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Passive exposure to electronic cigarette aerosol in pregnancy: A case study of a family

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ABSTRACT

Background: Passive exposure to the aerosols of electronic cigarettes (e-cigarettes) has been little studied. We assessed this exposure in late pregnancy in a woman and her 3-year-old child, exposed through e-cigarette use by another household member.

Methods: This prospective longitudinal case study involved a family unit consisting of an e-cigarette user, a pregnant woman who delivered an infant during the study, and the couple's older 3-year-old son. At 31, 36, and 40 weeks of the pregnancy, we measured biomarkers (nicotine metabolites, tobacco-specific nitrosamines, propanediols, glycerol, and metals) in the urine and hair of all three participants and in the saliva of the adults, in cord blood at delivery, and in the breast milk at the postpartum period.

Results: Samples from the e-cigarette user showed quantifiable concentrations of all analytes assessed (maximum urinary cotinine concentration, 4.9 ng/mL). Among samples taken from the mother, nicotine and its metabolites were found mainly in urine and also in saliva and hair, but not in cord blood. During the postpartum period, we found cotinine concentrations of 2.2 ng/mL in the mother's urine and 0.22 ng/mL in breast milk; 1,2-propanediol was generally detected in urine and saliva, but not in cord blood or breast milk. The maximum urinary cotinine concentration in the 3-year-old child was 2.6 ng/mL and propanediols also were detected in his urine. Nitrosamines were not detected in samples taken from the mother or the 3-year-old. Metals found in the refill liquid were detected at low levels in both the mother and the 3-year-old.

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Conclusions: We detected low but not negligible concentrations of e-cigarette–related analytes (including cord blood and breast milk) in an exposed pregnant non-user and in a 3-year-old child also living in the home. Passive exposure to e-cigarette aerosols cannot be disregarded and should be assessed in larger observational studies.

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Ethical considerations

The Research and Ethics Committee of the Bellvitge University Hospital provided ethical approval for the study protocol, including the informed consent form (Ref: PR266/17). This study met the code of the Declaration of Helsinki.

1. Introduction

Exposure to second-hand tobacco smoke (SHS) during pregnancy increases the risk of having an infant with congenital anomalies and reduced birthweight, length, head circumference, and placental weight (Abdullah et al., 2017;IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2004; Salmasi et al., 2010). Postnatal SHS exposure is linked to sudden infant death syndrome, upper and lower respiratory tract illnesses, hearing loss, cognitive deficits, and behavioral problems, including hyperactivity-inattention patterns (DiFranza et al., 2004; Luk et al., 2018; Padrón et al., 2012; Sailer et al., 2019).

New nicotine products, such as electronic cigarettes (e-cigarettes), have been suggested as tools for harm reduction, and e-cigarette manufacturers have claimed that these products produce a harmless vapor instead of the myriad toxic compounds emitted in smoke from conventional cigarettes. Although most pregnant women perceive e-cigarettes to be less harmful than conventional cigarettes (Bhandari et al., 2018; Bowker et al., 2018; McCubbin et al., 2017), the refill liquids and aerosol exhaled by e-cigarette users contain several harmful substances (Ward et al., 2020). The liquid used in e-cigarettes usually contains nicotine, 1, 2-propanediol (PD), and/or vegetable glycerin as humectants, along with flavorings. The aerosol exhaled by e-cigarette users (or secondhand aerosol, SHA) contains these and many other compounds, such as ultrafine particles, volatile organic compounds, heavy metals, and tobacco-specific nitrosamines (TSNAs), among others, which negatively affect indoor air quality (Schober et al., 2013). These substances usually occur in a much lower quantity than in smoke from conventional cigarettes, but SHA contains other substances usually not present in SHS, mainly 1,2-PD and glycerin (National Academies of Sciences, Engineering, 2018). Their quantity varies depending on use patterns and e-cigarette type and liquid, and wide differences among products have been observed (Williams and Talbot, 2011; Williams et al., 2016).

Studies assessing the effects of passive exposure to SHA in pregnancy are scarce. Only a small number of animal studies have examined potential effects in pregnancy and on the fetus, indicating some complications in fetal development. These findings include cardiovascular effects, such as altered umbilical and maternal uterine arterial flow and cardiac edema; pneumological effects, including disrupted lung development and decreased postnatal lung growth (increasing risk for later respiratory morbidities); neurological effects, such as altered metabolic pathways, epigenetic modifications, global DNA methylation and transcriptomic changes, hippocampal inflammation, and adult memory and behavioral effects; and metabolic effects, including disrupted hormones in offspring, increased risk for type 2 diabetes and obesity, and decreased birth length. Some of these effects were not linked to nicotine exposure specifically, as they sometimes appeared when an e-liquid without nicotine was studied (Chen et al., 2018; Larcombe, 2019; Lauterstein et al., 2016; McGrath-Morrow et al., 2015; Noel et al., 2020; Orzábal and Ramadoss, 2019; Palpant et al., 2015; Smith et al., 2015); a caveat is that nicotine has been found in liquids that were claimed to be nicotine-free (Girvalaki et al., 2020).

In humans, assessment of SHA exposure in pregnancy is lacking. Studies of SHS exposure in pregnancy and to the fetus have relied on different biological matrices such as urine, saliva, serum, hair, cord blood, and breast milk (Joya et al., 2014; Llaquet et al., 2010) to evaluate levels of nicotine and its metabolites (e.g., cotinine, 3-OH-cotinine) and TSNAs (N-nitrosonornicotine [NNN], 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone [NNK], and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol [NNAL]). Because these substances also are present in SHA, they are available biomarkers of exposure to SHA.

