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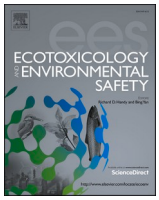

Migration of contaminants from printed masks for children to saliva simulant using liquid chromatography coupled to ion mobility-time of flight-mass spectrometry and gas chromatography-mass spectrometry

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ABSTRACT

The COVID-19 pandemic has led to children using polymeric FFP2 and polymeric surgical masks on a daily basis. Children often bite and suck on such masks as they wear them closed to their mouths. In this work, the migration of contaminants from printed and unprinted children`s masks to a saliva simulant has been studied. Liquid chromatography coupled to ion-mobility quadrupole time-of-flight mass spectrometry has been used for the detection and identification of non-volatile migrants. An orthogonal projection to latent structures – discriminant analysis (OPLS-DA) was applied to compare the data from the printed masks against the data from the unprinted ones. Headspace solid phase microextraction coupled to gas chromatography mass spectrometry was used to assess the migration of volatile compounds. Thirteen compounds were found in the masks with concentrations ranging from 5 ng/g to 254 ng/g. Toluene, chlorobenzene, irganox 1076 and 2-(2-butoxyethoxy)ethyl acetate were all found to migrate from the masks studied. Moreover, differences between the migrants from printed and unprinted FFP2 masks were found. Octocrylene, 4-(dimethylamine)benzoate, methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate and tris(3-methylphenyl)phosphate were found to migrate only from printed masks. Toluene that migrated from all the masks studied and tris(3-methylphenyl)phosphate, that migrated only from printed masks, have been listed as hazardous priority substances.

1. Introduction

The COVID-19 pandemic has led to the extensive use of polymeric masks on a daily basis. Children have been using masks at school and other indoor spaces for up to 8 h or more each day for many months over the course of the pandemic.

Facemasks are usually made from polymers such as polypropylene, polyurethane, polyacrylonitrile, polystyrene, polycarbonate, polyethylene, or polyester (Armentano et al., 2021). Many additives are incorporated with these polymers to improve their properties (Todesco and Ergenc, 2002; Bart, 2005; Blázquez-Blázquez et al., 2020). In addition, masks aimed at children often have an external ink layer to create a design that encourages children to wear the masks. The inks that are applied to polymers can also contain additives (RH, 1988; Oestreich and Struck, 2002). Moreover, in addition to intentionally added substances, non-intentionally added substances (NIAS) could also be present in both the polymers and inks. Such substances include impurities, degradation compounds and products formed by interactions between different compounds (Nerin et al., 2013; Ibarra et al., 2018; Vera et al., 2018; Canellas et al., 2019; Portesi et al., 2019; Coniglio et al., 2020).

There is a high probability that children will bite and suck on the masks and therefore the additives and NIAS in the polymers and inks could migrate to saliva. Studies on toys, have demonstrated that hazardous compounds within toys maybe ingested by children (Bouma and Schakel, 2002; Noguerol-Cal et al., 2011; Brandsma et al., 2022; Souza et al., 2022).

Liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) are conventionally used to determine the migration of non-volatile and volatile polymer additives respectively (Simal-Gandara et al., 2002; Silva et al., 2006). In this study in which the composition of the polymers is unknown, high-resolution mass spectrometry techniques are needed to detect and identify unexpected compounds. The processing of the data obtained through high-resolution mass spectrometry can be challenging since it requires

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the collection of accurate *m/z* values to derive elemental compositions, a bibliographic search to obtain the likely candidates and the high-energy mass spectrum to study fragments of the molecule. (Nerin et al., 2013; Aznar et al., 2015; Canellas et al., 2015; Vera et al., 2018; Canellas et al., 2019; Wrona and Nerin, 2020; Nerin et al., 2022).

The technique of ultra-high-pressure-liquid chromatography (UPLC) coupled to ion mobility-quadrupole time of flight (IMS-QTOF) mass analyzer enables the creation of libraries of compounds that contain collision cross section values (CCS), a parameter derived from the drift time (DT) that is consistent across different IMS platforms and laboratories (Larriba-Andaluz and Prell, 2020). CCS values together with accurate mass and the retention time of the molecules enable the creation of databases of compound information that facilitate the identification of unexpected compounds detected in samples. (Song et al., 2022a, 2022b) established a database comprising 675 compounds related to plastic additives and NIAS. This database includes experimental CCS values, retention times, and precise *m/z* values for all 675 compounds. Furthermore, they constructed a predictive model for both retention times (RT) and CCS, employing it to forecast these properties for over 10,000 compounds associated with food packaging (Song et al., 2022a, 2022b; Su et al., 2023).

In this work, UPLC-IMS-QTOF has been used to identify and quantify the migrants from plain and printed masks to a saliva simulant. Additionally, solid phase microextraction used in conjunction with gas chromatography mass spectrometry and a NIST library were used to identify and quantify the volatile migrants, originating from the masks, in the saliva simulant.

2. Materials and methods

2.1. Reagents and materials

High-performance liquid chromatography (HPLC) grade ethanol was supplied by Scharlau Chemie S.A (Sentmenat, Spain).

Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (99 %), Tris(2,4-di-tert-butylphenyl)phosphate (certified reference material), docosanamide (99 %), methyl palmitate (99 %), triphenylphosphine oxide (98 %), 2,6-Di-tert-butyl-4-methylphenol (BHT) (99 %), octocrylene (98 %), ethyl 4-(dimethylamino)benzoate (99 %), 2-(2-butoxyethoxy)ethyl acetate (99.2 %), tris(3-methylphenyl)phosphate (90 %), methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (97 %) were purchased from Merck.

Solid phase microextraction (SPME) fibers as 100 μm polydimethylsiloxane fiber, 85 μm polyacrylate fiber and 50/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxane fiber were supplied by Supelco (Spain).

Masks were purchased from various supermarkets and pharmacies. Table 1 shows the origin of the masks and their main characteristics. Three FFP2 masks and eight surgical masks were studied. Mask 1 was the unprinted version of mask 2 and mask 3. Mask 5 was the unprinted version of mask 6 and mask 7 and mask 8 was the unprinted version of mask 9, 10 and 11. Moreover, mask 4 was printed with different inks. Therefore, several pieces of mask 4 were studied separately to determine whether the different inks affected the compound migration. Fig. 1 shows the range of colors and patterns of the masks studied.

