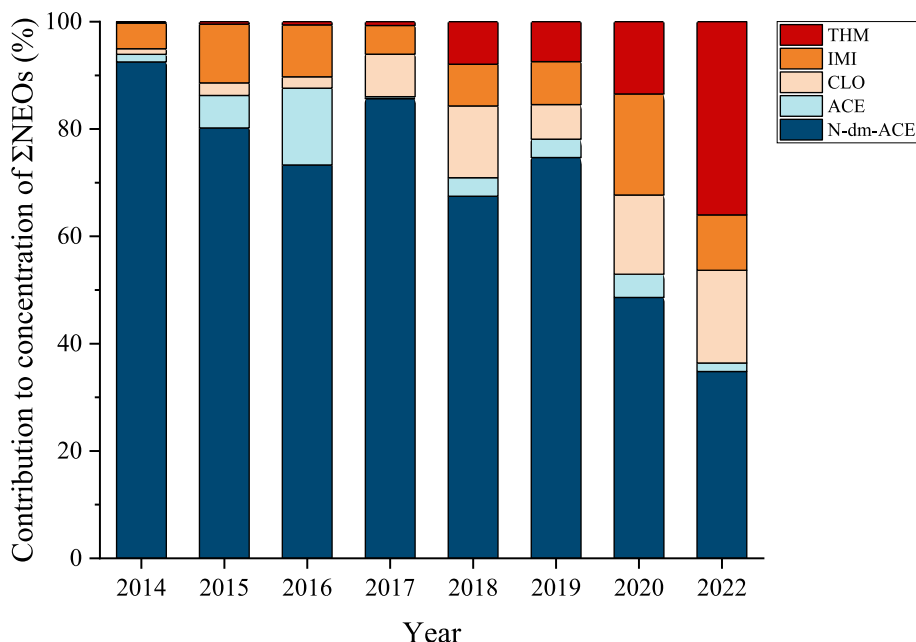


Fig. 2. Composition profiles of NEOs in breast milk samples between 2014 and 2022.

et al. reported that THM may act on central nicotinic acetylcholine receptors of rats, altering cholinergic transmission and affecting anxiety behavior, acetylcholinesterase levels and high-affinity choline uptake (Rodrigues et al., 2010). In addition to the selective stimulation of THM and CLO on the central nervous systems of pests (Rodrigues et al., 2010), their effects on the human nervous system are of increasing concern. Therefore, the effects of THM and CLO in breast milk on the neuro-development of infants should be urgently investigated.

3.3. Associations of NEOs between paired matrices

As presented in Table 3, moderate to strong significant positive correlations were found in the concentrations of N-dm-ACE ($r = 0.862, p < 0.01$), CLO ($r = 0.623, p < 0.01$), IMI ($r = 0.579, p < 0.01$) and THM ($r = 0.458, p < 0.01$) between maternal serum samples and umbilical cord serum samples, suggesting that both p-NEOs and N-dm-ACE can cross the placental barrier and transfer from mother to fetus. However, moderate significant positive correlations were only found in the concentrations of N-dm-ACE ($r = 0.627, p < 0.01$) and THM ($r = 0.527, p < 0.01$) between maternal serum samples and breast milk samples, and in the concentration of N-dm-ACE ($r = 0.646, p < 0.01$) between umbilical cord serum samples and breast milk samples, suggesting enrichment mechanism of NEOs in breast milk and serum samples are different. This may be attributed to different sampling dates for breast milk, maternal serum, and umbilical cord serum. It has been reported that compounds present in the mother are likely redistributed during lactation (Ettinger et al., 2004). Indeed, in early stages of lactation, concentrations of lipids

Table 3
Correlations of NEOs among paired maternal serum, umbilical cord serum and breast milk samples.

	Maternal serum vs. Umbilical cord serum	Maternal serum vs. Breast milk	Umbilical cord serum vs. Breast milk
N-dm-ACE	0.862**	0.627**	0.646**
ACE	0.175	0.026	-0.137
CLO	0.623**	0.047	0.143
IMI	0.579**	0.266	0.260
THM	0.458**	0.527**	0.332

** $p < 0.01$.

and proteins in colostrum are variable, which may affect the distribution of maternal compounds in breast milk (Jakobsson et al., 2012). Pedersen et al. observed that the concentrations of pesticides in breast milk samples collected on day 249 were approximately 2–20 times higher than those collected on day 42 (Pedersen et al., 2021). Therefore, collection of time-paired maternal serum and breast milk samples at different stages of lactation is necessary for further research on the dynamic exposure risk for breast-fed infants.