Although strong evidence demonstrates the deleterious effects of SHS exposure in pregnancy and childhood, to the best of our knowledge, no studies have assessed effects during pregnancy of potential passive exposure to SHA. This evidence gap has led some organizations to default to the conservative advice to avoid use of e-cigarettes around pregnant women and around children (American Thoracic Society, 2020; Departament de Salut de la Generalitat de Catalunya, 2021).

To provide the first evidence on this topic and test the feasibility of assessing this exposure, we conducted a case study using several biomarkers to prospectively evaluate passive SHA exposure during pregnancy, in breast milk, and in childhood.

2. Methods

2.1. Study design and participants

In this prospective longitudinal case study (BERNAT Study) to evaluate passive exposure to e-cigarette aerosol in a family unit, the participants were a couple and their older child. The child's father was a 47-year-old male e-cigarette user (height 178 cm, weight 98 kg). He had been a daily user of e-cigarettes for 7.8 years at the time of the study and had not used any other tobacco product or any other nicotine product during that period. The electronic cigarette, a first-generation device (model: KR-808) from a Chinese manufacturer (Shenzhen Kanger Technology Co., Ltd.), was bought in an online shop sited in the United States. The battery had a charge of 280 mAh and lasted for up to around 200 puffs.

Although his device was intended to be used as a closed system, he refilled the cartomizers by opening them. The refill liquid throughout the study period contained 11 mg of nicotine (according to the labelling), with menthol flavor (Totally Wicked™, bought in an online shop sited in the UK). He refilled the cartomizer six times a day on average with 11 drops of liquid each time, with no differences between working and non-working days. At approximately 20 drops of liquid per milliliter, 11 mg/mL of nicotine was about 0.55 mg nicotine per drop; thus, based on 66 drops per day, we calculated a daily nicotine intake of 36.3 mg. His e-cigarette use was regular and continuous throughout the day and throughout the home. Before turning to e-cigarettes, he had smoked 20 cigarettes a day for 22 years, starting at age 17. The user considered himself to be highly dependent on nicotine and as a user of conventional cigarettes had scored 8 on the Fagerström test of cigarette dependence.

The other adult in the study was a pregnant woman exposed to e-cigarette aerosols. She was 40 years old, with a nonpregnancy height of

169 cm and weight of 60 kg. During pregnancy, her weight was 73 kg at week 31, 77.7 kg at week 36, 78.2 kg at week 40 (delivery), and 67.8 kg at week 42 (17 days after delivery, the postpartum period). During the 12-week study, she was potentially exposed to secondhand aerosol from e-cigarettes an average of 7 h per day at home on workdays, 10 h each workday on the last 15 days before delivery, and 16 h a day during weekends. She was never exposed to secondhand tobacco smoke at work or in other environments with the rest of her family or friends. She had never smoked or used a tobacco or nicotine product. She had a routine delivery at term (40 weeks of gestation) of a newborn male who weighed 3.5 kg and was 49.5 cm in length at delivery.

The third study participant was the couple's older child, a boy aged 3 years (height 100 cm, weight 13.1 kg), who was exposed to secondhand aerosol from e-cigarettes. During the study period, he was potentially exposed an average of 8 h per day at home on workdays and 14 h per day during weekends (not including sleeping hours).

2.2. Main place of exposure

The family lived together in a 130-m^2 flat with ceilings 2.7 m high in the city center of Barcelona, Spain. The two sleeping rooms had windows (one interior), and the two living rooms, bathroom, and kitchen had exterior windows. The flat was ventilated every morning for 10 min, and the windows were kept closed the rest of the day. During the study period (spring and summer), the air conditioner was commonly used with the windows closed. The temperature indoors during the study ranged from 24.7 °C to 25.5 °C, and the relative humidity ranged from 28% to 44%. Smoking was forbidden inside the flat without exceptions, and no tobacco or nicotine products apart from the e-cigarette were used inside the flat.

2.3. Procedure

The adult participants expressed their understanding of the study and its purpose and signed informed consent before starting the study. Also, each adult gave written consent for their older son's participation in the study. One researcher went to their home to collect samples from the three participants from week 31 of pregnancy to 2 weeks postpartum.

The Research and Ethics Committee of the Bellvitge University Hospital provided ethical approval for the study protocol, including the informed consent form (Ref: PR266/17). This study met the code of the Declaration of Helsinki.

2.4. Sampling and analysis

2.4.1. Participants and timing

In this study, we evaluated several analytes in urine, saliva, hair, cord blood, and breast milk, including nicotine and its main metabolites (cotinine, 3′OH-cotinine, nornicotine), TSNAs (NNN, NNK, NNAL), 1,2-PD and 1,3-PD, and glycerol. The timing of the sampling was determined according to the pregnancy course: at weeks 31, 36, and 40 of pregnancy (the last sampling was a few hours before the delivery), urine and hair (all three participants) and saliva (both adults) were sampled. At delivery, cord blood sampling was performed. At week 42 (17 days postpartum), we analyzed breast milk from the mother (Fig. 1).

2.4.2. Sampling procedures

To collect saliva samples from the adults, they were asked to rinse their mouths to remove any residual chemicals present and then to suck a lemon candy (Smint™) to stimulate saliva production. They first spit out a small amount of saliva and then provided about 4 mL of saliva by spitting it into a funnel placed in a test tube. Saliva samples were frozen in 2-mL aliquots to −80 °C for storage until analysis. All funnels and test tubes were previously washed with ultrapure water obtained from a Millipore Milli-Q water purification system. Subsequent analysis of the lemon candies and the funnels and tubes used in the study confirmed the absence of PDs and glycerol. Prior to analysis, saliva samples were centrifuged at 4500 g for 15 min for sputum separation.

