EXPERIMENTAL REPORT

P1 CHARACTERIZATION

Related Project Numbers:

RLS-ZRH-2016-119 (GCxGC-TOFMS; TPM & GVP)
RLS-ZRH-2016-120 (LC-HRAM-MS; TPM)
RLS-ZRH-2016-126 (LC-HRAM-MS; TPM)
RLS-ZRH-2017-119 (LC-HRAM-MS; GVP)

Program Name	P1
Project Name	THS 2.2 Commercial Product Characterization
Covered Time Period	March 2016 – April 2018
Category of Experimental Work	Non-Targeted Screening (NTS) using GC×GC-TOFMS and LC-HRAM-MS
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1 ABSTRACT

The chemical composition of aerosol from THS 2.2 regular (Dorado II 'Ron') HeatSticks (THSR), using THD 2.4 devices generated under the Health Canada Intense (HC) smoking regimen, has been determined by non-targeted screening (NTS) using liquid chromatography with high resolution accurate mass spectrometry (LC-HRAM-MS) and comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-TOFMS).

For this investigation, only compounds with semi-quantified estimates of 100 ng/stick or greater have been reported. PMI used 100 ng/stick as a general reporting cut-off limit, which enabled an estimated 99.8% of the total aerosol mass to be determined.

A total of 529 compounds, excluding water, nicotine and glycerin, were identified as being present in the aerosol of THS 2.2 at a concentration of 100 ng/stick or greater. A previous estimate of ca. 750 compounds present at a concentration ≥ 100 ng/stick was communicated to the FDA at the Tobacco Products Scientific Advisory Committee meeting held on 24/25 January 2018[1]. This estimate has since been shown to be an overestimate, with a reduced number (529) demonstrated as being present. PMI has been able to confirm these compound identities using reference standard, revealing a number of overlapping identifications between LC-HRAM-MS and GCxGC-TOFMS methods, thereby reducing the overall number of compounds identified from ~750 to 529.

Of these 529 compounds, 363 (68.6%) were exclusively found in the particulate phase (NFDPM), 127 (24.0%) were exclusively found in the gas vapor phase (GVP) and 39 (7.4%) compounds were found to be partitioned between both particulate phase and GVP.

Over 80% of all compounds identified were confirmed by reference standard.

81.3% of the total mass determined by NTS was present in the particulate phase (total particulate matter - TPM) and 97.7% of this mass was identified with high certainty or confirmed by reference standard.

A recently performed non-targeted differential screening (NTDS) investigation comparing the same THS 2.2 aerosol with 3R4F smoke has been submitted by PMI to the FDA as supporting data for an MRTP application. In this NTDS investigation, a statistical approach was taken to identify only those compounds present in THS 2.2 aerosol that were significantly higher in abundance than in the smoke of the reference cigarette 3R4F. This study was performed in the context of identifying any new hazards associated with using THS 2.2 compared to cigarettes.

The data from this P1 NTS study confirms the absence of any additional compounds that would constitute a 'new hazard' when compared with cigarette smoke.

2 INTRODUCTION

Platform 1 (P1), also referred to as Tobacco Heating System 2.2 (THS 2.2), is a heat-not-burn system that generates an aerosol which is significantly less complex than the cigarette smoke. The THS 2.2 aerosol has been shown to reduce the formation of Harmful and Potentially Harmful Constituents (HPHCs) by more than 90% compared with the smoke from a 3R4F reference cigarette (Schaller et al, 2016)[2]. Targeted analysis for the presence of these HPHCs in smoke/aerosol is routinely performed after aerosol collection, which may comprise a separate collection of the particulate and gas vapor phases by passing the aerosol/smoke through a Cambridge filter pad (CFP), where the particulate phase is trapped and the gas vapor phase constituents pass through for subsequent trapping using solvent filled impingers. Whole aerosol may also be trapped by omitting the use of a CFP, for some methods.

For testing purposes, smoke/aerosol is generated using a defined smoking regime such as ISO[3] or HC[4] (Health Canada Intense). The total mass of the material trapped by the CFP is also referred to as the TPM and is determined by calculating the weight difference of the CFP before and after the smoke/aerosol generation process, is performed. Water and nicotine are extracted from the CFP and their amounts subtracted from the TPM to obtain a value for NFDPM (nicotine free dry particulate matter). For aerosol delivered by THS 2.2, which is primarily composed of water, an improved method was developed and is used internally within Philip Morris International (PMI) in order to accurately determine TPM deliveries and calculate the amount of NFDPM collected (NFDPM = TPM - water - nicotine) (Ghosh & Jeannet, 2014)[5]. In addition, the amounts of glycerin collected were also determined quantitatively since it is a key component of THS 2.2 aerosol.

