



A survey on published analytical methods for the analysis of HPHCs

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Background



- The U.S. Food & Drug Administration (FDA) has issued a list of 93 harmful and potentially harmful constituents (HPHCs) found in tobacco and nicotine products, cigarette smoke, e-liquids, and aerosols from electronic nicotine delivery systems (ENDS).
- The toxicity of HPHCs is classified as carcinogen (CA), respiratory toxicant (RT), cardiovascular toxicant (CT), reproductive or developmental toxicant (RDT), addictive (AD), banned in food (BF).
- A simple parameter used for the characterization of dose and response relation is the median lethal dose LD_{50} (lethal dose, 50%), which is the dose of a toxic substance (or radiation) required to kill half of the members of a tested population (usually of rats).
- IARC classification of carcinogens consists of: Group 1 - Carcinogenic to humans, Group 2A - Probably carcinogenic to humans, Group 2B - Possibly carcinogenic to humans, Group 3 - Not classifiable as to its carcinogenicity to humans, Group 4 - Probably not carcinogenic to humans.

Background



- Only a limited number of analytical methods for HPHCs are available in the literature, many of these methods being proprietary information and not available.
- Published methods for the analysis of HPHCs are available either in peer reviewed literature, or issued by organizations such as International Organization for Standardization (ISO), Cooperation Centre for Scientific Research Relative to Tobacco or CORESTA (CRM), Health Canada (T), DIN Standard Committee, etc.
- This presentation is a survey of published and available analytical methods for HPHCs and points out specific gaps in the available public information regarding the analysis of HPHCs.
- The methods can be classified in three groups:
 - 1) Methods well represented in the literature,
 - 2) Methods available only in peer reviewed literature and not in ISO or CORESTA,
 - 3) Methods not readily available, or 30 to 50 years old, which although may generate correct results, in most cases are rather complex and difficult to implement.

Nicotine



- Cigarette smoke (mainstream, sidestream, different smoking protocols such as ISO 3308, ISO 20778): **ISO 10315, ISO 22253, CRM 7, CRM 54, T-115, peer reviewed publications.**
- Other sources of tobacco smoke (e.g. cigars, heat-not-burned or HTP, hookah): **CRM 66, peer reviewed publications.** 
- Tobacco and tobacco products (including nicotine pouches): **ISO 15152, ISO 22980, CRM 85, CRM 62, CRM 87, peer reviewed publications.**
- ENDS: **ISO 20714, CRM 84, peer reviewed publications.**
- Environment: **CRM 50, peer reviewed publications.**
- Free base nicotine: **CORESTA Technical Report, peer reviewed publications.**
- Nicotine enantiomers: **peer reviewed publications.**
- Tobacco derived and synthetic nicotine: **radiocarbon analysis, several reports based on impurities.**

Methods: CFA, GC-FID, GC-NPD, GC-MS, GC-MS/MS, LC-UV, LC-MS, LC-MS/MS, other.

Carbon monoxide



- Cigarette smoke (mainstream, sidestream, different smoking protocols such as ISO 3308, ISO 20778): **ISO 8454, ISO 22947, CRM 5, CRM 55, T-115, peer reviewed publications.**
- Other sources of tobacco smoke (e.g. cigars, hookah): **CRM 68, peer reviewed publications.**

Methods: **NDIR.**

Acetamide and Acrylamide



- Cigarette smoke, heated tobacco products (HTP) smoke (for acetamide): **peer reviewed publications.**
- Tobacco (for acetamide): **peer reviewed publications.**

Methods: GC-MS, GC-MS after derivatization (e.g. with 9-xanthidol), LC-MS/MS.

- Cigarette smoke, HTP smoke (for acrylamide): **peer reviewed publications, TSRC presentations.**
- Tobacco, moist snuff (for acrylamide): **peer reviewed publications.**
- No acrylamide in ENDS: **peer reviewed publications.**