3.4. Partitioning between paired matrices

Partitioning ratios of the target compounds between umbilical cord

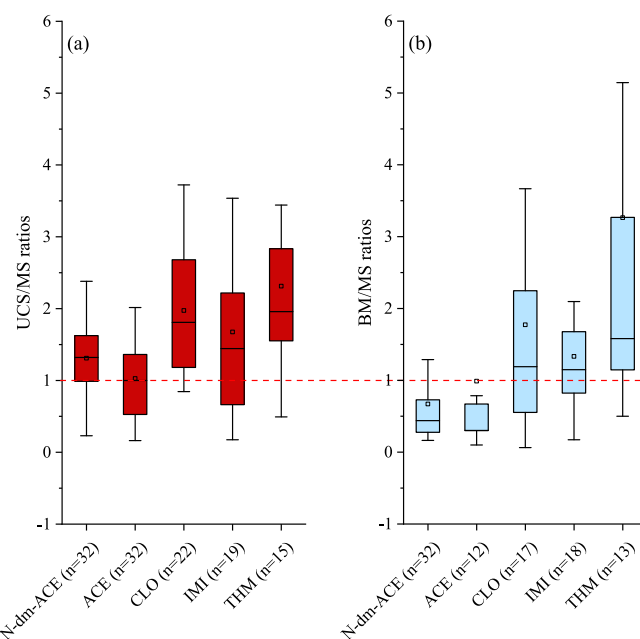


Fig. 3. Concentration ratios of NEOs (a) between umbilical cord serum and maternal serum samples (UCS/MS), and (b) between breast milk and maternal serum samples (BM/MS). n represents the number of paired samples that had concentrations $>$ LOD in both matrices.

serum and maternal serum (UCS/MS ratios) as well as between breast milk and maternal serum (BM/MS ratios) were also calculated from the analyte concentrations. As presented in Fig. 3, the median UCS/MS ratios of N-dm-ACE, ACE, CLO, IMI and THM were 1.32, 1.00, 1.80, 1.44 and 1.96, respectively, suggesting that these compounds were capable of transplacental transfer, in agreement with the results of the correlation analysis. Similar UCS/MS ratios of NEOs were recently reported by Zhang et al. (Zhang et al., 2022a, 2022b), indicating that NEOs may pass through the human placenta unimpeded. However, in the present study, the median BM/MS ratios of N-dm-ACE, ACE, CLO, IMI and THM were 0.44, 0.30, 1.19, 1.15 and 1.58, respectively, indicating that the enrichment capacity of CLO, IMI and THM in breast milk was stronger than that of N-dm-ACE and ACE, which may be due to the different structures of the nitroguanidine-type (CLO, IMI and THM) and cyanoamidine-type (N-dm-ACE and ACE) compounds. It has been reported that serum proteins (such as albumin) have a substantial binding capacity for some type of compounds (Han et al., 2003). Our results suggest that N-dm-ACE may be more likely to bind protein in blood than CLO, IMI and THM due to their different chemical structures, resulting in less free N-dm-ACE in blood to transfer to breast milk, although further research is needed to clarify this mechanism. Moreover, the metabolism and excretion rates of these compounds could also affect their behavior in human body. Further research is needed to clarify this mechanism. Compared to BM/MS ratios, the higher UCS/MS ratios suggest that the neonates' prenatal exposure to NEOs *in utero* was higher than their postnatal exposure through breast milk. In other words, the prenatal period might be a more critical window of NEO exposure.

Additionally, the median ratios of N-dm-ACE/ACE concentrations in maternal serum (10.9), umbilical cord serum (19.6) and breast milk (26.5) were > 1 (Fig. S4a). This result is consistent with a previous study, which reported a median ratio of 46.9 for N-dm-ACE/ACE concentrations in urine samples (Xu et al., 2021). These findings suggest that N-dm-ACE is the main form of ACE enriched in or excreted from the human body. CLO may also be enriched in the human body as a parent compound and metabolite of THM (Casida, 2011). However, the median ratios of CLO/THM concentrations in maternal serum (0.59), umbilical cord serum (0.65) and breast milk (0.52) were < 1 (Fig. S4b), indicating that CLO was not the major metabolite of THM. This may be because the mothers were exposed to less CLO than THM or most of the CLO was excreted through urine (Xu et al., 2021). Moderate significant positive correlations were found between CLO and THM in maternal serum ($r = 0.526, p < 0.01$), umbilical cord serum ($r = 0.380, p < 0.05$) and breast milk ($r = 0.470, p < 0.01$), suggesting multiple sources of CLO accumulation in these matrices.