2.2. Migration studies

Migration studies were performed using a saliva simulant. The saliva simulant was prepared as follows: 745.5 mg potassium chloride, 525.5 mg potassium carbonate anhydrous, 753.1 mg potassium phosphate dibasic anhydrous, 327.3 mg sodium chloride, 147.0 mg calcium chloride dehydrate and 166.7 mg magnesium chloride hexahydrate were dissolved in 1000 mL of ultrapure water adjusted to pH 6.8 with 3 M hydrochloric acid solution (Earls et al., 2003).

The migration studies were prepared by placing a 10 cm^2 mask sample in contact with saliva simulant. This sample size was selected to correspond to the surface area of a child's open mouth (Earls et al., 2003). 50 mL of the saliva simulant was placed into contact with each mask sample using migration cells (Noguerol-Cal et al., 2011). The face of the mask in contact with the mouth was in contact with the saliva simulant. The solution was retained in the migration cells for 4 h at 37 ºC. Three replicates of each sample were prepared and analyzed by UPLC-IMS-QTOF and HS-SPME-GC-MS in order to identify and quantify all migrant compounds from each mask.

2.3. Ultra-high-pressure-liquid chromatography coupled to an ion mobility-quadrupole time of flight analysis (UPLC-IMS-QTOF)

Screening analyses were carried out using an Acquity™ UPLC chromatography system coupled to an electrospray interface (ESI) and Vion® ion mobility-quadrupole time of flight (IMS-QTOF) mass spectrometer, from Waters (Manchester, UK). 5 μL of simulated saliva after each migration studies were directly injected. A UPLC BEH C_{18} column of 1.7 μm particle size (dimensions: 2.1 ×100 mm) was used with a flow rate of 0.3 mL/min and a column temperature of 35 ºC. The mobile phases were water (phase A) and methanol (phase B), both with 0.1 % formic acid. The gradient used was 95 % A to 100 % B within 13 min, with 2 min for re-equilibration to the initial conditions.

The electrospray interface (ESI) was used in positive ionization sensitivity mode with a capillary voltage of 3 kV and a sampling cone voltage of 30 V. The temperatures used for the source block and desolvation gas were 120 ºC and 500 ºC, respectively, and the desolvation gas flow rate was 800 L h^{-1}. The system was calibrated, and data were acquired in the *m/z* range 50–1000. Leucine-Enkephalin $[M+H]^+$, *m/z* 556.2766, was used as the lock-mass compound for real-time mass correction. Data were acquired in data independent analysis (DIA) using high definition MS^E mode ($HDMS^E$) in which low collision energy (6 eV) and high collision energy (ramp from 20 to 40 eV) data are acquired simultaneously. Argon was used as the collision gas and nitrogen was used as the ion-mobility gas. The IMS gas flow rate was 25 mL/min with a wave velocity of 250 m/s and an IMS pulse height of 45 V. Mass and

Table 1
Mask properties: name, type of mask, ink color, brand, manufacturer, origin and regulation that applied to each mask studied.

Mask number	Mask type	Ink color	Brand	Manufacturer	Origin	Regulation
1	FPP2	White	YWSH	Zhejiang Shaohua_medical equipment	China	EN149:2001-A1:2009 FPP2 NR
2	FPP2	Blue, yellow	YWSH	Zhejiang Shaohua_medical equipment	China	EN149:2001-A1:2009 FPP2 NR
3	FPP2	Black	YWSH	Zhejiang Shaohua_medical equipment	China	EN149:2001-A1:2009 FPP2 NR
4	Surgical	Blue, black, white	Xicen Shuwen	Zhejiang Shaohua_medical equipment	China	UNE 0064–2:2020
5	Surgical	White	Pimedical kids	Pi medical labs	Spain	EN 14683:2019
6	Surgical	White, pink	Pimedical kids	Pi medical labs	Spain	EN 14683:2019
7	Surgical	White, green, orange, blue, black	Pimedical kids	Pi medical labs	Spain	EN 14683:2019
8	Surgical	White	Mediroc	STL TEKNOLOJI LTD.STI	Turkey	EN 14683:2019
9	Surgical	Red	Mediroc	STL TEKNOLOJI LTD.STI	Turkey	EN 14683:2019
10	Surgical	Blue	Mediroc	STL TEKNOLOJI LTD.STI	Turkey	EN 14683:2019
11	Surgical	Yellow	Mediroc	STL TEKNOLOJI LTD.STI	Turkey	EN 14683:2019

Fig. 1. Photos of the masks studied. A) FPP2 masks 1, 2 and 3 from YWSH; B) mask 4 from *Xicen Shuwen*; C) masks 5, 6 and 7 from *Pimedical kids*; D) masks 8, 9, 10 and 11 from *Mediroc*.

CCS calibration was performed using the Major Mix kit (Waters Corp.). The acquisition rate was 10 Hz and data acquisition and processing were carried out using UNIFI v.1.8 software.

Thresholds set in the analysis method for the assignment of targets from the database developed by (Song et al., 2022a, 2022b), were 0.2 min for the retention time tolerance, 5 ppm for the mass and 5 % for the collision cross section tolerance.

After confirming the identification of all migrant compounds, their concentrations were quantified. For this purpose, calibrated curves were prepared in simulant saliva and analyzed directly by UPLC-IMS-QTOF with the method above described.

2.4. Solid phase microextraction-gas chromatography mass spectrometry (HS-SPME-GC-MS)

After conducting migration studies, 5 mL of saliva simulant were loaded into 20 mL vials and subjected to analysis.

The data acquisition process involved the use of a CTC Analytics CombiPal autosampler coupled to an Agilent 6890 N gas chromatography with a mass spectrometer MS 5975B detector. All equipment was from Agilent Technologies (Madrid, Spain).

The capillary column used was a HP-5MS (30 m x 0.25 μm x 250 μm) from Agilent Technologies (Madrid, Spain). The oven program was set as follows: 50 °C for 2 min, increasing at a rate of 10 °C/min up to a maximum of 300 °C which was maintained for 2 min. Splitless injections and a helium flow-rate of 1 mL/min were used, with the injector temperature set at 250 °C. The acquisition was performed using electron ionization (EI) and the SCAN acquisition mode (over the *m/z* range 45–350) was used for the identification of compounds,

The choice of fiber type was pre-optimized to ensure the selection of the most appropriate option for detecting the greatest number of compounds with optimal sensitivity.