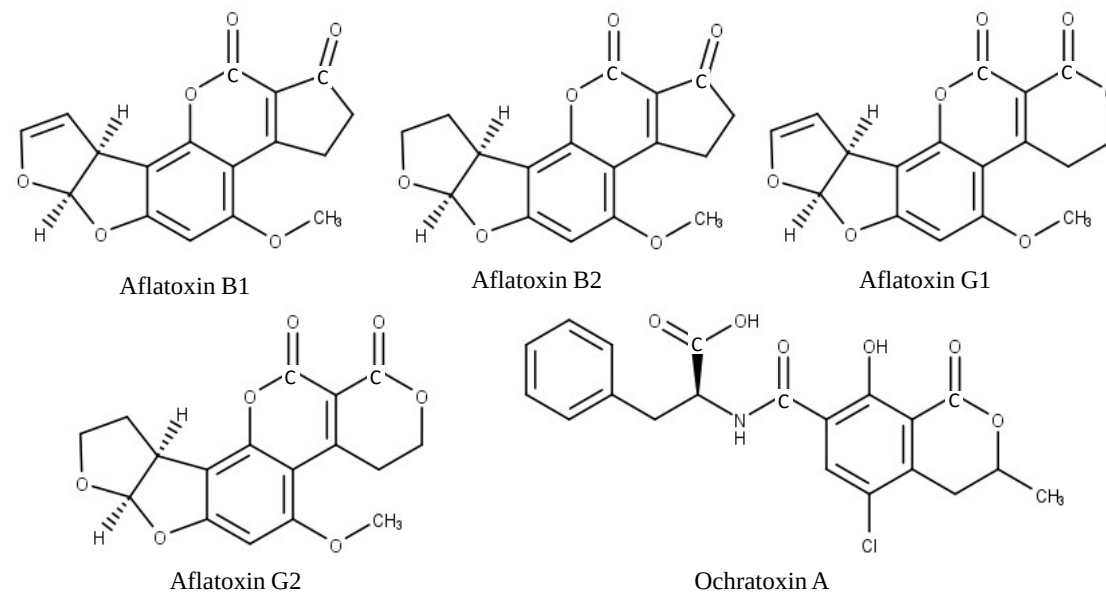
Methods: GC-MS, derivatization with Br, SPME with derivatization, LC-MS/MS as is, or after derivatization.

Aflatoxins



- No aflatoxin is transferred to cigarette smoke: **peer reviewed publications.**
- Tobacco, moist snuff, snus: **CORESTA presentation, peer reviewed publications.**

Methods: LC-MS/MS.



Ammonia



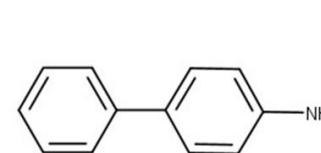
- Cigarette smoke (different smoking protocols), HTP smoke, cigars: CRM 83, T-101E, peer reviewed publications, TSRC presentations.
- Tobacco, moist snuff, snus: CRM 79, peer reviewed publications.
- Indoor air: peer reviewed publications.

Methods: Ion chromatography (IC) (conductivity detection), ion specific electrode, GC-MS after derivatization with dansyl chloride, CFA.

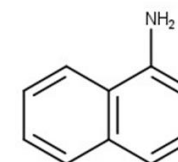
Aromatic amines



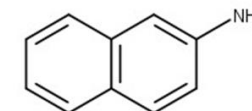
- Cigarette smoke (different smoking protocols), HTP smoke, cigars: CRM 95, T-102, peer reviewed publications, CORESTA collaborative study.
- Smokeless tobacco products: peer reviewed publications (aromatic amines not present).
- ENDS: peer reviewed publications (only low traces of o-toluidine detected by using a highly sensitive method).