3.5. Limitations of this study

To the best of our knowledge, this is the first investigation of the occurrence of NEOs in paired maternal serum, umbilical cord serum and breast milk samples, as well as of historical changes in NEO levels in breast milk. However, several limitations should be considered when interpreting the study findings. First, the breast milk samples were collected after the maternal and umbilical cord serum samples, which may have affected the chemical distribution between the matrices. Moreover, due to variations in the distribution of compounds in breast milk at different stages of lactation (Jakobsson et al., 2012; Pedersen et al., 2021), one sampling of breast milk was unlikely to represent exposure levels throughout lactation for infants. Nevertheless, all the breast milk samples used in this study were collected at the same stage of lactation to reduce uncertainty in the findings. Finally, the sample size was relatively small and confined to a small geographic region, limiting the statistical power and study precision. Therefore, a larger sample size covering multiple stages of lactation and a series of time-paired maternal serum and breast milk samples are needed for future studies to understand the temporal change in NEOs as well as extent and mechanism of transfer of NEOs from mother to infant.

4. Conclusions

In summary, the results of this study found decreasing trends in concentration for N-dm-ACE, ACE and Σ NEOs, and increasing trends for CLO and THM in breast milk samples, indicating the prevalence of NEOs continue to evolve and more attention should be paid to the exposure of vulnerable populations to new NEOs. Moreover, the results also suggested that NEOs can be transferred from mother to neonate through both the placenta and breast milk, and the transplacental transfer of NEOs was more important than breast milk transfer. Therefore, increasing exposure to new generation NEOs and the high efficiency of transplacental transfer suggest the use of NEOs should be regulated and strict limits should be placed on NEO residues in crops to safeguard the health of vulnerable populations, though the RI values for breast milk samples indicated no associated chronic adverse effects at present.

CRedit authorship contribution statement

Kaiqin Huang: Writing – original draft, Validation, Methodology, Formal analysis, Data curation. **Meiqing Lin:** Writing – review & editing, Writing – original draft, Validation, Funding acquisition, Conceptualization. **Jing Yi:** Resources, Formal analysis, Data curation. **Guocheng Liu:** Writing – review & editing, Supervision, Conceptualization. **Rui Hua:** Validation, Resources, Data curation. **Yangyang Liu:** Validation, Methodology, Formal analysis, Data curation. **Yanji Qu:** Writing – review & editing, Validation, Resources, Funding acquisition, Conceptualization. **Cairong Chen:** Resources, Investigation, Data curation. **Shengtao Ma:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.175386>.

References

- Anai, A., Hisada, A., Yunohara, T., Iwasaki, M., Arizono, K., Katoh, T., 2021. Urinary neonicotinoids level among pregnant women in Japan. *Int. J. Hyg. Environ. Health* 236, 113797.
- Arfat, Y., Mahmood, N., Tahir, M.U., Rashid, M., Anjum, S., Zhao, F., Li, D.-J., Sun, Y.L., Hu, L., Zhihao, C., Yin, C., Shang, P., Qian, A.-R., 2014. Effect of imidacloprid on hepatotoxicity and nephrotoxicity in male albino mice. *Toxicol. Rep.* 1, 554–561.
- Arican, E.Y., Gokceoglu Kayali, D., Ulus Karaca, B., Boran, T., Ozturk, N., Okyar, A., Ercan, F., Ozhan, G., 2020. Reproductive effects of subchronic exposure to acetamiprid in male rats. *Sci. Rep.* 10, 8985.
- Basley, K., Goulson, D., 2018. Neonicotinoids thiamethoxam and clothianidin adversely affect the colonisation of invertebrate populations in aquatic microcosms. *Environ. Sci. Pollut. Res. Int.* 25, 9593–9599.