Figue 1 shows the relative quantities of water, nicotine and lycerin present in the aerosol of THS 2.2, where NFDPM is represented as the sum of masses attributable to glycerin and 'others'.

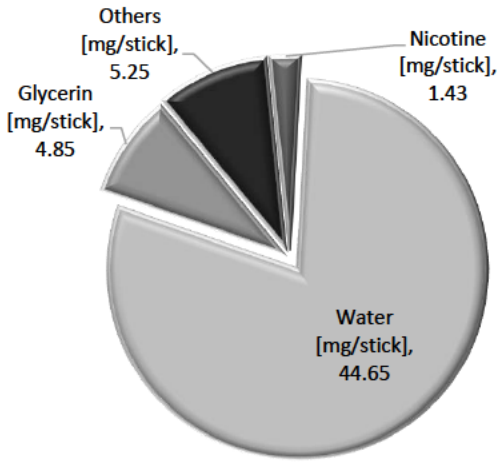

Figure 1: Gross composition of TPM from THS 2.2 aerosol under HC smoking regime

This document describes the experimental results for the determination of the aerosol chemical composition of THS 2.2 regular (Dorado II 'Ron') HeatSticks using Tobacco Heating Device (THD) 2.4 devices. The purpose of the study was (i) to identify all chemical constituents present in the aerosol at semi-quantified concentrations of 100 ng/stick or greater, (ii) to characterize the composition of the unknown ('others') portion of NFDPM (5.25 mg/stick) and (iii) to characterize the composition of the gas vapor phase (GVP).

Aerosols for all test items were generated using the HC smoking regimen and analyzed by the use of two different analytical techniques: liquid chromatography with high resolution accurate mass spectrometry (LC-HRAM-MS); and comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GCxGC-TOFMS). Four independent LC-HRAM-MS methods, as described in PMI-RRP-WKI-111571[6], and three independent GC×GC-TOFMS methods as described in PMI-RRP-WKI-111620[7], PMI-RRP-WKI-111621[8] and PMI-RRP-WKI-111622[9], for nonpolar, volatile and polar constituents, respectively, were used.

The combination of LC-HRAM-MS and GC×GC-TOFMS represents a complementary approach designed to encompass the chemical space related to tobacco based aerosols and enables a comprehensive characterization of the chemical composition of aerosols derived from the different test items. PMI had intended to include data from single dimension gas chromatography with high resolution mass spectrometry (GC-HR-MS) however, these data are not included in this report for two main reasons. First, the analytical coverage using GC-HR-MS was contained within the chemical space covered by the GCxGC-TOFMS suite of methods. Second, the high mass spectral purity afforded by a two-dimensional

separation combined with higher mass spectral scan rates provided a superior basis for accurate compound identification.The P1 assessment was performed on a single batch of regular THS 2.2 HeatSticks, using the current version of the THD 2.4 device. Since the mentholated HeatSticks are manufactured using the same cast leaf tobacco blend, these analyses provided a comprehensive understanding for the chemical composition of the aerosol generated by heating the base tobacco composition of THS 2.2.

Throughout this study, the 3R4F cigarette was included as a reference product.

3 RESEARCH OBJECTIVE

The primary objective of this study was to fully characterize the chemical composition of THS 2.2 regular aerosol by:

- providing semi-quantified concentrations for all chemical constituents identified as being present in THS 2.2 with an abundance ≥ 100 ng/item
- determining the chemical composition of the particulate phase (NFDPM) for mainstream aerosol (HC smoking regime) of regular THS 2.2 HeatSticks
- determining the chemical composition of the gas vapor phase (GVP) for mainstream aerosol (HC smoking regime) of regular THS 2.2 HeatSticks

4 EXPERIMENTAL PLAN

The study comprised the identification and semi-quantification of aerosol constituents derived from the regular version of THS 2.2. using LC-HRAM-MS[10] and GCxGC-TOFMS[11]. As data from the LC-HRAM-MS platform were to be combined with data from GCxGC-TOFMS platform, comparability between trapping approaches was essential. For this, PMI adopted a harmonized NFDPM and GVP collection approach in which separate trapping of the particulate and gas vapor phases was performed to individually characterize the composition of the respective gas/particulate phases.

PMI used the HC regime to generate the aerosols since it is commonly used and recognized as a standard smoking protocol by expert and official organizations for the assessment of cigarettes.

4.1 Research Materials

The aerosol was generated using the THS 2.2 tobacco heating system comprising a tobacco heating device (THD 2.4; PDIMS Device Batch B23172; Device Version DV.000174(7)) and the regular version of the THS HeatStick as described in **Table 1**.