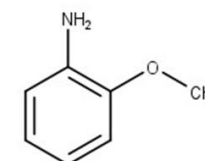
4-Aminobiphenyl



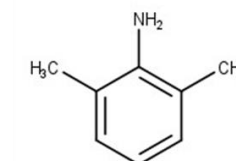
1-Aminonaphthalene



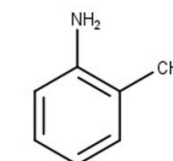
2-Aminonaphthalene



O-Anisidine



2,6-dimethylaniline



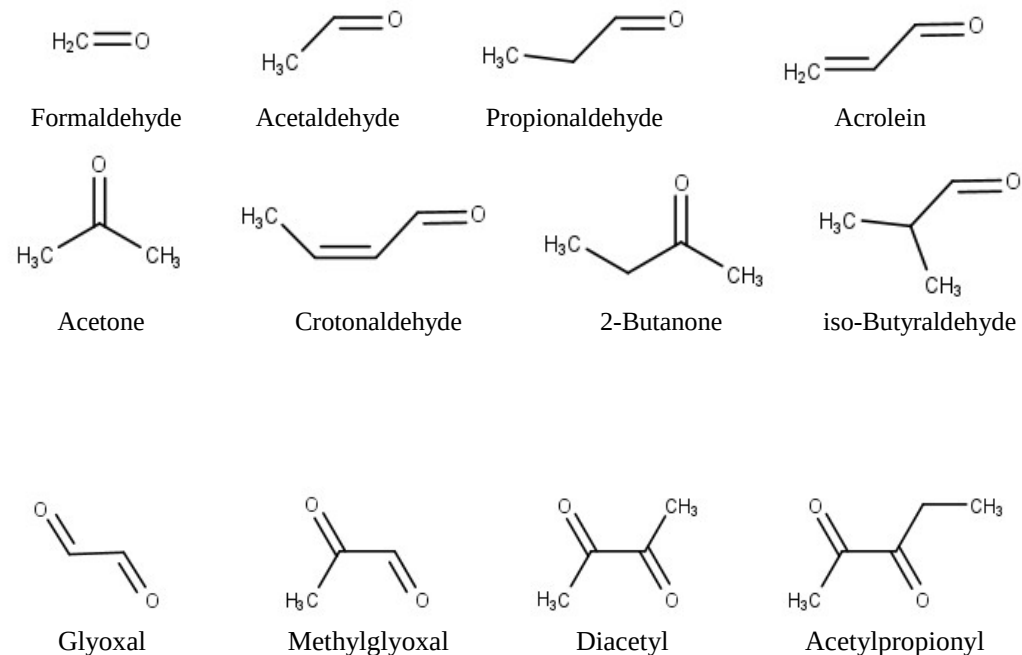
o-Toluidine

Methods: GC-MS or GC-MS-NCI after derivatization with HFBA, GC-MS/MS, SPME, LC-MS/MS, ion-mobility-LC-MS/MS.

Carbonyl compounds



- Cigarette smoke (mainstream, different smoking protocols), sidestream, HTP smoke, cigars: **CRM 74, T-104, peer reviewed publications (including CORESTA Technical Reports).**
- Tobacco and smokeless tobacco products: **CRM 86, T-104**
- **peer reviewed publications, CORESTA collaborative study.**
- **ENDS: CORESTA collaborative study, peer reviewed publications.**
- **ENDS (analysis of dicarbonyl compounds): peer reviewed publications.**



Methods: HPLC-UV after derivatization with DNPH, GC-MS, LC-MS, LC-MS/MS after derivatization with DNPH, other derivatization procedures.

Chlorinated dioxins/furans

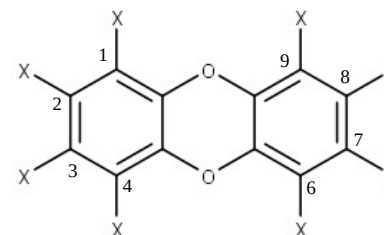


- Results expressed as toxic equivalent *TEQ* based on toxic equivalent factors *TEF*:

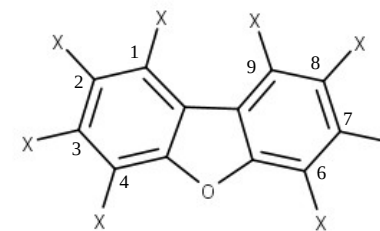
$$TEQ = \sum_j C_j \cdot TEF_j$$

(*C_j* are individual concentrations)

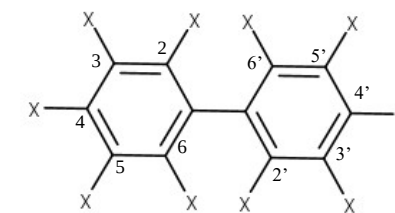
- Cigarette smoke, tobacco, cigarette paper:
CORESTA presentation, peer reviewed publications.
- ENDS: not expected to be present.**



Dibenzo-p-dioxins
X = H, Cl, or Br



Dibenzofurans
X = H, Cl, or Br



Biphenyls
X = H, Cl, or Br

Methods: HPLC-UV after derivatization with DNPH, GC-MS, LC-MS, LC-MS/MS after derivatization with DNPH, other derivatization procedures.

Hydrogen cyanide



- Cigarette smoke (mainstream different smoking protocols, sidestream), cigars, smoke from HTP: **CRM 106, CORESTA Technical Reports, T-107, T-205E, peer reviewed publications.**
- No hydrogen cyanide (HCN) in oral tobacco products.