Three types of SPME fibers were tested: 100 μm polydimethylsiloxane fibers, 85 μm polyacrylate fibers and 50/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxane fibers.

The extraction temperature was set at 80 °C and the extraction time was 30 min.

Calibration curves were established for quantifying the identified compounds, utilizing standards of these compounds prepared in the saliva simulant. The analysis process included filling 5 mL vials and subjecting them to HS-SPME-GC-MS, following the above-mentioned procedure, with the exception of data acquisition in selected ion monitoring (SIM) mode, using ions 91 and 92 *m/z* for Toluene and 112 and 77 *m/z* for Chlorobenzene.

2.5. Discriminant analysis

Multivariate statistical analysis (MVA) was applied to further explore the similarity and differences in the migration from printed and unprinted masks and meanwhile the compounds responsible for

discrimination were studied. The software EZInfo 3.0 from Waters, using Orthogonal Projection to Latent Structures– Discriminant Analysis (OPLS-DA) was applied to maximize the differences among the groups and contribute to the identification of potential markers for discrimination.

3. Results and discussion

A saliva simulant was selected for the study because children are likely to bite and suck masks when they are worn over an extended period of time. The migration conditions were set to reflect mask usage with a contact temperature of 37 ºC to mimic the temperature of human body in contact with the masks.

3.1. The identification and quantification of migrants in the saliva simulant using UPLC-IMS-QTOF

UPLC-IMS-QTOF was used to detect and identify the compounds that migrated from the masks into the saliva simulant. This technique yields a high peak capacity which results from a combination of retention time, drift time and the accurate mass of each compounds. Since $HDMS^E$ is used to acquire the data, the low and high energy spectra are obtained within a single injection. With a suitable calibration, CCS values can be derived from the drift time measurements.

The database of extractables and leachables from plastic materials, developed by (Song et al., 2022a, 2022b), was used to screen the test samples in this study. The database contains a total of 1038 CCS_{N2} values from 675 standards which include compounds commonly used additives and non intentionally added substances found in food contact materials. In addition to the CCS values, the database also contains retention time and accurate mass measurements. The assignment of compounds migrating from the masks into the saliva stimulant is performed automatically by the UNIFI software by comparing the retention time, accurate mass and CCS values of the measured data to the database content subject to the tolerances stated in Section 2.3. To aid the identification of migrating compounds alone, three replicates of the untreated saliva simulant were acquired as reference samples. Components found in both the migration extract samples and the reference samples were then filtered from the component lists generated for the migration extract samples prior to identification.

To compare the migration from printed masks to that from unprinted masks, the migration analyses from each of the 4 groups of masks were compared. Each group of masks is from the same manufacturer and the unprinted masks (or printed regions of one color) were compared to printed versions (or printed regions of other colors) of the same mask type. The comparison was performed using a multivariate analysis (MVA) approach. Markers across the sample set, each uniquely defined by a combination *m/z*, retention time and drift time, were determined by UNIFI with the intensity of each marker in each sample used to generate a marker matrix. The marker matrix was automatically transferred and the model is automatically fitted by the MVA software, EZInfo 3.0 for Waters, and investigated using Orthogonal Projection to Latent Structures – Discriminant Analysis (OPLS-DA) applied to two groups of sample, one group unprinted, the other printed. OPLS-DA yields two plots, a score plot which shows whether there is separation between groups of samples and an S-plot from which the markers responsible for any separation can be determined. The S-plot compares the covariance of each marker, the magnitude of change (x-axis), to the correlation of each marker, the consistency of the change (y-axis). Marker close to the extremities of the y-axis ($y = -1$ or $y = 1$) are either unique to, or elevated in, one of the sample groups. Therefore, discriminating compounds, sometimes at very low concentrations, that are only present or elevated in the printed masks (or for a certain color in the case of mask 4), can be determined. These following comparisons were made; mask 1 vs mask 2, mask 1 vs mask 3, mask 4 (blue part) vs mask 4 (black and white part), mask 5 vs mask 6, mask 5 vs mask 7, mask 8 vs mask 9, mask 8 vs mask 10 and mask 8 vs mask 11.

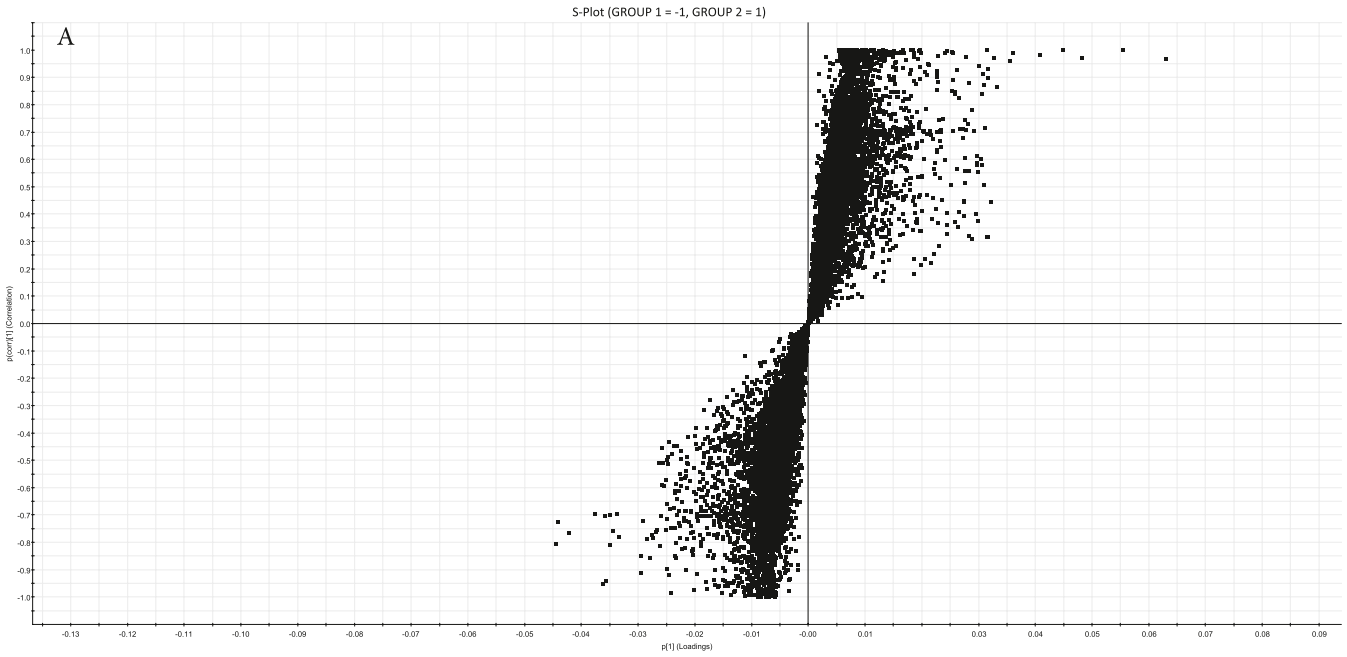
Fig. 2 shows the group Differences between mask 1 (group 1) and mask 3 (group 2) using OPLS-DA. S-plot, scores plot and model parameters are shown in the figure. Scores plot shows the within group variation (y axis) and between group variation (x axis). Moreover, S-plot obtained for the data from the mask 1 samples and mask 3 samples. The markers close to $y = 1$ on the S-plot are either unique to, or have elevated intensities in, mask 3, while those close to $y = -1$ are unique to, or have elevated intensities in, mask 1. Additionally, five markers unique to, or at elevated intensities, in the printed masks (mask 3) were selected from the S-plot as shown in Fig. 2. The markers were identified using the previously described databases. For the other masks, there were no significant differences between the compounds migrating from the printed and unprinted versions.