Table 1 Test Items

Short Name	Description	Batch Number
THSR	THS 2.2 Dorado II Ron	B-25906 CONS.02873.RD(5)
3R4F	3R4F Reference Cigarette	N/A

The THSR test items were produced at Philip Morris Manufacturing & Technology Bologna (PMMTB), Italy. The samples were stored in a climatic chamber in packs. The samples were stored at 22 ± 3°C and 60 ± 5% relative humidity (RH) prior to conditioning and aerosol collection. The packs were polypropylene wrapped mini princess packs consisting of two collations, each collation containing 10 HeatSticks.

The Reference Cigarette 3R4F was purchased from the University of Kentucky, Kentucky Tobacco Research and Development Center[12] and stored in a cooling chamber at 4 ± 3°C with uncontrolled humidity prior to conditioning and aerosol collection. Prior to aerosol generation, the test items THSR and 3R4F were conditioned according to ISO 3402[13] and PMI-RRP-WKI-111777[14] for a minimum of 48h and a maximum of 10 days at 22 ± 1 °C and 60 ± 3% RH. The conditioning was performed in open packages for all test items. Although, mass-produced test items and cigarettes are generally homogeneous, still most constituents involved in the manufacture are derived from natural products therefore, may contribute to the intrinsical products variability.

4.2 Aerosol Generation and Sample Collection

4.2.1 LC-HRAM-MS Analysis

The list of experimental groups defined and used for LC-HRAM-MS investigations are presented in **(Table 2)**.

Table 2 Experimental Groups of Study Samples for LC-HRAM-MS

Description	Smoking Regimen	Sample Trapping	Type	Short Name
THS 2.2 regular	HC	TPM	test	THSR
3R4F	HC	TPM	reference	3R4F
Blank	HC	TPM	reference	Blank
THS 2.2 regular	HC	GVP	test	THSR
3R4F	HC	GVP	reference	3R4F
Blank THS 2.2	HC	GVP	reference	BLKT
Blank 3R4F	HC	GVP	reference	BLK3

The aerosol was trapped and treated as required for each of the four analytical methods in accordance with PMI-RRP-WKI-111741[15], PMI-RRP-WKI-111801[16] and PMI-RRP-WKI-111729[17]. The smoking machines used are presented in **Table 3**.

Mainstream aerosol for THSR and 3R4F was generated under HC smoking conditions (**Table 4** for samples requiring reversed phase (RP) chromatography and **Table 5** for samples requiring hydrophilic interaction (HILIC) chromatography) and the particulate phase was trapped using a 44mm Cambridge glass fiber filter pad. The gas vapor phase samples (**Table 6**) were collected downstream from the filter pad using two consecutive micro-impingers containing methanol (10 mL) maintained at approximately -60°C, using a dry ice-isopropanol mixture.

For the HC smoking protocol (Health Canada, T-115, 1999)[4], 3R4F cigarettes were 100% vent-blocked by taping in accordance with PMI-RRP-WKI-111712[18]. THSR items were not taped due to absence of ventilation holes in the filter region. The room conditions for aerosol generation were 22 ± 2°C and 60 ± 5% RH.

Table 3 Smoking Machines

Smoking Machine ID	Regime	Method	Project Number
KC1 – 44	HLT-CANADA (HC)	RP	RLS-ZRH-2016-120
KC1 – 44	HLT-CANADA (HC)	HILIC	RLS-ZRH-2016-126
KC1 – 44	HLT-CANADA (HC)	GVP	RLS-ZRH-2017-119

Table 4 Smoking Regime for RLS-ZRH-2016-120 RP TPM Samples

Short Name	Sample Number	Puff Volume [mL]	Duration [s]	Puff interval [s]	Puff Count [n]
THSR	1517286	55	2	30	12
3R4F	1517295	55	2	30	10*
Blank	1517289	55	2	30	12

*smoked to a fixed butt length (35mm), achieved in approximately 10 puffs

Table 5 Smoking Regime for RLS-ZRH-2016-126 HILIC TPM Samples

Short Name	Sample Number	Puff Volume [mL]	Duration [s]	Puff interval [s]	Puff Count [n]
THSM	1519319	55	2	30	12
3R4F	1519328	55	2	30	10*
Blank	1519324	55	2	30	12

*smoked to a fixed butt length (35mm), achieved in approximately 10 puffs

Table 6 Smoking Regime for RLS-ZRH-2017-119 GVP Samples

Short Name	Sample Number	Puff Volume [mL]	Duration [s]	Puff interval [s]	Puff Count [n]
THSM	1678780	55	2	30	12
3R4F	1678797	55	2	30	10*
BLKT	1678795	55	2	30	12
BLK3	1678823	55	2	30	10

*smoked to a fixed butt length (35mm), achieved in approximately 10 puffs

For the characterization of NFDPM, each smoking replicate comprised the accumulated TPM from 2 sticks/cigarettes, which were then extracted from the CFP using methanol (2 x 5mL) for RP chromatography or acetonitrile (2 x 5mL) for HILIC chromatography. GVP samples were trapped directly into methanol using 5 sticks per replicate for THSR and 3 cigarettes per replicate for 3R4F (see **Table 7**). The blank samples were generated using the same aerosol collection configuration without test items.