For urine samples from all three participants, they were asked to provide about 30 mL of urine, following the same procedure as for any clinical urine collection, taken mid-stream in the container provided. The urine containers were previously washed with ultrapure water obtained from a Millipore Milli-Q water purification system and were confirmed to contain no PDs or glycerol. The samples were stored in a freezer at −80 °C until analysis.

Hair samples also were taken from all three participants by cutting off a lock from the root, with the sample stored in a sealed plastic bag at room temperature. To eliminate any superficial contamination before analysis, hair samples (about 20 mg) were extensively washed with dichloromethane. In total, about 1.5 cm of hair for each person was analyzed, corresponding with the last month and a half of pregnancy.

Cord blood was sampled at delivery. Two Vacutainer™ sample tubes with a separating gel were filled with blood from the cord, left 30 min for coagulation in the hospital laboratory (Hospital Clínic de Barcelona – Seu Maternitat), and then stored at 4 °C. Within 48 h of being taken, samples were allowed to thaw to room temperature and the tubes centrifuged to separate the serum (1700 g for 10 min at 20 °C). After this process, the samples were again stored at −20 °C.

Breast milk was sampled at 17 days after delivery. A total of 60 mL of

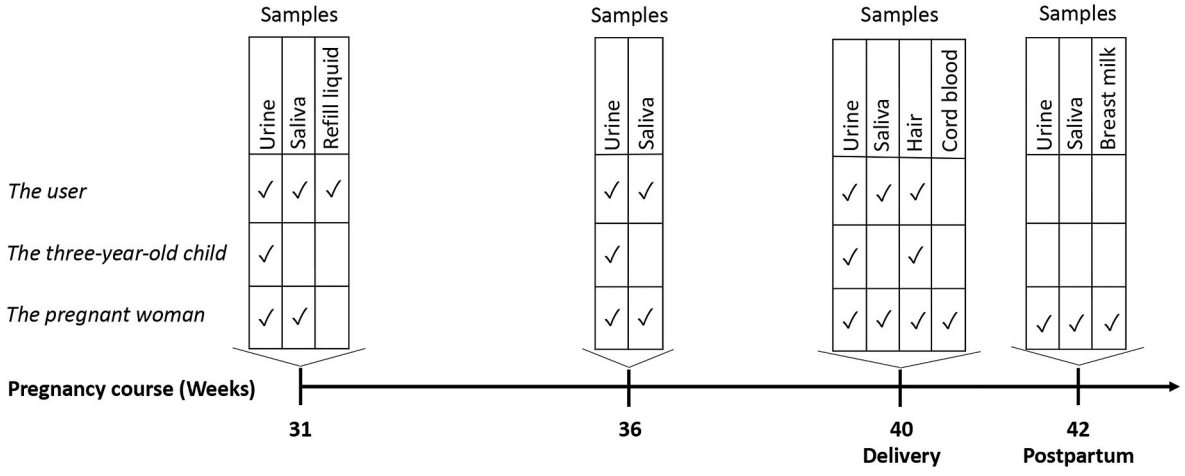

Fig. 1. Scheme for sample collection during the pregnancy and postpartum.

transitional breast milk was collected and stored at −80 °C until analysis.

During week 31 of the pregnancy, we also collected 2 mL of the refill liquid used for the e-cigarette and stored it at −20 °C until analysis.

2.4.3. Sample analyses

In the liquid matrices and hair samples, analyses of nicotine and its main metabolites, as well as TSNAs, were performed by liquid chromatography coupled to tandem mass spectrometry (MS) with multiple reactions monitoring, as previously described (Pérez-Ortuño, et al., 2016a, 2016b). For the determination of PDs and glycerol, in brief, all liquid matrices were treated in the same way: A 100-μL aliquot was fortified with 100 μL of the internal standard solution, alkalinized with 300 μL NaOH 8 M, derivatized with 100 μL BzCl, and extracted with 5 mL of n-hexane. After evaporation to dryness under N_2, the residue was redissolved in 100 μL n-hexane. Instrumental analysis for PDs and glycerol was performed with a gas chromatograph system connected to a mass spectrometer, through an electron impact ionization source.

We also analyzed metals (Al, Cr, Ni, Cu, Zn, Sn, and Pb) in urine, saliva, hair, cord blood, breast milk, and refill liquid (another 20 metals and substances were analyzed, and the results are presented as supplementary data). A calibration curve was prepared in ultrapure water (Milli-Q) with 2% HNO_3 (Merck) and 1% HCl (Merck) using appropriate metal standard solutions. Samples were diluted in ultrapure water (Milli-Q) with 2% HNO_3 (Merck), and appropriate blanks were analyzed to correct the results. The multi-element analyses were performed on an Agilent 8900 triple quadrupole inductively coupled plasma MS (Agilent Technologies, Santa Clara, CA, USA). The instrument was tuned and performance parameters checked prior to analysis. Suitable certified reference materials were reanalyzed together with a blank and an intermediate calibration standard every 12 samples.

Nicotine and its main metabolites, as well as TSNAs, PDs, and glycerol, were analyzed in the different matrices by the Hospital del Mar, Medical Research Institute, Barcelona. The analyses of metals in all matrices were performed at the University of Granada.

3. Results

The results obtained for the family are detailed in Table 1 and Table 2.

3.1. Assessment of samples from the e-cigarette user

Samples from the e-cigarette user showed quantifiable concentrations of all analytes assessed except NNAL.

As shown in Fig. 2, urine cotinine reached a concentration of 4900 ng/mL at week 31 of pregnancy and at the delivery collection point. Also, 3′OH-cotinine reached the maximum concentration of 18,000 ng/mL at the delivery collection point, whereas nornicotine reached its maximum concentration of 300 ng/mL at week 36. Nitrosamines were

Table 1
Concentrations of analytes in urine, saliva, hair, cord blood, and breast milk samples.