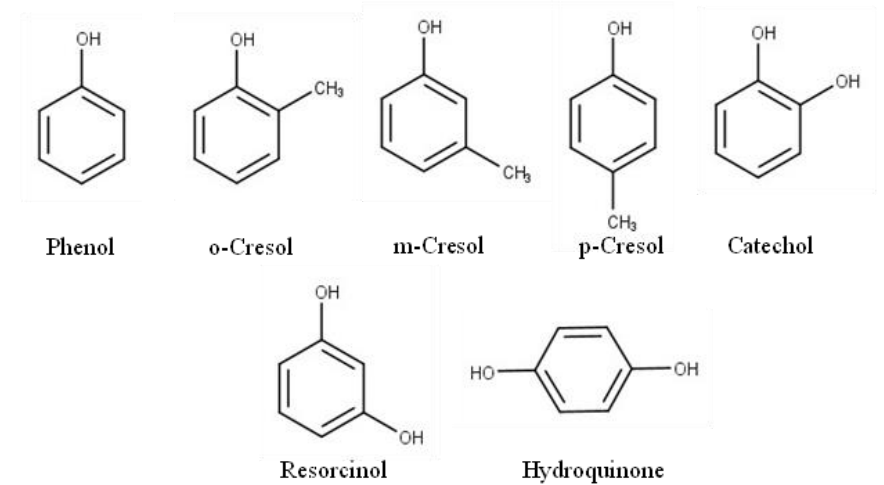
Methods: CFA after derivatization with chloramine T and a chromogenic reagent (e.g. isonicotinic acid and 1,3-dimethylbarbituric acid), other derivatization procedures.

Hydroxybenzenes



- Cigarette smoke (mainstream different smoking protocols, sidestream), cigars, smoke from HTP, water-pipe: **CRM 78, CORESTA Collaborative Studies, T-114, peer reviewed publications.**
- Tobacco and oral tobacco products: **peer reviewed publications.**
- E-liquids and aerosols from ENDS: **modification of CRM 78, peer reviewed publications.**

Methods: HPLC-FLD, GC-MS after silylation, LC-MS/MS



Metals and semimetals



- Cigarette smoke (mainstream different smoking protocols, sidestream), cigars, smoke from HTP, bidies, kreteks: **T-109, peer reviewed publications.**
- Cigarette smoke (Hg): **T-108, peer reviewed publications.**
- Tobacco and oral tobacco products: **CRM 93, T-306, CORESTA Technical Reports, peer reviewed publications.**
- E-liquids and aerosols from ENDS: **CRM 98, peer reviewed publications.**

As, Be, Cd, Cr, Co, Pb, Ni, Se

Hg

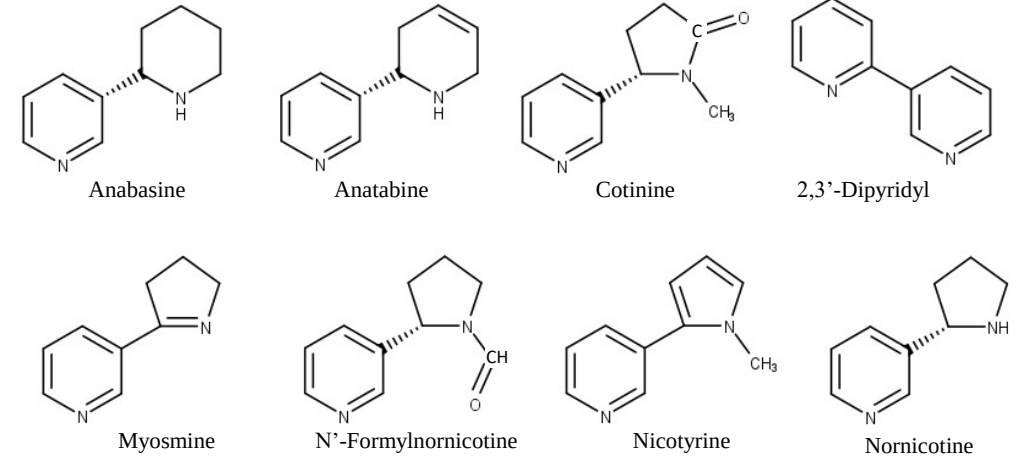
^{238}U , ^{235}U , ^{210}Po

Methods: AAS (graphite furnace) GFAAS, ICP-OES, ICP-MS, (cold vapor) CVAAS (for Hg), CV-ICP-MS, other techniques, radiometric methods for ^{210}Po .