Table 7 Experimental Details for the Preparation of Aerosol Samples

Short Name	Sample Number	Replicates [n]	Items per Replicate [accumulations]	Extraction Solvent	Extraction Volume [mL]
THSR	1517286	3	2	Methanol	10
3R4F	1517295	3	2	Methanol	10
Blank	1517289	3	-	Methanol	10
THSR	1519319	3	2	Acetonitrile	10
3R4F	1519328	3	2	Acetonitrile	10
Blank	1519324	3	-	Acetonitrile	10
THSR	1678780	3	5	Methanol	2 x 10
3R4F	1678797	3	3	Methanol	2 x 10
BLKT	1678795	3	-	Methanol	2 x 10
BLK3	1678823	3	-	Methanol	2 x 10

4.2.2 GCxGC-TOFMS Analysis

The list of experimental groups defined and used for GCxGC-TOFMS investigations are presented in **Table 8**.

Table 8 Experimental Groups of Study Samples for GCxGC-TOFMS

Description	Smoking Regimen	Sample Trapping	Type	Short Name
THS 2.2 regular	HC	TPM/GVP	test	THSR
Blank_THS	HC	TPM/GVP	reference	Blank_THS
3R4F	HC	TPM/GVP	reference	3R4F
Blank_3R4F	HC	TPM/GVP	reference	Blank_3R4F

Mainstream aerosol for THSR and 3R4F was generated under HC smoking conditions and trapped according to PMI-RRP-WKI-111741[15], PMI-RRP-WKI-111801[16] and PMI-RRP-WKI-111729[17] (see **Table 10** and **Table 11**) using the smoking machines presented in **Table 9**. For the HC smoking protocol (Health Canada, T-115, 1999)[4], 3R4F cigarettes were 100% vent-blocked by taping in accordance with PMI-RRP-WKI-111712[18]. THSR items were not taped due to absence of ventilation holes in the filter region. The room conditions for aerosol generation were 22 ± 2 °C and 60 ± 5% RH.

Table 9 Smoking Machines

Smoking machine ID	Regime	Method
KC1-44	HLT-CANADA (HC)	Volatile
KC1-44	HLT-CANADA (HC)	Nonpolar & Polar

Table 10 Smoking Regime for RLS-ZRH-2016-119 Volatile

Short Name	Sample Number	Puff Volume [mL]	Duration [s]	Puff interval [s]	Puff Count [n]
THSR	1515588	55	2	30	12
Blank_THS	1515613	55	2	30	12
3R4F	1516631	55	2	30	10*
Blank_3R4F	1516688	55	2	30	12

*smoked to a fixed butt length (35mm), achieved in approximately 10 puffs

Table 11 Smoking Regime for RLS-ZRH-2016-119 Nonpolar/Polar

Short Name	Sample Number	Puff Volume [mL]	Duration [s]	Puff interval [s]	Puff Count [n]
THSR	1515589	55	2	30	12
Blank_THS	1515616	55	2	30	12
3R4F	1516634	55	2	30	10*
Blank_3R4F	1516693	55	2	30	12

*smoked to a fixed butt length (35mm), achieved in approximately 10 puffs

Blank samples were generated using the same aerosol collection configuration without test items. The number of aerosol/smoke replicates for each method and the number of accumulations (sticks used) per replicate are defined in **Table 12**. The required trapping configurations are summarized in **Table 13**.

Table 12 Experimental Details for the Preparation of Aerosol Samples

Short Name	Methods	Items per Replicate [accumulations]	Replicates (n)
THSR	Nonpolar and Polar	5	3
	Volatile	5	4
Blank_THS	Nonpolar and Polar	-	3
	Volatile	-	4
3R4F	Nonpolar and Polar	3	3
	Volatile	3	4
Blank_3R4F	Nonpolar and Polar	-	3
	Volatile	-	4

Table 13 Trapping Setup for TPM and GVP Samples

	GC×GC-TOFMS Nonpolar + Polar	GC×GC-TOFMS Volatile
For trapping of total particulate matter (TPM) samples:		
Cambridge Glass Fiber Filter	44mm	44mm
For trapping of gas vapor phase (GVP) samples, consecutive:		
No. Impinger traps	2	2
Impinger solvent*	2x10mL DCM:acetone (80:20 v/v) - containing internal standard and retention index marker compounds	2x10mL N,N-DMF - containing internal standard and retention index marker compounds
Impinger temperature	dry ice / isopropanol (approx. -80°C)	dry ice / isopropanol (adjust to approx. -50 to -60°C)

* DCM: dichloromethane DMF: dimethylformamide

4.3 Instruments, Materials and Sample Analysis

4.3.1 LC-HRAM-MS Analysis

LC-HRAM-MS systems, additional instrumentation and materials are listed in **Table 14**, **Table 15** and **Table 16**.