	Week of pregnancy	Nicotine	Cotinine	3′–OH–Cotinine	Nornicotine	NNN	NNK	NNAL	1,2-PD	1,3-PD	Glycerol
Urine											
Unit		ng/mL	ng/mL	ng/mL	ng/mL	pg/mL	pg/mL	pg/mL	nmol/mL	nmol/mL	nmol/mL
LOQ		0.50	0.10	0.040	0.040	2.0	2.0	0.50	3.0	3.0	10
User											
	31	7500	4900	13000	290	7.7	<2.0	<0.50	1200	11	55
	36	9900	4100	14000	300	16	<2.0	<0.50	1300	10	45
	40 (Delivery)	9000	4900	18000	280	9.7	<2.0	<0.50	1200	<3.0	48
Mother											
	31	1.5	0.54	6.1	<0.040	<2.0	<2.0	<0.50	9.8	8.7	49
	36	1.2	1.0	7.3	<0.040	<2.0	<2.0	<0.50	6.4	7.4	22
	40 (Delivery)	1.5	0.33	3.8	<0.040	<2.0	<2.0	<0.50	3.4	3.3	29
	42 (Postpartum)	0.66	2.2	8.9	<0.040	<2.0	<2.0	<0.50	6.9	<3.0	25
Child											
	31	0.62	2.6	5.6	<0.040	<2.0	<2.0	<0.50	6.7	3.3	12
	36	1.0	1.7	6.8	<0.040	<2.0	<2.0	<0.50	6.4	4.6	20
	40 (Delivery)	3.5	0.82	2.1	<0.040	<2.0	<2.0	<0.50	4.8	5.9	13
Saliva											
Unit		ng/mL	ng/mL	ng/mL	ng/mL	pg/mL	pg/mL	pg/mL	nmol/mL	nmol/mL	nmol/mL
LOQ		0.50	0.10	0.040	0.040	2.0	2.0	0.50	3.0	3.0	10
User											
	31	5200	430	50	11	<2.0	<2.0	<0.50	4400	<3.0	150
	36	1900	240	30	15	<2.0	2.4	<0.50	3600	<3.0	590
	40 (Delivery)	1800	290	46	5.0	<2.0	<2.0	<0.50	430	4.0	<100
Mother											
	31	<0.50	<0.10	<0.040	<0.040	<2.0	<2.0	<0.50	7.2	<3.0	<40
	36	2.0	<0.10	<0.040	<0.040	<2.0	<2.0	<0.50	<3.0	<3.0	<10
	40 (Delivery)	0.84	<0.10	<0.040	<0.040	<2.0	<2.0	<0.50	13	<3.0	48
	42 (Postpartum)	2.3	<0.10	<0.040	<0.040	<2.0	<2.0	<0.50	9.4	<3.0	<10
Hair											
Unit		ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	–	–	–
LOQ		25	5.0	2.0	2.0	100	100	25	–	–	–
User	40 (Delivery)	15000	3700	950	580	100	130	<25	–	–	–
Mother	40 (Delivery)	140	<5.0	<2.0	<2.0	<100	<100	<25	–	–	–
Child	40 (Delivery)	180	<5.0	<2.0	<2.0	<100	<100	<25	–	–	–
Cord blood											
Unit		ng/mL	ng/mL	ng/mL	ng/mL	pg/mL	pg/mL	pg/mL	nmol/mL	nmol/mL	nmol/mL
LOQ		1.0	0.20	0.080	0.080	4.0	4.0	1.0	3.0	3.0	10
Mother	40 (Delivery)	<1.0	<0.20	<0.080	<0.080	<4.0	<4.0	<1.0	<3.0	<3.0	123
Breast milk											
Unit		ng/mL	ng/mL	ng/mL	ng/mL	pg/mL	pg/mL	pg/mL	nmol/mL	nmol/mL	nmol/mL
LOQ		0.50	0.10	0.040	0.040	2.0	2.0	0.50	3.0	3.0	10
Mother	42 (Postpartum)	3.2	0.22	0.10	0.068	<2.0	<2.0	<0.50	<3.0	<3.0	>22000

LOQ: Limit of quantification.

Table 2
Concentration of metals in the study samples and in the e-cigarette refill liquid.