Minor alkaloids



- Cigarette smoke (mainstream, sidestream), cigars, water pipe: **peer reviewed publications.**
- Tobacco and oral tobacco products: **collaborative studies, CORESTA Technical Reports, peer reviewed publications.**
- Nicotine pouches: **CRM 105, CORESTA Technical Report.**
- E-liquids and aerosols from ENDS: **peer reviewed publications.**
- Minor alkaloids enantiomers: **peer reviewed publications.**



Methods: GC-FID, GC-NPD, GC-MS, GC-MS/MS, LC-UV, LC-MS/MS, chiral GC-MS, chiral LC-MS/MS, SFC-UV.

Nitro compounds



- Cigarette smoke (mainstream): **peer reviewed publications.**
- Tobacco (not present): **peer reviewed publications.**
- E-liquids and aerosols from ENDS (not present): **peer reviewed publication.**

nitrobenzene,
nitromethane,
2-nitropropane

Methods: GC-FID (older method) GC-MS, bidimensional GC-MS, GC-MS/MS.

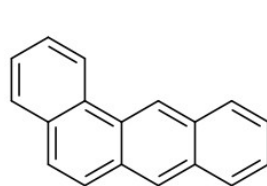
NO and NO_x



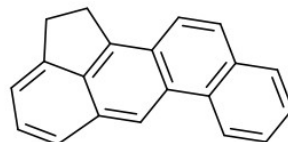
- Cigarette smoke (mainstream): CRM 104, T-110E, CORESTA Technical Report, collaborative studies, peer reviewed publications.
- HTP smoke, peer reviewed publications.

Methods: Chemiluminescence, non-dispersive NIR (not recommended).

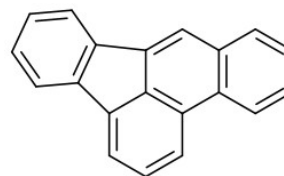
Polycyclic aromatic hydrocarbons (PAHs)



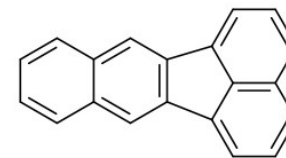
Benz[a]anthracene



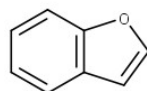
Benz[j]aceanthrylene



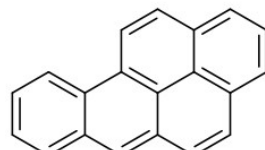
Benzo[b]fluoranthene



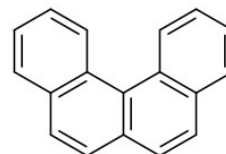
Benzo[k]fluoranthene



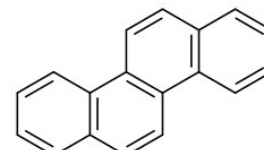
Benzo[b]furan



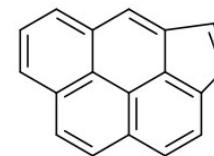
Benzo[a]pyrene



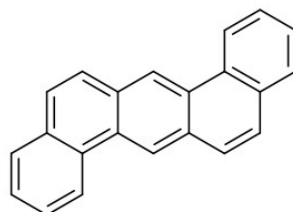
Benzo[c]phenanthrene



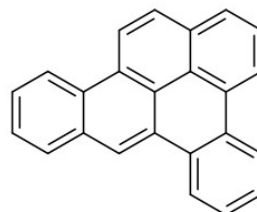
Chrysene



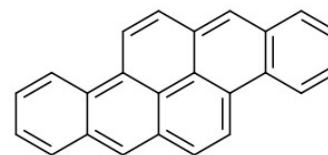
Cyclopenta[c,d]pyrene



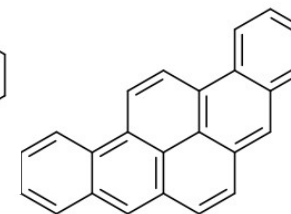
Dibenzo[a,h]anthracene



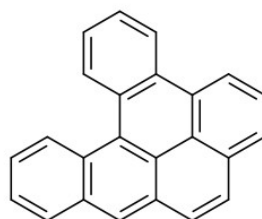
Dibenzo[a,e]pyrene



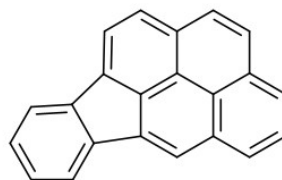
Dibenzo[a,h]pyrene



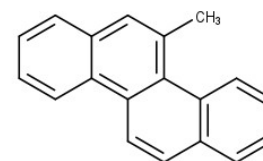
Dibenzo[a,i]pyrene



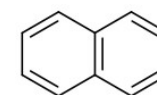
Dibenzo[a,l]pyrene



Indeno[1,2,3-cd]pyrene



5-Methylchrysene



Naphthalene

PAHs (cont.)



