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Comparative study of comprehensive gas chromatography-nitrogen chemiluminescence detection and gas chromatography-ion trap-tandem mass spectrometry for determining nicotine and carcinogen organic nitrogen compounds in thirdhand tobacco smoke

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ABSTRACT

Thirdhand tobacco smoke (THS) constitutes a poorly understood pathway of exposure of non-smokers, especially toddlers, to tobacco-related carcinogens. However, to date most of the carcinogens present in tobacco smoke have not been detected in THS and, therefore, the significance of THS health risk is still unknown. In this study, we have compared the performance of two analytical methods – one based on gas chromatography coupled to ion trap mass spectrometry detection (GC-IT-MS) and the other on comprehensive two-dimensional gas chromatography coupled to a nitrogen chemiluminescence detector (GC × GC-NCD) – for simultaneously determining, in settled house dust, the presence of 16 organic nitrogen carcinogens already detected in tobacco smoke. The target compounds included four aromatic amines, two nitrocompounds, eight N-nitrosamines and two tobacco-specific nitrosamines, as well as nicotine as a tobacco marker. Dust samples were extracted using in-cell clean up pressurized liquid extraction with silica as clean up sorbent and ethyl acetate as the organic solvent, with average recovery of 89%. Although GC-IT-MS, using chemical ionization with methanol and tandem MS, performed well, the optimized GC × GC-NCD gave lower limits of detection (from 4 to 22 ng g^{-1}) and better repeatability and reproducibility a low concentration levels (%RSD < 8%) and, therefore, was applicable for determining these different groups of carcinogens without the need for derivatization prior to the GC analysis.

The performance of the optimized PLE/GC × GC-NCD method was tested by quantifying the target compounds in house dust samples from smokers' and non-smokers' homes. The median carcinogen compounds detected was 3.8 μg g^{-1} and 1.1 μg g^{-1} in smokers' and non-smokers' house dust, respectively. In this study, we have detected highly carcinogenic aromatic amines and nitro compounds for the first time in settled house dust complementing the state of knowledge of THS composition and providing fresh evidence about THS health risks.

1. Introduction

Tobacco smoke is considered one of the major sources of inorganic and organic carcinogens in indoor environments including arsenic, polycyclic aromatic hydrocarbons (PAH), and several kinds of organic nitrogen compounds (ONs), such as amines, nitroaromatics, N-nitrosamines and tobacco-specific nitrosamines (TSNAs) [1]. Recent studies have shown that many components of tobacco smoke are not removed by ventilation and can persist indoors long after the cigarette is extinguished, subsequently reacting to form more toxic species [2]. This residual tobacco smoke and particles tend to deposit on indoor surfaces and settled dust forming the so-called thirdhand tobacco smoke (THS) [3]. This constitutes a further pathway of exposure for non-smokers to tobacco carcinogens through inhalation, involuntary ingestion and dermal contact. The

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risk is particularly relevant for children, especially toddlers, principally because they are more vulnerable to chemical exposure as a consequence of their immature metabolism, the fact that they spend more time indoors and close to the floor, and their hand-to-mouth behaviours.

Although the role of THS in tobacco-induced illnesses has been recently demonstrated [4–6], to date only a few THS toxicants have been identified, making it difficult to assess the health impact of THS-polluted environments. Several studies have found nicotine, PAHs and N-nitrosamines [7–12] in THS and, recently, a wide range of inhalable toxicants [13]. Furthermore, carcinogenic TSNAs, formed during tobacco burning and also after nicotine has deposited in THS [2], have recently been detected in settled dust from smokers' and non-smokers' homes [12], highlighting THS relevance to human exposure and health. However, the occurrence of most of the mainstream smoke carcinogens in THS is still unknown, in part due to a lack of techniques capable of measuring trace amounts of these species in such a complex matrix.

The International Agency for Research on Cancer (IARC) recognized the presence of over 60 organic carcinogens in tobacco smoke [1]. Among these tobacco-related carcinogens, ONs are of special interest because of their high toxicity. Since the group of carcinogenic ONs emitted by tobacco smoke include a wide variety of functionalities, finding a method to simultaneously determine different classes of compounds is a challenging task. Moreover, settled dust is a heterogeneous complex matrix composed of hundreds of inorganic and organic materials including a large number of organic contaminants [14]. The chemical complexity and high adsorption capacity of settled dust particles require an efficient and selective extraction method prior to the chromatographic analysis. In this sense, pressurized liquid extraction (PLE) in combination with in-cell clean-up has been successfully applied for the extraction of organic contaminants from settled dust, with enhanced recoveries and reducing the time and steps needed for sample treatment prior to the analysis [11,15,16].