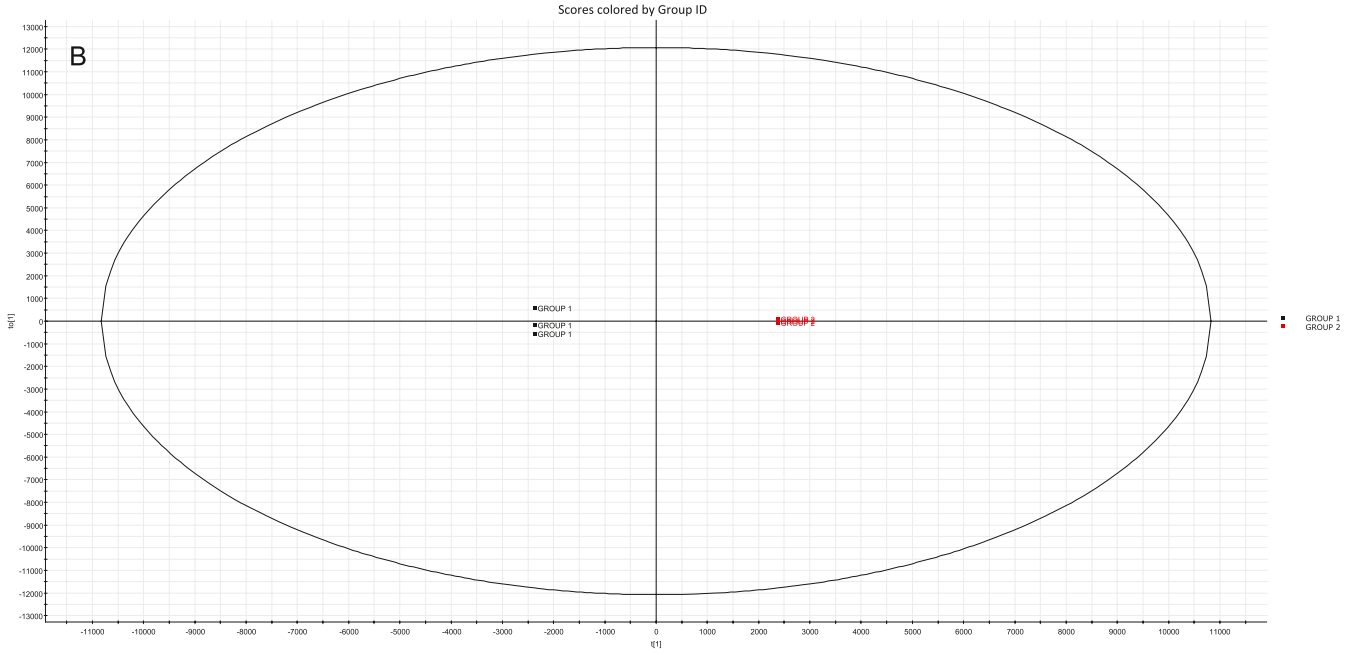
One advantage of using $HDMS^E$ to acquire the data is that potential fragment ions in the high-energy spectrum can be compared to theoretical fragments of an assigned compound. The *Fragment Match* tool integrated in the UNIFI software performs a series of chemically-intelligent bond disconnections on the structure of a proposed compound and automatically compares the *m/z* values of the generated substructures to the *m/z* values of ions in the high-energy spectrum. Being able to assign substructures of an identified compound ions in the high-energy spectrum increases confidence in the identification and complements the confirmation made using retention time, accurate mass and CCS values.

As an example, Fig. 3 shows the low and high energy spectra of the component identified as tris(2,4-di-tert-butylphenyl) phosphate in mask 3. The spectra shown in Fig. 3 have little interference from coeluting species or background ion and this is another advantage of using ion mobility in $HDMS^E$ since precursors and fragment ions are aligned in both the retention time and drift time dimensions. The CCS value obtained experimentally was 283.3 $Å^2$ while the CCS value in the library for this compound is 282.1 $Å^2$ (a difference below 1 %). The measured retention time was 9.37 min which was the same as that in the database. The accurate *m/z* values of the protonated and sodiated adducts were measured as 663.4554 and 685.4376, respectively, and compared to the theoretical values of 663.4537 and 685.4356 respectively. Therefore, the CCS value, retention time and accurate masses were all within the thresholds established for the automatic identification. Additionally, Fig. 3 shows the assignment of the compound to the low and high energy spectra of the component with a protonated *m/z* of 663.4554. The sodiated adduct of the compound can also be observed in the spectrum (*m/z* 685.4376). Sodium adducts do not fragment readily, as shown by the presence of the 685 *m/z* ion in the high energy spectrum, however they can be used as diagnostic features for the confirmation of an identification. The fragmentation of the protonated adduct is clearly displayed in high-energy spectrum and two accurate *m/z* values were assigned to fragments of tris(2,4-di-tert-butylphenyl) phosphate. The identification was ultimately confirmed by measuring a standard of this compound.