Table 14 LC-HRAM-MS System 1, PMI ID 11636

Instrument	Instrument ID	PMI ID
Autosampler	Thermo Accela PAL	11637
Column Oven	Thermo HotDog 3000	3533
UHPLC Pump	Thermo Accela 1250	3638
Mass Spectrometer	Thermo Q Exactive	11637
Data Unit	Dell Precision 7810	PMICHNEUW80812

Table 15 Additional Instrumentation

Instrument	Instrument ID	PMI ID
Analytical Balance	Mettler Toledo XP205 Delta	3489
Data Unit	Dell Precision 5500	PMICHNEUW80670

Table 16 Materials

Material	Material ID	Serial Number	Lot
Column RP	Thermo Hypersil GOLD (150 x 2.1 mm, 1.9µm)	10409667	14251
Column HILIC	Thermo Accucore HILIC (150 x 2.1, 2.6µm)	10372266	13335
Column RP (GVP)	Thermo Hypersil GOLD (150 x 2.1 mm, 1.9µm)	10530501	14613

Lot numbers for chemicals and solvents used were recorded in forms and lab notebooks and stored in the study binders RLS-ZRH-2016-120, RLS-ZRH-2016-126 and RLS-ZRH-2017-119.

Sample preparation was carried out in accordance with PMI-RRP-WKI-111571[6]. Aliquots (200 µL) of the CFP extracts were diluted with methanol (700 µL) for RP analysis or acetonitrile (700 µL) for HILIC analysis (CFP extracts only) and 100 µL WS IS1 (internal standard working solution) was added and, after vial closure, were mixed for 5 minutes using an Eppendorf ThermoMixer (5°C; 2000 rpm). For GVP samples aliquots (300 µL) were diluted with methanol (700 µL), since the trapping solvent already comprised the internal standard solution. Aliquots (1.5 µL) of the diluted extracts were injected and analyzed by LC-HRAM-MS in full scan mode and in data-dependent fragmentation mode for compound identification (see **Table 17** and **Table 18**). Each diluted extract was analyzed 5 times (analytical replicates) in both full scan mode and data-dependent fragmentation mode.

A pool sample was created and used as a reference for data processing (see **Section 4.4**), in order to have a single sample representing the entire trapped chemical space (TPM or GVP). Aliquots (100 µL) of each aerosol sample generated were equally combined and mixed well. Aliquots (200 µL) of this pooled aerosol sample were prepared for chromatographic analysis as detailed above.

The samples were analyzed by LC-HRAM-MS using a Thermo QExactive™ high resolution mass spectrometer in both full scan mode and data dependent mode in accordance with PMI-RRP-WKI-111571[6] and PMI-RRP-WKI-111570[19]. In total, 4 different methods were applied in order to cover a wide range of substances with different ionization properties and compound classes. Samples were analyzed using RP chromatography with heated electrospray ionization (HESI) in both positive and negative modes and with atmospheric pressure chemical ionization (APCI) in positive mode, and using HILIC chromatography in HESI positive ionization mode. For RP chromatography mode, samples extracted with methanol were used. For HILIC chromatography mode, samples extracted with acetonitrile were used.

For the subsequent identification of relevant constituents, samples were measured using a data dependent fragmentation method, complementary to the full scan analysis. Instrumental parameters are presented in **Table 17** and **Table 18**.

Table 17 Instrument Scan Events

Scan Event	Scan Event Details	Detection	Fragmentation	Resolution
(1)	Full Scan	FTMS	-	70000
(2)	MS/MS Top 3 most intense from (1)	FTMS	HCD	17500

Table 18 Instrument Parameters

Parameter	Setting
General Parameters	
In-source CID [eV]	Off (0.0 eV)
Default Charge State	1

(table continues)

Full MS	
Microscans	1
Resolution	70000
AGC Target	3e6
Maximum IT [ms]	100
Scan Range [Da]	80 - 800
Spectrum Data Type	Profile
dd-MS²	
Microscans	1
Resolution	17500
AGC Target	1e5
Maximum IT [ms]	150
Loop Count	3
TopN	3
Isolation Window [m/z]	4
Scan Range [Da]	80 - 800
Stepped NCE [eV]	25, 50, 75
dd Settings	
Underfill Ratio [%]	1.00
Intensity Threshold	6.7e3
Apex Trigger	Off
Dynamic Exclusion [s]	10

The accurate mass measurements allowed the determination of elemental composition (proposed sum formula) for precursor ions derived from the full scan analyses, and the elemental composition of the fragments using data dependent fragmentation experiments. Combining these information results in a high certainty for the proposed elemental composition of a compound and additionally identified structural features. Semi-quantification was carried out by means of derived consecutive replicates per sample.