	Week of pregnancy	Aluminum	Chromium	Nickel	Copper	Zinc	Tin	Lead
Urine								
Unit		μg/L	μg/L	μg/L	μg/L	μg/L	μg/L	μg/L
LOQ		20.00	0.11	0.41	0.42	27.37	0.22	0.08
LOD		6.66	0.03	0.13	0.14	9.12	0.07	0.02
User								
	31	**0**	**0.40**	**0.88**	**9.78**	**290.05**	**1.36**	**1.13**
	36	**0**	**0.40**	**1.16**	**11.60**	**307.93**	**2.16**	**1.06**
	40 (Delivery)	**0**	**0.28**	**1.39**	**11.54**	**802.75**	**1.78**	**0.88**
Mother								
	31	**0**	**0.34**	**3.42**	**26.52**	**138.18**	**0.80**	**1.52**
	36	**0**	**0.14**	**1.11**	**10.40**	**76.09**	**0.35**	**0.42**
	40 (Delivery)	**0**	**0.18**	**2.00**	**15.07**	**163.35**	**0.36**	**0.33**
	42 (Postpartum)	**0**	**0.30**	**2.22**	**10.65**	**157.60**	**0.63**	**0.46**
Child								
	31	**0**	**0.09**	**0.87**	**1.47**	**86.68**	**0.04**	**0.08**
	36	**0**	**0.23**	**1.91**	**7.02**	**249.63**	**0.24**	**0.49**
	40 (Delivery)	**0**	**0.23**	**1.10**	**5.09**	**205.37**	**0.10**	**0.12**
Saliva								
Unit		μg/L	μg/L	μg/L	μg/L	μg/L	μg/L	μg/L
LOQ		20.00	0.11	0.41	0.42	27.37	0.22	0.08
LOD		6.66	0.03	0.13	0.14	9.12	0.07	0.02
User								
	31	**0**	**0.49**	**0.60**	**5.86**	**23.42**	**1.52**	**0.07**
	36	**0**	**0.56**	**0.68**	**7.99**	**50.80**	**0.13**	**0.04**
	40 (Delivery)	**20.11**	**0.81**	**1.01**	**13.41**	**45.06**	**2.65**	**0.94**
Mother								
	31	**0**	**0.32**	**0.56**	**4.99**	**26.30**	**0**	**0.06**
	36	**0**	**0.17**	**0.33**	**2.61**	**20.36**	**0**	**0**
	40 (Delivery)	**42.90**	**0.46**	**0.78**	**3.70**	**16.99**	**0**	**0.88**
	42 (Postpartum)	**0**	**0.44**	**0.16**	**1.67**	**17.09**	**0**	**0**
Hair								
Unit		μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
LOQ		0.58	0.003	0.01	0.01	0.80	0.006	0.002
LOD		0.19	0.001	0.004	0.004	0.26	0.002	0.0007
User	40 (Delivery)	**14.70**	**0.44**	**0.08**	**15.61**	**134.25**	**0.16**	**3.41**
Mother	40 (Delivery)	**16.89**	**0.24**	**0.50**	**33.37**	**218.72**	**0.12**	**1.74**
Child	40 (Delivery)	**13.29**	**0.34**	**0.06**	**15.96**	**60.65**	**0.20**	**1.53**
Cord blood								
Unit		μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
LOQ		0.06	0.0003	0.001	0.001	0.08	0.0007	0.0002
LOD		0.02	0.0001	0.0004	0.0004	0.02	0.0002	0.00008
Mother	40 (Delivery)	**1.46**	**0.05**	**0.002**	**0.43**	**2.80**	**9.07E-06**	**0.004**
Breast milk								
Unit		μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
LOQ		0.04	0.0002	0.0008	0.0008	0.05	0.0004	0.0001
LOD		0.01	0.00007	0.0002	0.0002	0.01	0.0001	0.00005
Mother	42 (Postpartum)	**0**	**0.0005**	**0.0006**	**0.44**	**3.04**	**0.0009**	**0.001**
Refill liquid								
Unit		ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
LOQ		72.38	0.40	1.49	1.53	99.05	0.81	0.29
LOD		24.12	0.13	0.49	0.51	33.01	0.27	0.09
Result		**0**	**1.03**	**1.01**	**25.48**	**66.80**	**1.99**	**0**

LOQ: Limit of quantification.
LOD: Limit of detection.

scarcely detected in any matrix: NNN was detected in urine (between 7.7 and 16 pg/mL) and in hair (0.10 pg/mL), NNK was detected in saliva and hair, and NNAL was not detected at any collection point in any matrix. Quantifiable concentrations of 1,2-PD were found in urine (maximum concentration, 1300 nmol/mL at week 40), and saliva (maximum concentration, 4400 nmol/mL at week 36). The maximum concentration of 1,3-PD was 11.0 nmol/mL in urine at week 31 and 4.0 nmol/mL in saliva at week 40. Regarding glycerol, the maximum concentration was found in saliva at week 36 (590 nmol/mL), and in urine, the concentration range was 45–55 nmol/mL across time points.

We also found several metals in urine, saliva, and hair, including aluminum, chrome, nickel, copper, zinc, tin, and lead. Only aluminum was not found in urine (Table 2).

Finally, analyses of the refill liquid showed nicotine at 16 mg/mL although the liquid was labeled as containing 11 mg/mL. The 1,2-PD concentration was 9.8 mmol/mL, glycerol was 1.5 mmol/mL, and 1,3-PD was undetectable. Regarding nitrosamines, the refill liquid contained 1.1 pg/mL NNN and 0.79 pg/mL NNK. Similar to the biomarkers measured in samples from the e-cigarette user, we found several metals in the refill liquid, including chrome, nickel, copper, zinc, and tin, but not aluminum or lead (Table 2).

3.2. Assessment of samples from the pregnant woman

During pregnancy, nicotine and its metabolites were found mainly in urine. Nicotine was quantifiable in urine and in saliva, hair, and breast milk but not in cord blood. Cotinine was under the level of quantification in saliva, hair, and cord blood, showing a maximum concentration of 1.0 ng/mL in urine at week 36 (Fig. 1), but increased to 2.2 ng/mL at postpartum. At the postpartum measurement, cotinine was found in breast milk at a concentration of 0.22 ng/mL. Other nicotine metabolites, such as 3′OH-cotinine, could be quantified only in urine and breast

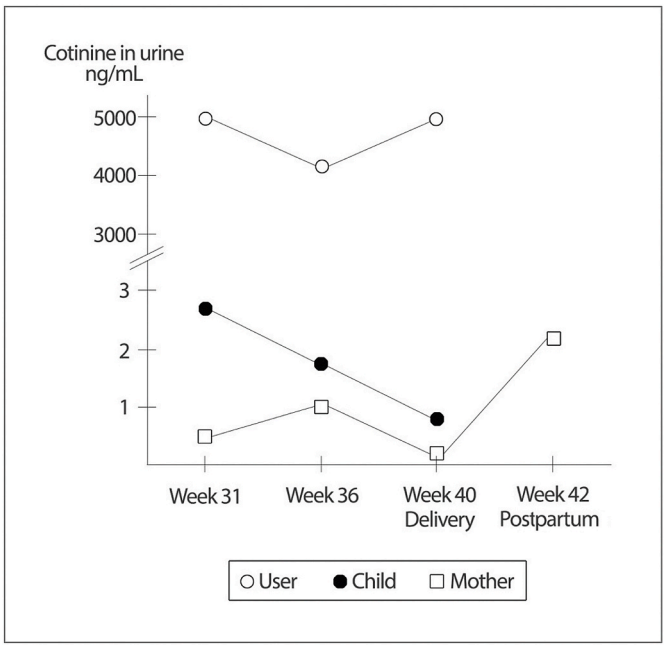

Fig. 2. Cotinine concentration (ng/mL) at weeks 31, 36, 40 (delivery), and 42 (postpartum).