The procedure described in the previous paragraph was followed to identify 11 compounds that had migrated from the printed masks to the saliva simulant. Table 2 lists the compounds together with the measurements supporting their identification. The difference between measured neutral masses and those retrieved from the database and was below 3 ppm for all the compounds identified. The variation in expected retention times against the observed retention times of the identifications was below 0.15 min and the difference between observed CCS values and expected CCS values ranged between 0.6 % and 2.61 %.

Every identified compound was validated by conducting standard tests, thereby affirming the robustness of the identification procedure based on the database. Table 3 shows the limits of detection (LOD) and quantification (LOQ) determined by considering the minimum amount of the analyte of interest that produces a chromatographic peak with a signal-to-noise (S/N) ratio of 3:1 and 10:1, respectively, in the presence of background signal noise. Very low limit of detection and





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Model	GROUP 1 vs. GROUP 2 (OPLS-DA)
Scaling	Pareto
Transform	None
Variance explained - R2Y (Cum)	99%
Variance predicted - Q2 (Cum)	99%

Fig. 2. Group Differences between mask 1 (group 1) and mask 3 (group 2) using OPLS-DA, A) S-plot, B) scores plot and C) goodness of fit obtained by MVA software, EZInfo.

quantification (ranging between 2.2 and 11.3 ng/g) and good linearity ($R2 > 0.99$) were obtained. Validation tests provided good results of these tested parameters.

Fig. 4 shows the migration concentration of each of the compounds identified from each mask.

The compounds found in higher concentrations were BHT, Irganox 1076, Tris(2,4-di-tert-butylphenyl) phosphate and 2-(2-Butoxyethoxy) ethyl acetate. The first two were common plasticizers in plastic. The third compound was a non-intentionally added substance (NIAS) produced by the oxidation of the compound Irgafos 168, a common

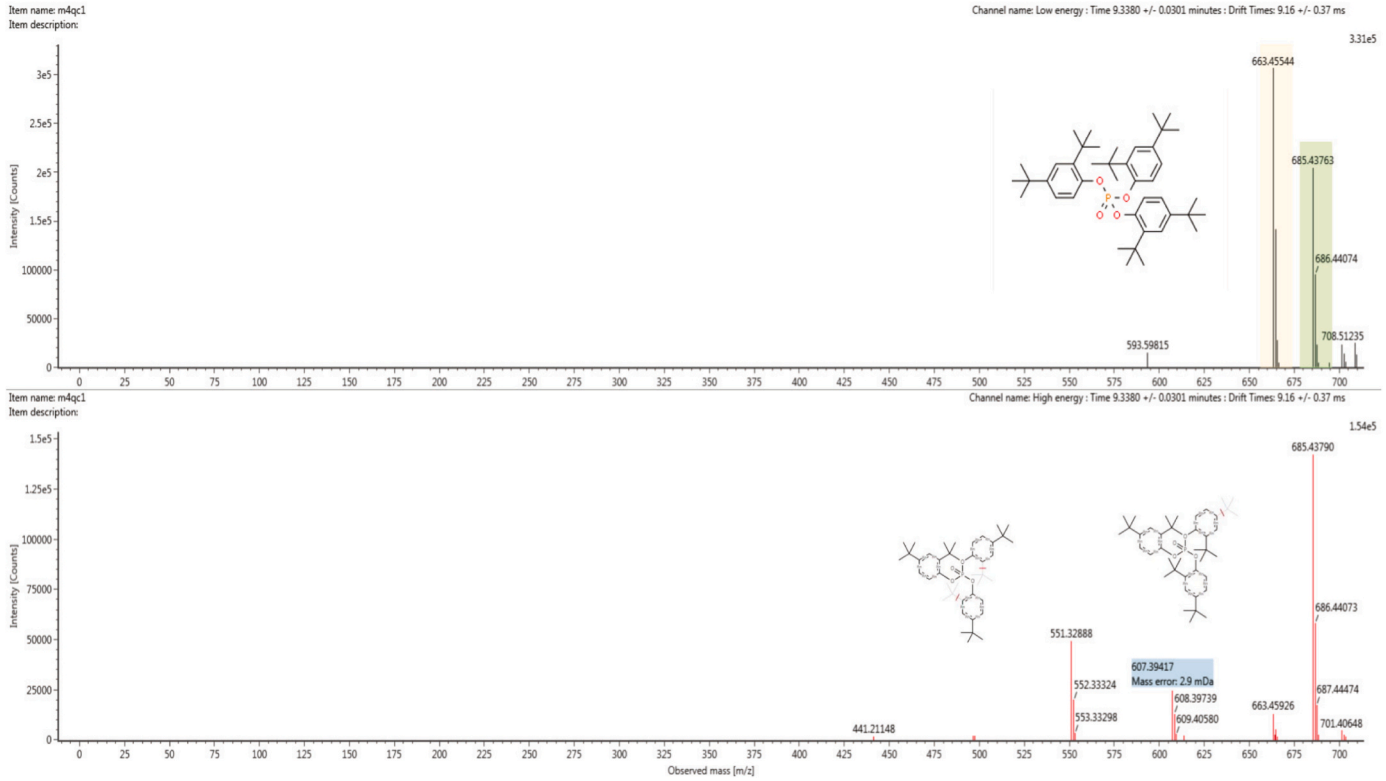

Fig. 3. Low energy (A) and high energy (B) mass spectra of the compound tris(3-methylphenyl) phosphate.

Table 2
Compound names, calculated mass, observed mass, mass error, expected and observed retention time, expected and observed CCS, delta CCS percentage and type of adduct(highlighted the most abundant) of the compounds identified by UPLC-IMS-Q-TOF.