- Bass, C., Denholm, I., Williamson, M.S., Nauen, R., 2015. The global status of insect resistance to neonicotinoid insecticides. *Pesticide Biochemistry and Physiology, Insecticide and Acaricide Modes of Action and their Role in Resistance and its Management*. 121, 78–87.
- Boekelheide, K., Blumberg, B., Chapin, R.E., Cote, L., Graziano, J.H., Janesick, A., Lane, R., Lillycrop, K., Myatt, L., States, J.C., Thayer, K.A., Waalkes, M.P., Rogers, J. M., 2012. Predicting later-life outcomes of early-life exposures. *Environ. Health Perspect.* 120, 1353–1361.
- Carmichael, S.L., Yang, W., Roberts, E., Kegley, S.E., Padula, A.M., English, P.B., Lammer, E.J., Shaw, G.M., 2014. Residential agricultural pesticide exposures and risk of selected congenital heart defects among offspring in the San Joaquin Valley of California. *Environ. Res.* 135, 133–138.
- Casida, J.E., 2011. Neonicotinoid metabolism: compounds, substituents, pathways, enzymes, organisms, and relevance. *J. Agric. Food Chem.* 59, 2923–2931.
- Chen, D., Liu, Z., Barrett, H., Han, J., Lv, B., Li, Y., Li, J., Zhao, Y., Wu, Y., 2020. Nationwide biomonitoring of neonicotinoid insecticides in breast milk and health risk assessment to nursing infants in the Chinese population. *J. Agric. Food Chem.* 68, 13906–13915.
- Chen, Q., Zhang, Y., Li, J., Su, G., Chen, Q., Ding, Z., Sun, H., 2021. Serum concentrations of neonicotinoids, and their associations with lipid molecules of the general residents in Wuxi City, Eastern China. *J. Hazard. Mater.* 413, 125235.
- Dorea, J.G., 2021. Exposure to environmental neurotoxic substances and neurodevelopment in children from Latin America and the Caribbean. *Environ. Res.* 192, 110199.
- Ettinger, A.S., Tellez-Rojo, M.M., Amarasiwardena, C., Gonzalez-Cossio, T., Peterson, K. E., Aro, A., Hu, H., Hernandez-Avila, M., 2004. Levels of lead in breast milk and their relation to maternal blood and bone lead levels at one month postpartum. *Environ. Health Perspect.* 112, 926–931.
- Ford, K.A., Casida, J.E., 2006. Unique and common metabolites of thiamethoxam, clothianidin, and dinotefuran in mice. *Chem. Res. Toxicol.* 19, 1549–1556.
- Galdikova, M., Sivikova, K., Holeckova, B., Dianovsky, J., Drazovska, M., Schwarzbacherova, V., 2015. The effect of thiacloprid formulation on DNA/chromosome damage and changes in GST activity in bovine peripheral lymphocytes. *J. Environ. Sci. Health B* 50, 698–707.
- Green, T., Toghiani, A., Lee, R., Waechter, F., Weber, E., Noakes, J., 2005. Thiamethoxam induced mouse liver tumors and their relevance to humans. Part 1: mode of action studies in the mouse. *Toxicol. Sci.* 86, 36–47.
- Halawa, E., Ryad, L., El-Shenawy, N.S., Al-Eisa, R.A., El-Hak, H.N.G., 2021. Evaluation of acetamiprid and azoxystrobin residues and their hormonal disrupting effects on male rats using liquid chromatography-tandem mass spectrometry. *PLoS One* 16, e0259383.
- Han, X., Snow, T.A., Kemper, R.A., Jepson, G.W., 2003. Binding of perfluorooctanoic acid to rat and human plasma proteins. *Chem. Res. Toxicol.* 16, 775–781.
- Harada, K.H., Tanaka, K., Sakamoto, H., Imanaka, M., Niisoe, T., Hitomi, T., Kobayashi, H., Okuda, H., Inoue, S., Kusakawa, K., Oshima, M., Watanabe, K., Yasojima, M., Takasuga, T., Koizumi, A., 2016. Biological monitoring of human exposure to neonicotinoids using urine samples, and neonicotinoid excretion kinetics. *PLoS One* 11, e0146335.