4.3.2 GCxGC-TOFMS Analysis

GCxGC-TOFMS systems and additional instrumentation are listed in **Table 19**, **Table 20**, **Table 21** and **Table 22**.

Table 19 GC×GC-TOFMS System 1, PMI ID 7764

Instrument	Instrument ID	PMI ID
Autosampler	Agilent 7683 Series	3484
Injector	Agilent 7683B Series	11650
Gas chromatograph	Agilent 7890A	7765
Mass spectrometer	LECO Pegasus 4D SN 3390	7766
Dewar	Cryotherm Apollo 350	6478

Table 20 GC×GC-TOFMS System 2, PMI ID 10606

Instrument	Instrument-ID	PMI ID
Autosampler	Agilent 7683 Series	11651
Injector	Agilent 7683B Series	0896
Gas chromatograph	Agilent 6890N	2938
Mass spectrometer	LECO Pegasus 4D SN 3284	3103
Dewar	Cryotherm Apollo 200	7771

Table 21 GC×GC-TOFMS System 3, PMI ID 6472

Instrument	Instrument-ID	PMI ID
Autosampler	Agilent 7683 Series	3494
Injector	Agilent 7683B Series	12457
Gas chromatograph	Agilent 6890N	6474
Mass spectrometer	LECO Pegasus 4D SN 3242	6473
Dewar	Cryotherm Apollo 350	9878

Table 22 Additional Instrumentation

Instrument	Instrument-ID (or equivalent)	PMI ID
Analytical balance	Mettler Toledo XP205 Delta Range	3489
Centrifuge	Beckman Coulter Avanti J-E	2132

NTS using GC×GC-TOFMS was performed to determine the chemical composition of aerosol from the regular version of THS 2.2 smoked under the HC smoking regime. The NTS assay consisted of three separate analytical methods focused on nonpolar, polar and highly volatile compounds.

GC×GC-TOFMS Nonpolar & Polar: Whole aerosol was collected using a Cambridge filter pad followed by two micro-impingers connected in series. Each micro-impinger was filled with 10 mL dichloromethane/acetone (80:20, v/v) containing internal standard (ISTD) and

retention index marker (RIM) compounds. The micro-impingers were cooled using a dry ice/isopropanol mixture at a temperature of approx. -80°C.

GC×GC-TOFMS Nonpolar (NFDPM): The TPM collected on the Cambridge filter pad was extracted using dichloromethane/acetone (80:20 v/v; 10 mL) containing internal standard (ISTD) and retention index marker (RIM) compounds. Water was added to an aliquot (5 mL) of the resulting extract in equal volume amounts. After the sample was shaken and centrifuged, the dichloromethane layer was separated, dried with sodium sulfate and analyzed by GC×GC-TOFMS in full scan mode.

GC×GC-TOFMS Nonpolar (GVP): After collection, the contents of the two micro-impingers were combined. Water was added to an aliquot (10 mL) of the resulting extract in equal volume amounts. After the sample was shaken and centrifuged, the dichloromethane layer was separated, dried with sodium sulfate and analyzed by GC×GC-TOFMS in full scan mode.

GC×GC-TOFMS Polar (NFDPM): The remaining water layer from the nonpolar (NFDPM) sample preparation was used for the analysis of the polar compounds. ISTD and RIM compounds were added to the water layer, which was then directly analyzed by GC×GC-TOFMS in full scan mode.

GC×GC-TOFMS Polar (GVP): The remaining water layer from the nonpolar (GVP) sample preparation was used for the analysis of the polar compounds. ISTD and RIM compounds were added to the water layer, which was then directly analyzed by GC×GC-TOFMS in full scan mode.

GC×GC-TOFMS Volatile: Whole aerosol was collected using a Cambridge filter pad followed by two micro-impingers connected in series. Each micro-impinger was filled with N,N-dimethylformamide (DMF, 10 mL) containing ISTD and RIM compounds. The micro-impingers were cooled using a dry ice/isopropanol mixture adjusted to a temperature between -50 and -60°C.

GC×GC-TOFMS Volatile (NFDPM): The TPM collected on the Cambridge filter pad, was extracted using DMF (10 mL) containing ISTD and RIM compounds and analyzed by GC×GC-TOFMS in full scan mode.

GC×GC-TOFMS Volatile (GVP): After collection, the contents of the two micro-impingers were combined and analyzed by GC×GC-TOFMS in full scan mode.