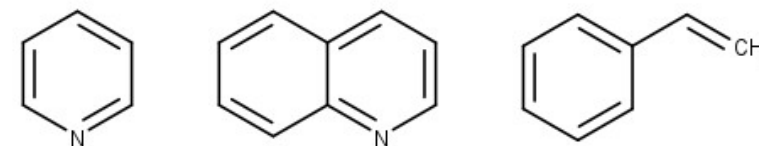
- Cigarette smoke (mainstream different smoking protocols, sidestream), cigars, HTP, water pipe: **ISO 22634, CRM 58, T-103, collaborative studies, CORESTA Technical Reports, peer reviewed publications.**
- Tobacco and oral tobacco products: **CRM 82, CRM 91, collaborative studies, peer reviewed publications.**
- Nicotine pouches: **CRM 82 (modif.), CORESTA Technical Report.**
- E-liquids and aerosols from ENDS (not present): **peer reviewed publications.**

Methods: GC-MS, GC-MS/MS, LC-FLD, LC-MS/MS, SFC-FLD.

Pyridine, quinoline, and styrene



- Cigarette smoke (mainstream different smoking protocols):
T-112, collaborative studies, peer reviewed publications.



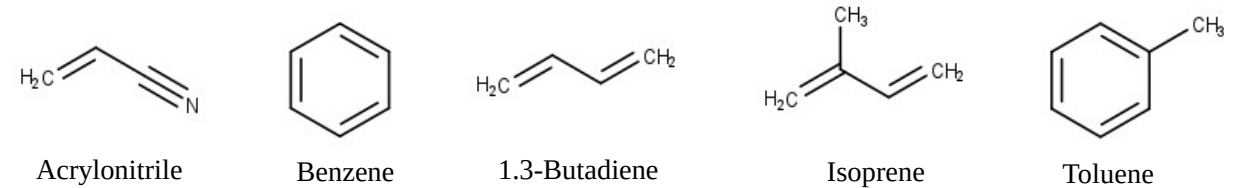
Several substituted pyridines

Methods: GC-MS, LC-MS/MS.

Selected volatiles



- Cigarette smoke (mainstream different smoking protocols, sidestream), cigars, HTP: CRM 70, T-116, collaborative studies, CORESTA Technical Reports, peer reviewed publications.
- Electronic cigarettes aerosols: peer reviewed publications.
- Environment: peer reviewed publications.



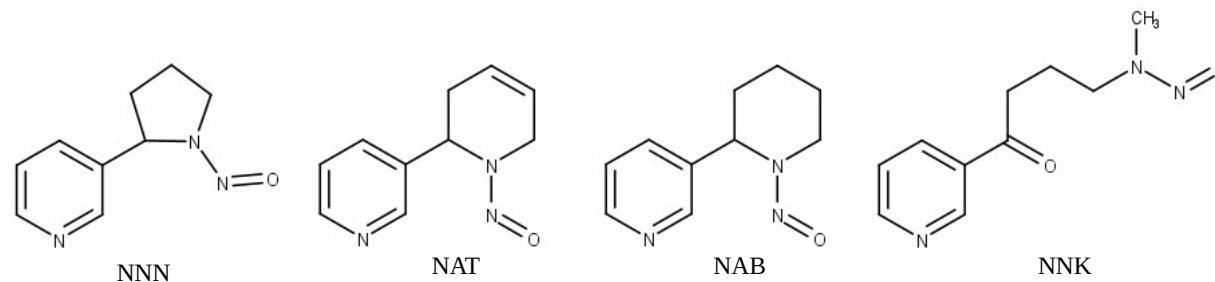
Other volatiles such as styrene, ethylene oxide, vinyl chloride, vinyl acetate, furan, propylene oxide, etc.

Methods: collection using: impinger(s), Tedlar bag, solid-phase trap, SPME, direct injection in measuring instrument, GC-MS.