milk, and nornicotine was not detected in any matrix except breast milk. No nitrosamines (NNN, NNK, NNAL) were detected in any matrix. 1,2-PD was generally detected in urine and saliva but not in cord blood or breast milk. Glycerol was present in low concentrations in urine, saliva, and cord blood but present at high concentrations in breast milk, above the upper limit of quantification (>22,000 nmol/mL; Table 1).

Finally, several metals were found in cord blood and breast milk. There was no detectable aluminum in urine or cord blood or tin in saliva (Table 2).

3.3. Assessment of samples from the 3-year-old

Nicotine was detected in both urine and hair at the three collection points during the pregnancy. The metabolites cotinine and 3′OH-cotinine were detected in urine but not in hair, and nornicotine was not detected in any of the child's samples. The maximum concentration of cotinine in the child's samples was found at the 31-week collection point (2.6 ng/mL). Nitrosamines were also undetected in any matrix analyzed (urine and hair). 1,2-PD was detected in urine, however, reaching a maximum concentration of 6.7 nmol/mL at the pregnancy week 31 collection, along with peak levels of 1,3-PD (5.9 nmol/mL) at the delivery collection point. Glycerol also was detected in low concentrations in urine (Table 1).

The metals found in the child's urine and hair were similar to those found in samples from the pregnant woman but usually in lower concentrations. Metals occurring at higher concentrations in samples from the child compared with the mother were zinc in urine and chrome and tin in hair (Table 2).

Results for the other 20 metals and elements analyzed in all participants and for all of the biological matrices are shown in the supplementary table.

4. Discussion

This longitudinal case study provides the first evidence of passive exposure to e-cigarette aerosols of people from vulnerable populations, such as children and pregnant women. The main analytes identified in samples from all three participants were nicotine and its metabolites, cotinine and 3′OH-cotinine.

The concentrations of nicotine and these metabolites in samples from the user of e-cigarettes were very high. Salivary cotinine reached concentrations of 240–430 ng/mL, which are by no means negligible, compared with a median cotinine concentration of 146.5 ng/mL (interquartile range, 86.8–220.5) in saliva of smokers of conventional cigarettes from the general population of Barcelona (Fu et al., 2009).

Nitrosamines are potent carcinogens, and we found low concentrations of NNN in urine and NNK in saliva of the e-cigarette user. NNAL, a nitrosamine associated with lung cancer (Stepanov et al., 2014), was not detected. Other studies have shown low concentrations of NNAL in urine samples from users of e-cigarettes (median 2.6 pg/mL) (Martínez-Sánchez et al., 2019) and around 300 pg/mL in smokers (Xia et al., 2011). Here, we found nitrosamines in the refill liquid, as reported in SHA in some studies (Goniewicz et al., 2014; McAuley et al., 2012) and in some but not all of other brands of refill liquids (FDA US Department of Health and Human Services, 2014; Kim H. J. and Shin, 2013), implying important differences among brands (Schober et al., 2013).

Glycerol, an endogenous human metabolite, was present in urine samples from the e-cigarette user at concentrations twice that found in urine samples from the pregnant woman. In addition, he had very high concentrations of 1,2-PD, a main ingredient of the refill liquids. 1,2-PD has been recognized as generally safe as a food substance (US Food and Drug Administration, 2021), although short exposures to its mist may cause ocular and upper airway irritation (Wieslander et al., 2001).

Metals, mainly zinc but also tin and copper, typically were found in higher concentrations in e-cigarette user samples than in samples from the pregnant woman and the child. These metals occurred in higher concentrations in refill liquid. Compared with other active users of e-cigarettes and dual users (tobacco and e-cigarettes) in Spain, the e-cigarette user in the current study had urine concentrations of zinc and copper that were markedly higher, along with slightly higher concentrations of chrome, nickel, lead, and tin (Olmedo et al., 2021).

We detected nicotine and cotinine in urine samples taken from the pregnant woman. In her saliva samples, we detected only nicotine at low concentrations and no cotinine. Testing for salivary cotinine is less sensitive than for urinary cotinine (Llaquet et al., 2010), and cotinine concentrations in urine samples from the pregnant woman ranged from 0.54 to 2.2 ng/mL, indicating passive exposure to SHA. These concentrations were all below cut-offs suggested for passive smoking from conventional cigarettes. Some authors set this cut-off to less than 5 ng/mL in urine to indicate non-exposure to SHS in adult non-smokers, whereas 100 ng/mL has been proposed to differentiate an active smoker from a non-smoker (Aranda Regules et al., 2008; Man et al., 2006).

Despite the low concentrations of cotinine in samples from the pregnant woman, we must bear in mind that the implementation of smoke-free policies has lowered the cut-offs for non-smokers or passive exposures (Pérez-Martín et al., 2022; Sánchez-Rodríguez et al., 2015). Additionally, there is a marked acceleration in metabolism of both nicotine (60% increase) and cotinine (140% increase) during pregnancy compared with postpartum levels (Dempsey et al., 2013), and that must be considered in interpreting low concentrations. In our study, cotinine concentrations in urine during pregnancy ranged from 0.33 to 1.0 ng/mL and increased to 2.2 ng/mL at the postpartum measure. Saliva samples are more commonly used in large studies because they are easier to obtain. As shown in our results, however, urine cotinine concentrations are generally much higher than those found in saliva; for this reason, urine analysis offers greater sensitivity for assessing low-level exposure (Jarvis et al., 1987).

