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NON-TARGET AND SUSPECT SCREENING FOR ORGANIC SUBSTANCES IN INDOOR DUST FOR HUMAN EXPOSURE ASSESSMENT

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Introduction

The indoor air quality and human exposure assessment of chemicals have become subject of multiple studies during the last decades, because people reside for the greatest part of life indoors. People in Europe and the US spend on average around 90% of their time indoors (homes, workplaces, cars and public transport means, etc.)[1]. The role that dust plays in human exposure assessment is of paramount importance. In terms of exposure pathways it can represent an important exposure medium (medium by which a contaminant moves from its source). Previous studies have shown that dust can carry organic contaminants[2] such as brominated and organophosphate flame retardants, fluorinated compounds, phthalates, PCBs, PAHs, pesticides and drugs[3]. Most of these chemicals are released through evaporation, leaching and ageing of common consumer goods present indoors (electronic devices, furniture, textiles, cleaning and health care products, building materials). Because a broad range of compounds is present in dust, we developed an analytical method that reduces the matrix complexity and facilitate the data interpretation. We created a library of around 11000 chemicals in which compound formula, name, IUPAC name, nominal mass, CAS number and structure have been included. The database comprises reported chemicals in dust and consumer products (e.g. plasticizers and flame retardants), but also potential persistent and bioaccumulative (P&B) compounds, P&B transformation and by-products, impurities and pharmaceuticals. A combination of various analytical techniques in combination with suspect and non-target screening and statistical multivariate approaches made it possible to identify a large range of chemicals in indoor dust.

Materials and methods

A total of 58 dust samples from vacuum cleaner bags was collected within the A-TEAM project (Advanced Tools for Exposure Assessment and bioMonitoring) between November 2013 and April 2014. The vacuum cleaner bags were wrapped in aluminum foil and stored in a plastic bucket at room temperature during the sampling. The collected dust was sieved with a 500 μm sieve and 2 g aliquot from each bag were stored in 30 mL containers[4].

A sub-sample of 100 mg of sieved dust from each sample was spiked with an internal standard mixture and extracted with n-hexane/acetone (1:2, v/v) in an ultrasound bath for 10 min after 1 min vortexing. Samples were then centrifuged and the supernatant was transferred into a clean tube. The extraction process was repeated two times. Isooctane was added to each tube and the extracts were evaporated to 800 μL under a gentle steam of nitrogen. This extract was fractionated in order to reduce the complexity of the sample using a Gilson GX-271 ASPEC™ system. Five fractions of increasing polarity (n-hexane, n-butyl chloride, dichloromethane, ethyl acetate:methanol (1:1, v/v), methanol:water (95:5, v/v)) were collected. Silica SPE cartridges as a non-destructive clean-up were used to ensure that degradation of chemicals did not occur. The collected 3 mL fractions were finally evaporated to almost dryness and re-suspended in 100 μL isooctane (only fractions A, B, C) for injection in GC, or methanol for LC analysis (all the 5 fraction in methanol were analyzed using the ESI source and fractions A-B-C were also analyzed with APCI).

The dust fractions were analyzed by GC and LC coupled to high resolution time- of-flight MS (microTOF II Bruker Daltonics, Bremen, Germany with mass accuracy <2ppm and resolution >16,500). Internal mass calibration was used resulting in an accuracy below 5 ppm. Concerning the LC analysis, both

electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) sources were used whereas, for the GC analysis, only the APCI source was applied.

The analytes in the GC analysis were separated on a Select PAH column whereas for the LC analysis a Kinetex core shell LC C18 column was used with eluents H_2O and CH_3OH.

The software Data analysis 4.0 from Bruker Daltonics (Bremen, Germany) was used to process data. Internal calibration was performed on all the spectra with the enhanced quadratic mode and chromatograms were processed with the Find Molecular Feature (FMF) algorithm which combines isotopes, charge state, adducts and common neutral losses belonging to the same compound into one feature. Retention time, m/z value and intensity define each molecular feature. Parameters were set as follows: S/N = 5, correlation coefficient threshold = 0.8, minimum compound length = 10 spectra. The molecular features were then aligned by retention time using an algorithm (non linear retention time shift), processed in bucketing and normalization with the software Profile Analysis 2.1 (Bruker Daltonics, Bremen, Germany). The generated bucket table was imported to SIMCA-P+ 13.0 (Umetrics, Umeå, Sweden) and processed for multivariate data analysis (MVDA). Principal component analysis (PCA) was performed using Pareto scaling (the intensity of each variable was scaled by the square root of that variable's standard deviation).

All the calibrated spectra were also processed with the MetaboliteDetect 2.0 SR4 software (Bruker Daltonik, Bremen, Germany) which allows to perform blank subtraction by the eXpose algorithm. Every data point in the mass spectrum of the LC-MS chromatogram is described by retention time, m/z value and intensity; the eXpose algorithm compares every single data point in sample and reference (blank) with a defined tolerance for a shift in mass position (DeltaMass) and retention time (DeltaTime). Parameters were set as followed: Ratio (3.00), Deltatime (±0.20 min), DeltaMass (±0.50 m/z). The obtained chromatograms were then processed with the Compass TargetAnalysis software (Bruker Daltonik, Bremen, Germany) using the aforementioned suspect database of around 11,000 compounds. Results from the Compass Target and Multivariate data analysis were eventually combined in order to identify chemicals including identification of unknown compounds based on exact mass and isotope pattern. Multivariate data analysis was also performed for the non-target screening using all molecular features.