Full details of the analytical methods are documented in PMI-RRP-WKI-111620[7], PMI-RRP-WKI-111621[8] and PMI-RRP-WKI-111622[9].

4.4 Data Processing

4.4.1 LC-HRAM-MS Data Processing

The data evaluation process for LC-HRAM-MS consisted of several steps:

- Data import into metabolomics data mining software Nonlinear Dynamics Progenesis QI™
- Alignment
- Experimental design setup (defining one or more groups for aligned runs)
- Peak picking
- Normalization using internal standards
- Deconvolution
- Compound identification (accurate mass and adduct search against database)
- Compound review (managing compound identities and exploring identities and expression between conditions)
- Processing of aligned and normalized (csv-)dataset using Microsoft Excel
- Semi-quantification of compounds
- Manual verification of results

Data were reported in a condensed result table containing, as a minimum, the proposed compound name, structural information, empirical formula, determined accurate molecular weight, identification confidence, PMICODE, ionization mode, chromatography mode and semi quantitative yield. The data evaluation process using Progenesis QI™ software was performed in accordance with PMI-RRP-WKI-111571[6].

Compound identification was performed using a semi-automatic stepwise approach employing an experimental MS^2 fragmentation database and *in silico* predicted fragmentation of constituents from public databases. In **Step 1**, all detected constituents were matched and assigned against an in-house database comprising experimental data for approximately 450 reference compounds with accurate mass data, stepped NCE (normalized collision energy), MS^2 first order fragmentation and retention times (precursor and fragment tolerance 5 ppm, retention time tolerance 0.7 min). In **Step 2**, the fragmentation patterns for all detected constituents were compared with *in silico* predicted fragments for chemical compounds registered in UCSD[20], HMDB 3.6[21] and via

ChemSpider search plugin with data sources of ChemIDplus[22] and FDA[23] (precursor and fragment tolerance 5ppm). In **Step 3**, fragmentation spectra for detected constituents were compared with experimental fragmentation spectra contained within the NIST14MS/MS library[24] (precursor and fragment tolerance 5ppm). All putative hits were scored using Progenesis QI™ algorithms, which considered mass accuracy, isotope similarity, fragmentation score and retention time. Threshold criteria were applied to combined match scores to assign confidence levels for compound identification, and proposed compound hits were designated as being identified with 'high', 'medium' or 'low' confidence. Chemical constituents confirmed as being present by means of direct comparison with purchased reference standards were designated as having 'confirmed' identification status.

Although normalization of the peak abundance against an internal standard was performed within Progenesis QI™, semi-quantification of the compounds was calculated using Microsoft Excel. Semi-quantification was performed on the basis of the peak area ratio between the analytes and an appropriate internal standard of known concentration, which was chosen depending upon the ionization mode used. Since no calibration was performed, the results were semi-quantitative. The ability for compounds to ionize varies strongly in atmospheric pressure ionization (API), since it is a soft ionization technique, therefore values derived should only be used as a rough estimation of abundance. If absolute quantitative data are needed, the respective analytes should be calibrated using certified reference standards.

4.4.2 GCxGC-TOFMS Data Processing

GC×GC-TOFMS data acquisition was followed by advanced raw data processing using ChromaTOF software version 4.50.8.0 for automatic peak finding, spectral deconvolution and peak alignment, resulting in an aligned peak table. Data processing was divided into multiple steps, (1) ChromaTOF Processing, (2) CASI Pre-Processor, (3) CASI, (4) CASI Post-Processor and (5) Pipeline Pilot Processing (see **Figure 2**). The processing tools automated a sequence of important data evaluation steps, e.g. batch processing, data alignment, compound identification, semi-quantification and result table generation. Full details regarding the data processing steps are documented in PMI-RRP-WKI-111620[7], PMI-RRP-WKI-111621[8] and PMI-RRP-WKI-111622[9].

For each compound, a structural proposal was generated using a platform developed in-house by PMI known as Computer Assisted Structure Identification (CASI), which is

dedicated for the identification of chemical structures using Mass Spectrometry (MS) data in combination with 2-dimensional chromatographic data.

CASI automatically searched mass spectral libraries for matches using a NIST MS Search algorithm, which proposed structural candidates for experimental spectra from comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry measurements, each with an associated match factor. Next, quantitative structure-property relationship (QSPR) models implemented in CASI predicted three specific parameters to enhance the confidence for correct compound identification, which were the Retention Index (RI) of the first dimension (1D) separation, the relative retention time of the second dimension separation (2DrelRT) and the boiling point (BP). In order to reduce the impact of chromatographic variability on the second dimension retention time, a concept based upon hypothetical reference points from linear regressions of a deuterated n-alkanes reference system was introduced for the nonpolar and volatile methods, providing a more stable relative retention time measurement.