Compound name	Calculated mass (Da)	Observed mass (Da)	Mass error (mDa)	Expected retention time	Observed retention time	Observed CCS (Å^2)	Expected CCS (Å^2)	CCS delta (%)	Adduct
Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate	553.4591	553.4583	0.8	10.22	10.27	245.9	248.6	-1.06	Na+
Tris(2,4-di-tert-butylphenyl) phosphate	663.4537	663.4544	0.7	9.37	9.37	283.3	282.1	0.44	**H+**,
	685.4356	685.4376	0.2						Na+
Docosanamide	340.3574	340.3574	0.0	8.67	8.56	205.9	209.9	-1.92	H+,
	362.3393	362.3392	0.1						**Na+**
Methyl palmitate	271.2632	271.2633	-0.5	8.42	8.42	182.5	187.0	-2.39	H+
Triphenylphosphine oxide	279.0933	279.0953	-2.0	5.59	5.60	159.7	162.0	-1.44	H+
2,6-Di-tert-butyl-4-methylphenol	221.1900	220.1928	-2.8	4.52	4.43	158.6	159.4	-0.52	H+
Octocrylene	384.1934	384.1913	2.1	7.43	7.41	204.8	207.3	-1.23	Na+
Ethyl 4-(dimethylamino)benzoate	194.1176	194.1149	2.7	5.90	5.74	144.6	149.4	-3.23	H+
2-(2-Butoxyethoxy)ethyl acetate	227.1254	227.1236	1.8	5.03	5.02	151.1	151.4	-0.20	Na+
Tris (3-methylphenyl)phosphate	369.3727	369.3715	1.2	7.21	7.03	181.7	186.6	-2.61	H+,
	391.3545	391.3533	1.2						**Na+**
Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate	315.1931	315.1941	-1.0	7.03	7.18	176.3	177.4	-0.6	Na+

antioxidant (Vera et al., 2018) and the last one was a common used solvent.

Regulation 10/2011/EC (commission, 2011) pertains to the permissible migration limits of substances emanating from materials in contact with food. In this context, the regulation has been applied tentatively, serving as a guiding framework because no established regulations govern the migration of substances from masks. Furthermore, the migration studies allowed a timeframe of 4 h for compounds to migrate from the masks into a simulated saliva medium. Yet, masks are often worn for more extended periods, and children might use multiple masks in a single day. As a result, it is challenging to precisely ascertain a child's exposure to the migrating substances. Consequently, the specific migration limits detailed in the subsequent sections should be viewed as indicative rather than definitive, offering insight into the potential impact of exposure. Additionally, the Food Contact Chemicals database (FCCdb) has been used to evaluate the evaluate the hazard derived. It is a comprehensive repository of intentionally added food contact chemicals (FCCs), encompassing hazard and regulatory data where available. Its coverage extends worldwide.The FCCdb aggregates data from 67 sources, including public regulatory lists and industry inventories, to compile its information. In its current iteration (version 5.0), the database identifies 12,285 distinct FCCs that have the potential for global use in the production of food contact materials and articles (FCMs and FCAs). Following a meticulous review of all substances in the database, 608 of these FCCs have been prioritized for further evaluation and potential substitution in FCMs and FCAs, with authoritative sources

Table 3
Compound names, limits of detection (LOD), limits of quantification (LOQ), linear regression (r2), linear range and relative standard deviation (RSD%) of the methods developed to quantify the compounds detected.

Compound name	LOD (ng/g)	LOQ (ng/g)	R^2	Linear range	RSD %
Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate	2.5	8.2	0.998	8.2–300	3.2
Tris(2,4-di-tert-butylphenyl) phosphate	3.4	11.3	0.999	11.3–350	3.4
Docosanamide	1.9	6.3	0.998	6.3–200	4.2
Methyl palmitate	1.3	4.4	0.997	4.4–200	2.2
Triphenylphosphine oxide	1.4	4.5	0.989	4.5–200	2.8
2,6-Di-tert-butyl-4-methylphenol	4.6	15.3	0.996	15.3–350	3.0
Octocrylene	1.4	4.6	0.999	4.6–200	4.1
Ethyl 4-(dimethylamino) benzoate	2.2	2.2	0.996	2.2–200	2.2
2-(2-Butoxyethoxy)ethyl acetate	0.7	5.6	0.998	5.6–350	1.9
Tris(methylphenyl)phosphate	1.2	4.1	0.991	4.1–200	3.5
Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate	1.3	4.2	0.999	4.2–200	2.1
toluene	2.6	8.6	0.979	8.6–100	4.8
chlorobenzene	1.6	5.3	0.981	5.3–100	5.9

of hazard information, such as the Globally Harmonized System, serving as a guide in this process (Groh et al., 2021; Groh et al., 2020). Finally, Threshold of Toxicological Concern (TTC) was also used. TTC is a concept used in toxicology to estimate a safe exposure level for chemical substances when little or no toxicity data is available. It is a pragmatic approach to assess the potential risks associated with low levels of exposure to a wide range of chemicals. The TTC is based on the idea that there are exposure levels so low that they pose negligible risk to human health. By comparing the exposure to a specific chemical with its established TTC value, it's possible to make a preliminary assessment of the potential safety of that exposure. If the exposure level is well below the TTC, it is generally considered to be of low concern.

TTC values vary depending on the chemical's structure, and they are usually categorized into different levels (e.g., high, intermediate, and low) based on potential hazards and structural features. Chemicals that fall into the high TTC category typically have more toxicological data available, whereas those in the low category have minimal data. The Threshold of Toxicological Concern is often used in regulatory and risk assessment contexts, such as evaluating the safety of chemicals used in food packaging, cosmetics, or other consumer products when detailed toxicity data is limited. However, it's important to note that the TTC is a conservative tool, and additional safety assessments may be needed if more information becomes available or if exposure levels are close to the established TTC value (TTC, 2005).

. Tris(2,4-di-tert-butylphenyl) phosphate and 2-(2-Butoxyethoxy) ethyl acetate were found to migrate from all the FFP2 masks (masks 1,2 and 3) studied. Tris(2,4-di-tert-butylphenyl) phosphate is a NIAS produced by the oxidation of the compound Irgafos 168 as mentioned above. The compound's migration ranged from 88 to 169 ng/g, depending on the masks. This antioxidant is allowed to be added to plastics that are used for food contact without a specific migration limit (SML) (commission, 2011). Therefore, following this guidance, the compound does not pose a human health hazard.

2-(2-Butoxyethoxy)ethyl acetate is a frequently incorporated substance in plastics, coatings, adhesives and inks. Nevertheless, European regulations have yet to define a migration threshold for this compound. Furthermore, it is noteworthy that the Food Contact Chemicals database (FCCdb), as reported by (Groh et al., 2021; Groh et al., 2020). Moreover, when applying the TTC criterion to this substance, it falls within Class I. Consequently, its migration levels (ranging from 56 to 165 ng/g) would be substantially lower than the recommended 1800 ng/g limit.Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (Irganox 1076), docosanamide, methyl palmitate, triphenylphospine oxide and butylated hidroxytoluene (BHT) were identified in all the surgical masks (mask 4–11) irrespective of the supplier of the mask, the printing ink use or the region of maufacture (China, Spain and Turkey).