- Huang, K.Q., Yi, J., Liu, G.C., Liu, Y.Y., Jiang, K.X., Li, Z.W., Qu, Y.J., Lin, M.Q., Ma, S.T., 2024. Automatic Magnetic Solid Phase Extraction for Rapid and High-Throughput Determination of Neonicotinoid Insecticides and their Metabolites in Serum, Breast Milk and Urine Samples. Submitted to *Anal. Methods*. (AY-ART-07-2024-001244).
- Ichikawa, G., Kuribayashi, R., Ikenaka, Y., Ichise, T., Nakayama, S.M.M., Ishizuka, M., Taira, K., Fujioka, K., Sairenchi, T., Kobashi, G., Bonmatin, J.-M., Yoshihara, S., 2019. LC-ESI/MS/MS analysis of neonicotinoids in urine of very low birth weight infants at birth. *PLoS One* 14, e0219208.
- Jakobsson, K., Fång, J., Athanasiadou, M., Rignell-Hydbom, A., Bergman, A., 2012. Polybrominated diphenyl ethers in maternal serum, umbilical cord serum, colostrum and mature breast milk. Insights from a pilot study and the literature. *Environ. Int.* 47, 121–130.
- Jeschke, P., Nauen, R., 2008. Neonicotinoids—from zero to hero in insecticide chemistry. *Pest Manag. Sci.* 64 (11), 1084–1098.
- Jeschke, P., Nauen, R., Schindler, M., Elbert, A., 2011. Overview of the status and global strategy for neonicotinoids. *J. Agric. Food Chem.* 59, 2897–2908.
- Kapoor, U., Srivastava, M.K., Srivastava, L.P., 2011. Toxicological impact of technical imidacloprid on ovarian morphology, hormones and antioxidant enzymes in female rats. *Food Chem. Toxicol.* 49, 3086–3089.
- Karaca, B.U., Arican, Y.E., Boran, T., Binay, S., Okyar, A., Kaptan, E., Özhan, G., 2019. Toxic effects of subchronic oral acetamiprid exposure in rats. *Toxicol. Ind. Health* 35, 679–687.
- Kataria, S.K., Chhillar, A.K., Kumar, A., Tomar, M., Malik, V., 2016. Cytogenetic and hematological alterations induced by acute oral exposure of imidacloprid in female mice. *Drug Chem. Toxicol.* 39, 59–65.
- Laubscher, B., Diezi, M., Renella, R., Mitchell, E.A.D., Aebi, A., Mulot, M., Glauser, G., 2022. Multiple neonicotinoids in children's cerebro-spinal fluid, plasma, and urine. *Environ. Health* 21, 10.
- Li, A.J., Si, M., Yin, R., Qiu, R., Li, H., Yao, F., Yu, Y., Liu, W., Wang, Z., Jiao, X., 2022b. Detection of neonicotinoid insecticides and their metabolites in human cerebrospinal fluid. *Environ. Health Perspect.* 130, 127702.
- Li, L., Liang, H., Zhao, T., Liu, Y., Yan, S., Zhu, W., 2022a. Differential effects of thiamethoxam and clothianidin exposure on their tissue distribution and chronic toxicity in mice. *Chem. Biol. Interact.* 366, 110149.
- Li, Y., Su, P., Li, Y., Wen, K., Bi, G., Cox, M., 2018. Adsorption-desorption and degradation of insecticides clothianidin and thiamethoxam in agricultural soils. *Chemosphere* 207, 708–714.
- Lonare, M., Kumar, M., Raut, S., Badgujar, P., Doltade, S., Telang, A., 2014. Evaluation of imidacloprid-induced neurotoxicity in male rats: a protective effect of curcumin. *Neurochem. Int.* 78, 122–129.
- Lu, C.S., Chang, C.H., Palmer, C., Zhao, M.R., Zhang, Q., 2018. Neonicotinoid residues in fruits and vegetables: an integrated dietary exposure assessment approach. *Environ. Sci. Technol.* 52, 3175–3184.
- Mahai, G., Wan, Y., Xia, W., Wang, A., Qian, X., Li, Y., He, Z., Li, Y., Xu, S., 2022. Exposure assessment of neonicotinoid insecticides and their metabolites in Chinese women during pregnancy: a longitudinal study. *Sci. Total Environ.* 818, 151806.
- Marfo, J.T., Fujioka, K., Ikenaka, Y., Nakayama, S.M.M., Mizukawa, H., Aoyama, Y., Ishizuka, M., Tair, K., 2015. Relationship between urinary n-desmethyl-acetamiprid and typical symptoms including neurological findings: a prevalence case control study. *PLoS One* 10, e142172.