In hair, analysis of nicotine has been traditionally preferred over cotinine because of the much higher concentrations of nicotine (Al-Delaimy, 2002). Low concentrations of nicotine were found in the hair samples from the pregnant woman, indicating a light but stable passive exposure to nicotine during the pregnancy. The result (140 ng/g) is below cut-offs proposed for passive exposure to SHS (500–700 ng/g; Llaquet et al., 2010; Matt et al., 2004). We identified no nicotine

metabolites in hair samples from the pregnant woman, in contrast to other results reported in pregnant women passively exposed to SHS (mean cotinine concentration, 60–90 ng/g) (Florescu et al., 2007).

We also detected low concentrations of PDs in her urine and saliva samples, although samples from the e-cigarette user had higher concentrations of both 1,2-PD and 1,3-PD. Moreover, no nitrosamines were found in any sample from the pregnant woman.

Finally, regarding metal concentrations, we found lower concentrations than in other studies of pregnant women from the general population (non-users of e-cigarettes or conventional cigarettes), including markedly lower concentrations than in a study in the United States (Kim et al., 2019) and lower or similar values for lead, zinc, and copper. An exception was nickel, which was present at higher levels than previously reported (Lozano et al., 2022; Stojsavljevic et al., 2022).

Although we did not directly measure samples from the infant, for evaluating passive exposure of the fetus to e-cigarette aerosol, cotinine in cord serum has been claimed to be among the best biomarkers for discriminating exposure to SHS and exposure vs non-exposure at the end of pregnancy (Llaquet et al., 2010). The cut-off of cotinine in cord serum to distinguish nonexposed from exposed non-smokers ranges from 1 ng/mL (Bearer et al., 1997; Chazeron et al., 2008; Franchini et al., 2008) to 1.78 ng/mL (Pichini et al., 2000). In their study of pregnant women passively exposed to conventional cigarettes, Wu et al. (2008) reported mean cotinine concentrations of 2.39 ng/mL (standard deviation, 3.20), but to the best of our knowledge, no reference values for cotinine under these conditions have been established. In cord blood, we found no quantifiable concentrations of nicotine, cotinine, or the other metabolites and analytes studied, such as 1,2-PD and nitrosamines, in agreement with low concentrations of nicotine and its metabolites found in samples from the pregnant woman. Finally, the concentration of metals found in our study (aluminum, nickel, copper, zinc, and lead) were lower than reported in other studies of pregnant women (nonusers of e-cigarettes or conventional cigarettes) (Baeyens et al., 2014; Stojsavljevic et al., 2022), but slightly higher (chrome, nickel, lead, and zinc) than those found in a study with this matrix in a Spanish sample (Cabrera-Rodríguez et al., 2018).

Breast milk also can be a source of passive exposure to SHA for a newborn. Reported short-term effects of absorbing nicotine from breast milk are restlessness, nausea, insomnia, vomiting, diarrhea, and rapid pulse (Llaquet et al., 2010). Cotinine is the most prevalent biomarker of tobacco smoke in the breast milk of smoking mothers, and nicotine is present only in the milk samples of active smokers, as just 10% of the maternal nicotine dose is excreted into breast milk (Atkinson et al., 1988; Llaquet et al., 2010). Use of nicotine patches has no significant influence on milk intake by the breast-fed infant (Ilett et al., 2003). Nevertheless, we found nicotine and its metabolites in the breast milk sample in our study, which implies exposure of the newborn to this toxicant.

Tobacco-specific carcinogens also can be transferred to milk, as shown in animal models (LaVoie et al., 1987; Zanieri et al., 2007), but we did not find nitrosamines and other toxicants in our sample, and they were not detected in maternal samples, either. 1,2-PD was not detected in breast milk, and we found lower levels of metals in breast milk when compared with other studies of recent mothers from the general population (non-users of e-cigarettes or conventional cigarettes) (Freire et al., 2022; Szukalska et al., 2021), with the exception of copper and zinc, which were present in higher concentrations than in a Spanish population (Motas et al., 2021).

Results for samples from the 3-year-old child were similar to those for his mother in terms of toxicants detected and concentrations. Some studies have suggested a urinary cut-off of 0.25 ng/mL for cotinine and 0.50 ng/mL for 3′-OH cotinine to characterize passive exposure to conventional cigarettes in children (Parks et al., 2021). In our study, we found higher concentrations, ranging from 0.82 to 2.6 ng/mL for cotinine and 2.1–6.8 ng/mL for 3′-OH cotinine, suggesting significant exposure to emissions of an e-cigarette, in the absence of exposure to other sources of nicotine. No nicotine metabolites or TSNAs were found in hair samples from the child, but the concentrations of nicotine (180 ng/g) were slightly higher than in his mother's hair. One study found a cut-off value of 200 ng/g of cotinine in hair to discriminate between children exposed or not to SHS, whereas cotinine was not detected in the child in this study (Florescu et al., 2007). We also detected low concentrations of PDs in urine and saliva in the 3-year-old. 1,2-PD is considered safe for ingestion, but its health effects when it is aerosolized and inhaled are largely unknown, except that it is an upper airway irritant and could be especially concerning for children. Finally, the results for metals in urine were also lower than those found in other studies with samples of children (Pérez et al., 2018; Vogel et al., 2021).