Additionally, the selection of the appropriate determination technique is also crucial in the analytical process. Whilst GC has been the preferred method for determining volatile N-nitrosamines, e.g. [17–19], and it has also showed a good performance for the analysis of TSNAs [11,20], the dipole created by the amino group requires a derivatization step prior to the GC analysis of aromatic amines adding an extra step to the analytical method [21,22]. Further, other techniques suitable for amines, such as HPLC followed by electrospray ionization and tandem mass spectrometry – widely used for determining aromatic amines in cigarette smoke [23,24] – are not appropriate for the determination of the more volatile N-nitrosamines.

The aim of this study is the optimization and validation of a highly sensitive and selective analytical method for simultaneously determining a wide range of polarity and volatility tobacco-related carcinogenic ONs in settled house dust samples. We have compared the performance of two analytical methods for simultaneously determining the presence in settled house dust of 16 organic nitrogen carcinogens already detected in tobacco smoke. One method was based on gas chromatography coupled to ion trap mass spectrometry detection (GC-IT-MS), working with two ionization modes [electron impact and (EI) and chemical ionization (CI)], and two ion analysis modes [micro selected ion storage (μSIS) and tandem mass spectrometry (MS/MS)]. The other method was comprehensive two-dimensional gas chromatography coupled to a nitrogen chemiluminescence detector (GC × GC-NCD). The sixteen selected compounds have been previously detected in tobacco smoke and are considered carcinogens by the IARC [1]. These target carcinogens include four aromatic amines and two nitroaromatic compounds (not identified yet in THS), and eight N-nitrosamines and two TSNAs. Nicotine has been also included as a marker of tobacco smoke. The complete list of the target compounds and their health risks are summarized in Table 1. In-cell clean-up pressurized liquid extraction (PLE) with ethyl acetate [11] was used as extraction method.

Table 1
Target compounds included in our study, their IARC classification and oral slope factors and the source of this information.

Target compound	IARC classification[a]	Oral slope factor
N-Nitrosodimethylamine (NDMA)	2A	51[b]
N-Nitrosomethylethylamine (NMEA)	2B	22[b]
N-Nitrosodiethylamine (NDEA)	2A	150[b]
N-Nitrosodi-n-propylamine (NDPA)	2B	7[b]
o-Toluidine (OT)	1	0.18[c]
Nitrobenzene (NB)	2B	–
N-Nitrosomorpholine (NMor)	2B	6.7[c]
N-Nitrosopyrrolidine (NPyr)	2B	2.1[b]
N-Nitrosopiperidine (NPip)	2B	9.4[c]
o-Anisidine (OA)	2B	0.14[c]
N-Nitrosodi-n-butylamine (NDBA)	2B	5.4[b]
Nicotine	–	–
2-Aminonaphthalene (2AN)	1	1.8[c]
4-Aminobiphenyl (4ABP)	1	2.1[c]
N′-Nitrosonornicotine (NNN)	1	1.4[c]
4-(Methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK)	1	49[c]
1-Nitropyrene (1NP)	2A	1.2[c]

[a] IARC classifications: group 1, carcinogen to humans; group 2A, possible carcinogen to humans; group 2B, probably carcinogen to humans [29].
[b] Data from IRIS [30].
[c] Data from OEHHA [31].

The applicability of the developed method was tested by analysing settled house dust samples from 18 smoking and non-smoking homes. As far as we know, this is the first time that a GC method without derivatization is used for the simultaneous determination of a wide range of polarities of tobacco-related carcinogenic ONs.

2. Experimental

2.1. Chemical standards

The standards of the 17 target compounds included a methanol solution of 8 nitrosamines (2000 mg L^{-1} of each) [EPA 8270/Appendix IX Nitrosamines Mix, from Sigma–Aldrich, (Steinheim, Germany) including N-nitrosodimethylamine (NDMA), N-nitrosomethylethylamine (NMEA), N-nitrosodiethylamine (NDEA), N-nitrosodi-n-propylamine (NDPA), N-nitrosomorpholine (NMor), N-nitrosopyrrolidine (NPyr), N-nitrosopiperidine (NPip) and N-nitrosodi-n-butylamine (NDBA)] and the individual standards for nicotine, nitrobenzene (NB), o-anisidine (OA), o-toluidine (OT) and 1-nitropyrene (1NP) (Sigma–Aldrich), 2-aminonaphthalene (2AN) and 4-aminobiphenyl (4ABP) from LGC standards (Teddington, UK) and the TSNAs N′-nitrosonornicotine (NNN) and 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK) from Fluka (Buchs, Switzerland). The minimal purity of the standards was 98%. The standard solutions of the target compounds were prepared in methanol and the diluted mixtures in ethyl acetate (GC grade with purity >99.9%, Fisher Scientific, Loughborough, UK).

2.2. Sample collection and extraction

Settled dust samples were collected from 18 non-smokers' and smokers' private homes in the area of Tarragona (Spain), using conventional vacuum cleaners in regular use by the households. Samples were classified as smokers' if at least one occupant was a tobacco smoker, including those whose occupants do not smoke