- Ozdemir, H.H., Kara, M., Yumurtas, O., Uckardes, F., Eraslan, E., Demir, C.F., Bal, R., 2014. Determination of the effects on learning and memory performance and related gene expressions of clothianidin in rat models. *Cogn. Neurodyn.* 8, 411–416.
- Pedersen, T.L., Smilowitz, J.T., Winter, C.K., Emami, S., Schmidt, R.J., Bennett, D.H., Hertz-Picciotto, L., Taha, A.Y., 2021. Quantification of nonpersistent pesticides in small volumes of human breast milk with ultrahigh performance liquid chromatography coupled to tandem mass spectrometry. *J. Agric. Food Chem.* 69, 6676–6689.
- Rodrigues, K.J., Santana, M.B., Do Nascimento, J.L., Picanço-Diniz, D.L., Maués, L.A., Santos, S.N., Ferreira, V.M., Alfonso, M., Durán, R., Faro, L.R., 2010. Behavioral and biochemical effects of neonicotinoid thiamethoxam on the cholinergic system in rats. *Ecotoxicol. Environ. Saf.* 73, 101–107.
- Toor, H., Sangha, G., Khera, K., 2013. Imidacloprid induced histological and biochemical alterations in liver of female albino rats. *Pestic. Biochem. Physiol.* 105, 1–4.
- U.S. EPA, 2010. Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures. U.S. EPA, Washington, DC.
- Ueyama, J., Harada, K.H., Koizumi, A., Sugiura, Y., Kondo, T., Saito, I., Kamijima, M., 2015. Temporal levels of urinary neonicotinoid and dialkylphosphate concentrations in Japanese women between 1994 and 2011. *Environ. Sci. Technol.* 49, 14522–14528.
- Wang, A., Mahai, G., Wan, Y., Yang, Z., He, Z., Xu, S., Xia, W., 2020a. Assessment of imidacloprid related exposure using imidacloprid-olefin and desnitro-imidacloprid: neonicotinoid insecticides in human urine in Wuhan, China. *Environ. Int.* 141, 105785.
- Wang, H., Yang, D., Fang, H., Han, M., Tang, C., Wu, J., Chen, Y., Jiang, Q., 2020b. Predictors, sources, and health risk of exposure to neonicotinoids in Chinese school children: a biomonitoring-based study. *Environ. Int.* 143, 105918.
- Xu, M., Zhang, Z., Li, Z., Kan, S., Liu, Z., Wang, D., Liu, Q., Zhang, H., 2021. Profiles of neonicotinoid insecticides and characteristic metabolites in paired urine and blood samples: partitioning between urine and blood and implications for human exposure. *Sci. Total Environ.* 773, 145582.
- Yang, W., Carmichael, S.L., Roberts, E.M., Kegley, S.E., Padula, A.M., English, P.B., Shaw, G.M., 2014. Residential agricultural pesticide exposures and risk of neural tube defects and orofacial clefts among offspring in the San Joaquin Valley of California. *Am. J. Epidemiol.* 179, 740–748.
- Zhang, H., Shen, K., Wu, R., Li, Z.Y., Wang, X., Wang, D.S., Zhan, M.X., Xu, W.G., Gao, Y. F., Lu, L.G., 2021. Occurrence and distribution of neonicotinoids and characteristic metabolites in paired urine and indoor dust from young adults: implications for human exposure. *Environ. Res.* 199, 111175.
- Zhang, H., Zhu, K.R., Du, J., Ou, M., Hou, J.L., Wang, D.S., Wang, J., Zhang, W.C., Sun, G.D., 2022b. Serum concentrations of neonicotinoids and their characteristic metabolites in elderly population from South China: association with osteoporosis. *Environ. Res.* 203, 111772.
- Zhang, H.L., Bai, X.Y., Zhang, Tao, Song, S.M., Zhu, H.K., Lu, S.Y., Kannan, K., Sun, H.W., 2022a. Neonicotinoid insecticides and their metabolites can pass through the human placenta unimpeded. *Environ. Sci. Technol.* 56, 17143–17152.
- Zhu, J., Phillips, S.P., Feng, Y.L., Yang, X., 2006. Phthalate esters in human milk: concentration variations over a 6-month postpartum time. *Environ. Sci. Technol.* 40, 5276–5281.