Tobacco specific nitrosamines (TSNAs)



- Cigarette smoke (mainstream different smoking protocols, sidestream), cigars, heat-not-burned, water-pipe: **ISO 23921:2020(en), ISO 19290-2021E, CRM 63, T-111E, CRM 75, collaborative studies, CORESTA Technical Reports, peer reviewed publications.**
- Tobacco and oral tobacco products: **ISO 21766, CRM 72, collaborative studies, CORESTA Technical Reports, peer reviewed publications.**
- Electronic cigarettes aerosols, e-liquids, nicotine, nicotine pouches: **CRM 102, CORESTA Technical Report, peer reviewed publications.**
- Environment: **peer reviewed publications.**



Methods: GC-TEA, GC-MS, LC-MS/MS.

Volatile nitrosamines (VNAs)



- **Cigarette smoke (mainstream, different smoking protocols): peer reviewed publications.**

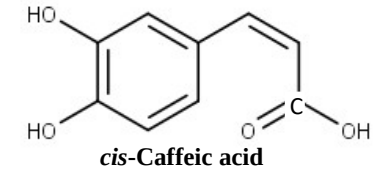
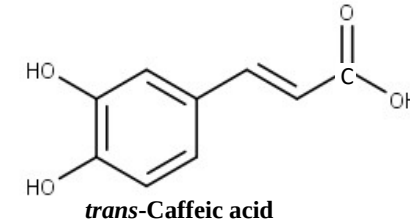
N-nitrosodiethylamine (NDEA), N-nitrosodiethanolamine (NDELA), N-nitrosodimethylamine (NDMA), N-nitrosodi-n-propylamine (NPRA), N-nitrosodi-n-butylamine (NBUA), N-nitrosomethylethylamine (NMEA), N-nitrosomorpholine (NMOR), N-nitrosopiperidine (NPIP), N-nitrosopyrrolidine (NPYR), and N-nitrososarcosine (NSAR)

Methods: GC-TEA, LC-MS/MS.

Caffeic acid, coumarin, ethyl carbamate, hydrazine



- Caffeic acid - cigarette smoke (mainstream, different smoking protocols), tobacco products: **CORESTA meeting reports, peer reviewed publications.**
- Coumarin - cigarette smoke (mainstream), tobacco products: **peer reviewed publications.**
- Ethyl carbamate - cigarette smoke (mainstream), tobacco products: **peer reviewed publications.**
- Hydrazine - cigarette smoke (mainstream), tobacco products: **peer reviewed publications.**



Methods: GC-MS, derivatization + GC-MS, LC-MS/MS, derivatization + GC-ECD.

Pesticides, free radicals, unknowns



- **Pesticides (not listed as HPHCs): Various publications by organizations such as FAO-WHO, OECD, FDA, CORESTA Guides (1, 2, 5, 19, 27), and peer reviewed publications.**

Methods: GC-MS, LC-MS/MS.

- **Free radicals (not listed as HPHCs) in cigarette smoke (mainstream, sidestream), HTP, aerosols from ENDS: peer reviewed publications.**

Methods: EPR, LC-MS/MS.

- **Unknown detected by non-targeted analysis (not listed as HPHCs): peer reviewed publications.**

Methods: GC-MS, LC-MS/MS, etc.

Details on HPHCs analysis in a new book



Serban Moldoveanu
Robert Owen Bussey

Analytical Methods for Tobacco and Nicotine Harm Reduction

 Springer

Conclusions



- Methods of analyses for all HPHCs were described in the literature, but the extent of attention paid to different groups differ considerably.
- Certain analytes (groups of analytes) are well represented in the available literature. This includes for example: **Nicotine, CO, aromatic amines, carbonyl compounds, hydroxybenzenes, certain metals, PAHs, selected volatiles, TSNAs, HCN, NH₃.**
- Methods for the other analytes (groups of analytes) are also available, but mainly in peer reviewed literature. This include for example: **Acetamide, acrylamide, minor alkaloids, etc.**
- Methods for certain analytes are not readily available or are 30-40 years old. This include for example: **aflatoxin, chlorinated dioxins/furans, certain metals, nitro compounds, volatile nitrosamines, caffeic acid, coumarin, hydrazine, ethyl carbamate, certain specific PAHs, heterocyclic amines.**
- Further attention to compounds indicated as lacking sufficient available publications would be desirable.