Results and discussion

In all samples the majority of peaks were present in the hexane fractions with every technique applied. In general, hundreds of molecular features were found in fraction A. As an example the number of molecular features found and the number of peaks tentatively identified with the suspect database for five samples analyzed by HPLC-APCI-TOF-MS in positive mode are shown in Table.1. As shown, about a third of the molecular features could be identified with the suspect list.

Principal component analysis (PCA) was performed on all molecular features found. Samples were sorted by fraction, source and ionization mode. PCA was used to highlight differences among samples and compounds. The score plot of the samples analyzed with APCI in positive mode (Fig. 1) shows the variation between samples, and suggests little differences in the chemical profile among the samples. It seems that a number of samples, such as 39A and 40A, have a different chemical pattern.

As can be seen from the loading plot (Fig. 2), which shows the compounds, many chemicals cluster together which supports the observation that most samples have similar chemical patterns in fraction A. Especially chemicals which are not in the origin of the loading plot are interesting as these are enhanced in some of the samples. As an example, the peak at RT 23.1 with m/z 352.2374 in sample 31 (fraction A) (indicated with a red spot in Fig. 2) corresponds to the molecular formula $C_{22}H_{29}N_3O$. The exact mass and isotopic pattern is compatible with 2-(2H-benzotriazol-2-yl)-4,6-ditertpentylphenol (Tinuvin 328), an UV absorber of the hydroxyphenyl benzotriazole class. Its purpose is to enhance the light stabilization of coatings. It has a high log K_{ow} of 7.25 and it is soluble at 20°C in hexane (16%w/w); both parameters fit with the hexane fraction. In case of the compound with m/z 245.3771 at RT 21.1 in sample 40 (fraction A) with molecular formula $C_3H_3N_6$, Melamine (blue spot in Fig. 2) is a candidate. This compound is used worldwide in a variety of applications in consumer products: used as flame retardant in paints, plastic

and papers, as superplasticizer for high-resistance concrete, stabilizer in wood coatings and paintings, in resins for laminate flooring and formica, additive in insulation for pipes, heating and ventilation system and it is the principal component of Pigment yellow 150 (a colorant for inks and plastics). The large use of this compound in consumer goods makes it reasonable to be present in dust; on the contrary its presence in the hexane fraction is not compatible with the log K_{ow} of -1.14. Therefore, this compound needs further verification by an analytical standard.

In conclusion, the extraction and fractionation method developed resulted to be efficient, producing "clear" chromatograms, simplifying the identification of the compounds using suspects databases. The use of physical-chemical properties of the tentatively identified compounds is another step to be used to verify and reduce the number of identified unknown compounds based on exact mass and isotopic pattern. The blank subtraction algorithm of the software package MetaboliteDetect made it possible to eliminate interfering compounds from the procedure during sample preparation. The combined approach of molecular features and multivariate data analysis showed to be a good solution for identifying interesting samples and chemicals. The confirmatory analysis based on analytical standards and MS/MS experiments can further help to elucidate the identity of unknown compounds. With multivariate data further correlations with questionnaire information from the houses where the dust was collected will be made to link the identified chemicals to consumer products present in the homes. This approach will help to fill the gap between the vast number of chemicals in consumer goods and human exposure.

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Table 1: As an example the number of Molecular Features and the number of suspects found in 5 dust samples analyzed by LC-APCI-TOF-MS (+).

Sample	Molecular Features	Suspects
05 A	529	112
05 B	51	16
05 C	65	28
18 A	485	334
18 B	181	168
18 C	101	39
20 A	322	143
20 B	33	90
20 C	14	24
32 A	344	144
32 B	56	55
32 C	39	33
41 A	438	97
41 B	49	75
41 C	66	63

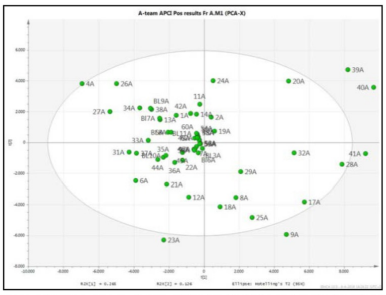

Fig. 1: *PCA score plot*. Summary of the relationship among the Fraction A of all dust samples analysed using HPLC-APCI-TOF-MS (+).

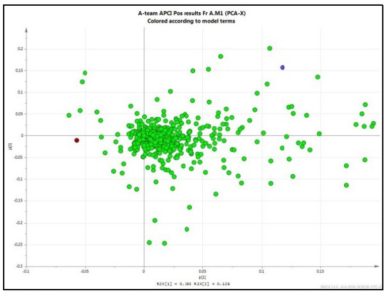

Fig. 2: *PCA loading plot*. Summary of the compounds in Fraction A of all dust samples analysed using HPLC-APCI-TOF-MS (+).