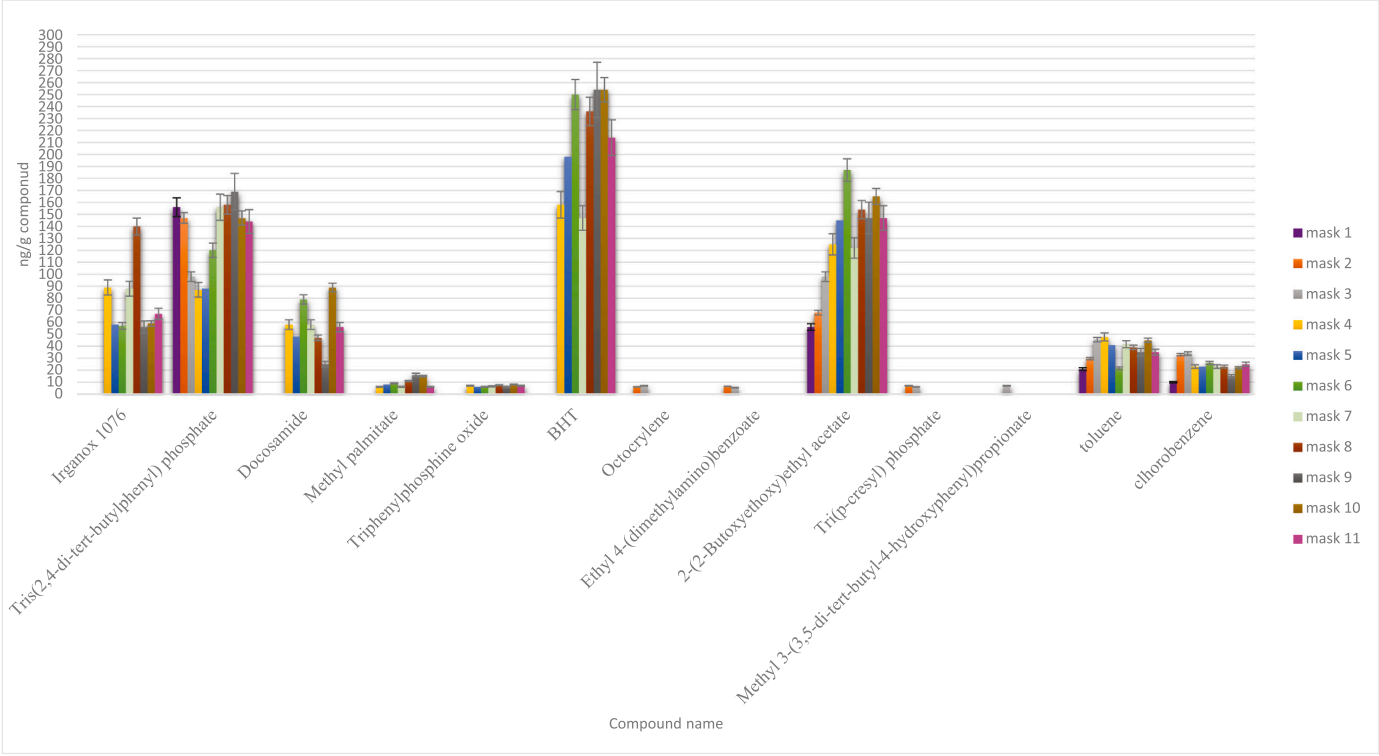

Fig. 4. The concentration of the compounds identified as migrating from the masks to the saliva simulant expressed in ng/g compound.

Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (Irganox 1076), BHT, docosanamide and methyl palmitate are all listed in the food contact plastics regulation (commission, 2011) and they are commonly added to plastics. The regulation established a specific migration limit of 6 mg/kg for Irganox 1076, however, no limits have been set for docosanamide and methyl palmitate. The concentration found in saliva simulant after migration was far lower than this limit (Fig. 4).

According to FCCdb, the compound triphenylphospine oxide is an additive used in plastics and printing inks. The compound is listed in part B of the Swiss 2019 list for packaging inks (no migration of the substance is authorized, compliance shall be established using the appropriate test methods selected in accordance with Article 11 of Regulation (EC) No 882/2004 which can confirm the absence of migration beyond a specified detection limit. If specific detection limits have not been established for specific substances or groups of substances, a detection limit of 0.01 mg/kg shall apply). Therefore, if the Swiss 2019 list is taken as tentative guidance, the migration of this compound (ranging from 5 ng/g to 8 ng/g) was below the limit established.

The MVA analysis discussed earlier established that the only significant differences in the migration from printed and unprinted masks were observed for the FFP2 masks (masks 1, 2 and 3). Four compounds were identified in mask 3 (FFP2 printed) that were not detected in mask 1 (FFP2 unprinted). They were the compounds octocrylene, ethyl 4-(dimethylamine)benzoate, ethyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate and tris(3-methylphenyl)phosphate. Additionally, three of these compounds were detected in mask 2 (FFP2 printed) but were not detected in mask 1 (FFP2 unprinted). They were the compounds octocrylene, ethyl 4-(dimethylamine)benzoate and tris(3-methylphenyl) phosphate.

The FCCdb (Groh et al., 2021; Groh et al., 2020) lists octocrylene as an additive used as a UV filter in plastics, coatings and printing inks which is allowed by the plastics regulation (commission, 2011) with a specific migration limit of 0.05 mg/kg. The migration concentration from masks 2 (6 ng/g) and 3 (7 ng/g) was below this migration limit.

The compound ethyl 4-(dimethylamine)benzoate is a substance commonly used in printing inks according to FCCdb (Groh et al., 2021; Groh et al., 2020). The compound has been designated as a high-priority health risk due to its potential for carcinogenicity, mutagenicity, or reproductive toxicity, as determined by the Danish EPA's GHS-aligned classifications derived from in silico modeling.