In general, we detected nicotine and its metabolites in samples from the pregnant woman and in the breast milk, although at significantly lower concentrations than in pregnant women passively exposed to conventional cigarettes. The concentrations of these analytes, including metals, were low, but the literature shows no safe level of nicotine consumption during pregnancy (Suter et al., 2015). Healthcare providers, therefore, should warn women about the potential adverse maternal and fetal health implications associated with nicotine consumption in any form (McCubbin et al., 2017) and recommend that people avoid both using e-cigarettes (Whittington et al., 2018) and passive exposure to SHA during pregnancy. Pregnant women should be asked not only if they smoke or use e-cigarettes but also if they are passively exposed to SHA, as this exposure may be underreported because of lack of information about whether exposure is harmful (Mark et al., 2015).

As noted, some studies using animal models have suggested that exposure to SHA during pregnancy may be of concern because of its toxicants, although dose-response studies are lacking (Breland et al., 2019). Unfortunately, no studies have addressed exposure to SHA during pregnancy. One publication describes active use of e-cigarettes by five pregnant women, although only one was a daily/regular user, and the results showed an association between maternal e-cigarette use and small-for-gestational-age infants (Cardenas et al., 2019). No data support e-cigarettes as a safe alternative for nicotine use in pregnant women (Sailer et al., 2019; Whittington et al., 2018).

The present study has the typical limitations of case reports, including limited generalization. Individual differences in the use of e-cigarettes (puff duration, depth of inhalation, time keeping the vapor inhaled) and devices used (device power, nicotine concentration of the liquid refill) can influence outcomes (Breland et al., 2019). Devices differ in terms of nicotine delivery, with some delivering little to no nicotine (Yan and D'Ruiz, 2015) and others delivering nicotine at levels similar to those of a conventional cigarette (St Helen et al., 2016). The e-cigarette used in this study was a first-generation device, which the user preferred for its more discreet profile compared with other bigger options. Newer e-cigarettes are more efficient in supplying nicotine to the users and produce more aerosol. The voltage for first-generation e-cigarettes is about 3.7 V, whereas more recent devices, like Mods, can range from 3 to 8 V; thus, the current findings might be an underestimation of potential passive exposure to SHA. However, the user in this study was a heavy user, with high concentrations of nicotine in urine and saliva because of continuous use, so the exposure of bystanders to this aerosol is likely not negligible. Moreover, the refill liquid contained a concentration of nicotine as high as 16 mg/mL, despite labeling citing 11 mg/mL.

Regarding the results for metals content in the different matrices, we did not take into account any information about the diets of the participants, which could have influenced metal concentrations. Moreover, the pregnant woman was always cohabitating with the e-cigarette user, so that baseline values could not be obtained. As this was a case study, there was no control group to support interpretation of metals concentrations.

Another limitation is the lack of data about the air in the home, such as airborne nicotine and fine and ultra-fine particulate matter, to

complement biomarker data.

Data regarding exposure to SHA are insufficient for setting a cut-off point of passive exposure in the general population or even for special populations such as pregnant women or children. For this reason, we had to compare our results to data regarding exposure to SHA in adults or to data from studies of passive exposure to conventional cigarettes, which is not optimal because SHS and SHA differ in composition. There is no standard classification to clearly identify the hazards, and the toxicological profile has not been fully investigated (SCHEER (Scientific Committee on Health, Environmental and Emerging Risks) 2021). Also, the potential for third-hand exposure to SHA in toddlers is not negligible (Goniewicz and Lee, 2015), as they put their hands in their mouths constantly. In general, much more research in large observational studies is needed to elucidate the potential harms of exposure to SHA and third-hand aerosol exposures. Moreover, some research should focus on the pharmacokinetics of nicotine in breast milk after active exposure to e-cigarettes and after passive exposure to e-cigarette aerosol.

The strengths of this study include the novelty of the results, as few studies have targeted objectively assessed passive exposure to SHA in real-life scenarios, and to our knowledge, none have addressed this exposure in vulnerable populations such as pregnant women or children or *in utero* exposures. Other strengths of this study are its prospective design and the comprehensive number of analytes evaluated.

6. Conclusions

The concentrations of analytes found in samples from the mother during pregnancy and postpartum (including cord blood and breast milk) and from the 3-year-old child were low. However, the levels of nicotine and its metabolites found in many of their samples suggest passive exposure to SHA to some extent during pregnancy and that exposures of the fetus and the child should be avoided.

Credit author statement

Montse Ballbè: conceptualization, methodology, investigation, data curation, writing original draft, supervision, project administration. **Marcela Fu:** methodology, investigation, data curation, draft writing and review, editing original draft. **Guillem Masana:** investigation, draft writing and review, editing original draft. **Raúl Pérez-Ortuño:** methodology, formal analysis, investigation, resources, data curation, draft writing and review, editing original draft. **Antoni Gual:** investigation, draft writing and review, editing original draft. **Fernando Gil:** methodology, formal analysis, investigation, resources, data curation, draft writing and review, editing original draft. **Pablo Olmedo:** methodology, formal analysis, investigation, resources, data curation, draft writing and review, editing original draft. **Óscar García-Algar:** investigation, draft writing and review, editing original draft. **Jose Antonio Pascual:** methodology, formal analysis, investigation, resources, data curation, draft writing and review, editing original draft. **Esteve Fernández:** conceptualization, methodology, draft writing and review, editing original draft, supervision, funding acquisition.

Declaration of competing interest

The members of the family in this study were the primary investigator (Montse Ballbè, the mother), one of the co-authors (Guillem Masana, the e-cigarette user), and their older son (the 3-year-old child). MB and GM have no conflict of interests regarding the results or their implications. Both MB and GM strictly followed the study protocol and were not involved in any of the analytical procedures. MB and GM objectively discussed all the results with the rest of investigators. All the authors declare no conflicts of interests.

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.envres.2022.114490.

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