Additionally, the third compound found to migrate from the printed FFP2 masks, tris(3-methylphenyl)phosphate, is listed in the (Groh et al., 2020; Pomatto et al., 2018) and is classified as a priority hazardous substance based on selected authoritative sources. The FCCdb indicates that the substance is included in the list of 608 hazardous substances prioritized as the most urgent candidates for further evaluation. Additionally, the FCCdb states the compound is a as potential endocrine disruptor as assessed in the European Union (Groh et al., 2021; Groh et al., 2020).

Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate was found to have only migrated from mask 3. The FCCdb lists this compound as an additive used in plastics and printing inks. This compound is listed in part B of the Swiss 2019 regulation for packaging inks (no migration of the substance is authorized. Compliance shall be established using the appropriate test methods selected in accordance with Article 11 of Regulation (EC) No 882/2004 which can confirm the absence of migration beyond a specified detection limit. If specific detection limits have not been established for specific substances or groups of substances, a detection limit of 0.01 mg/kg shall apply). The migration of this compound (7 ng/g) was below the detection limit of 0.01 mg/kg.

In summary, it has been demonstrated that the most concerning compounds migrated only from printed FFP2 masks and they were compounds related to inks. This is a remarkable outcome from the point of view of public health, as all the more concerning as these masks are mainly used by children, a very vulnerable population. Furthermore, this summary highlights the importance of the protocol used in this study; comparing the printed and unprinted samples through the use of statistical tools applied to IMS-QTOF data and subsequent identification of compounds through the use of a well curated database.

3.2. The identification and quantification of volatile migrants in the saliva simulant using HS-SPME-GC-MS

HS-SPME-GC-MS was used to identify volatile compounds that had migrated from the masks to the saliva simulant.

A preliminary SPME method was created to compare three SPME fiber coatings in order to select the most appropriate fiber coating for this analysis. The extraction temperature and the extraction time for the preliminary method were set to 80 °C and 30 min, respectively. The time and temperature were established by reviewing previous methods used for the identification of unknown volatile compounds (Nerin et al., 2009; Canellas et al., 2014, 2015; Vera et al., 2020). The first fiber tested was a 85 μm polyacrylate fiber used for the analysis of polar, semi-volatile compounds (MW 80–300) (Merck, 2021). No migration products were detected in the simulant when using this fiber. The two other fibers tested were a 100 μm polydimethylsiloxane fiber, which is commonly used to analyze volatile compounds (MW 60–275), and a 50/30 μm divinylbenzene/Carboxen/polydimethylsiloxane fiber, which is used for trace compound analysis (MW 40–275). Two migration products were detected using these two fibers, toluene and chlorobenzene. The 50/30 μm divinylbenzene/Carboxen/polydimethylsiloxane fibre was the more sensitive of the two fibres (the limit of detection (LOD) of toluene was 2.3 times lower and the limit of detection of chlorobenzene was 1.5 times lower when using this fiber) and was therefore selected for subsequent quantitation analyses. The LOD of toluene using this fibre was 8.6 ng/g and that of chlorobenzene was 5.3 ng/g. The relative standard deviation (RSD %) values were 4.8 % and 5.9 % for toluene and chlorobenzene, respectively.

Compound identification was performed by comparing the measured spectrum to the NIST 2014 GCMS (Version 2.2) library with a forward-fit acceptance criterion of at least 900.

Fig. 5 compares the chromatogram obtained for the saliva simulant following the migration study for mask 1 against that for the blank saliva simulant. The figure shows the chromatographic peaks of both compounds unique to the saliva simulant following the migration study. Fig. 5 also shows the spectrum of the peak obtained at 5.46 min which is compared to the spectrum obtained from the NIST 2014 database for chlorobenzene. There is extremely good agreement between the two spectra for all m/z values above the lower limit of the acquired experimental range of 45 Da (Section 2.4).

Fig. 4 shows that chlorobenzene and toluene migrated from all the masks studied, regardless of the color, supplier or origin of the mask. The FCCdb (Groh et al., 2021; Groh et al., 2020) lists chlorobenzene and toluene as solvents in the production of coatings, plastics, adhesives and printing inks. Although the FCCdb does not list chlorobenzene as hazardous, toluene has been listed as a hazardous priority substance and a putative endocrine disruptor. Applying TTC values, chlorobenzene would be classified as Class III, and toluene as Class I. Both of the migrating compounds, toluene and chlorobenzene, would remain within the recommended limits of 90 ng/g and 1800 ng/g, respectively, as the migration of toluene ranged from 21 to 48 ng/g, and the migration of chlorobenzene varied from 10 to 34 ng/g.

4. Conclusions

The identification of compounds migrating from a range of masks to a saliva simulant has been achieved using data acquired with UPLC-IMS-QTOF in conjunction with a FCM database containing CCS values, accurate masses and retention times of the compounds was successfully done. The multivariate analysis technique of OPLS-DA was used to

Fig. 5. A) Chromatogram of the mask 1 obtained by GC-MS. B) spectrum of the peak obtained at 5.46 min and spectrum of chlorobenzene.

compare printed and unprinted masks. It allowed identifying four migrants coming only from the printed masks.

Moreover, high sensitivity was reached for the techniques of UPLC-IMS-QTOF and SPME-GC-MS used in this study.

This work has revealed that is it possible for hazardous substances to migrate from the masks to the saliva simulant, and printed masks tended to release a higher concentration of these compounds compared to the unprinted masks. However, it's essential to acknowledge that this study represents one facet of the broader safety considerations associated with mask usage. While the migration of substances into a saliva simulant is a critical aspect, there are other variables that must be taken into account for a comprehensive evaluation. One such variable is metabolization that plays a pivotal role in determining the overall safety profile of masks, as it addresses the fate of substances once they are exposed to saliva and other physiological fluids.

CRediT authorship contribution statement

Elena Canellas: Conceptualization, Methodology, Investigation, Resources, Wrtting Paula Vera: Methodology, Investigation, Wrtting Cristina Nerin: Methodology, Investigation, Resources, Wrtting Jeff Goshawk and Nicola Dreolin: Resources, writing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Elena Canellas reports financial support was provided by University of Zaragoza. Elena Canellas reports a relationship with University of Zaragoza that includes: funding grants (Project JIUZ-2020-CIE-03 funded by University of Zaragoza/Ibercaja). Elena Canellas reports a relationship with the grant RYC2021–034150-I funded by MCIN/AEI/ 10.13039/501100011033 and by the "European Union Next Generation EU/PRTR that includes: funding grants and paid expert testimony.

Data availability

No data was used for the research described in the article.

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