Predicted values for RI and 2DrelRT were calculated and matched with experimentally derived values. Boiling points derived from 1D separations were matched with predicted boiling points, calculated from the chemical structures of the proposed candidates.

As a last step, CASI combined the NIST MS Search match factors (NIST MF) with up to three predicted parameter matches from the QSPR models to generate a combined CASI Score, which represented a measure of overall confidence for compound identification. Threshold values were applied to the CASI Scores assigned to proposed structures, which improved the accuracy for the classification of true/false positives and true/false negatives. Chemical constituents confirmed as being present by means of direct comparison with purchased reference standards were designated as having ‘confirmed’ identification status. This process for the identification of compounds has been validated (Knorr et al, 2013)[25].

Classification of the CASI results together with an expert verification scheme is presented in **Table 23**.

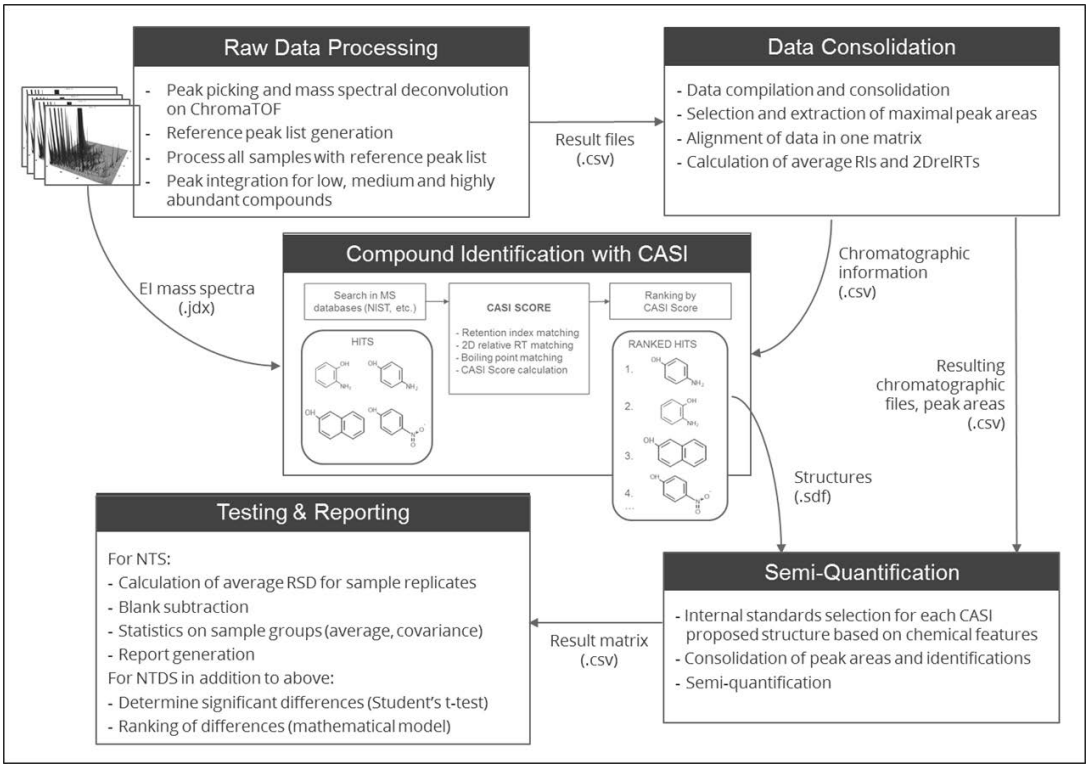

Figure 2: Overview of the data processing steps for GCxGC-TOFMS using CASI

Table 23 Classification of Identification Status

CASI Score	Confidence Level CASI Proposal + Expert Verification	Confirmation by Reference Standard	Identification Status (Report)
≥795	High + Agreed	Yes	**Confirmed**
		Not performed	**High**
		Negative	**Not Identified**
700≤X<795	Medium + Agreed	Yes	**Confirmed**
		Not performed	**Medium**
		Negative	**Not Identified**
<700	Low + under consideration	Yes	**Confirmed**
		Not performed	**Low**
		Negative	**Not Identified**

5 EXPERIMENTAL RESULTS

Data from non-targeted screening using both LC-HRAM-MS and GC×GC-TOFMS to determine the chemical composition of THS 2.2 aerosol are presented in **Table 24**, in descending order of abundance.

For constituents identified by both liquid and gas chromatographic based approaches, mean semi-quantified concentrations have been reported. Identification confidence and aerosol fraction origin (NFDPM, GVP or both) have also been indicated. For the constituents found to be partitioned between particulate and gas vapor phases, the sum of concentrations from both phases has been reported.

Due to a high abundance in THS 2.2 aerosol, water, nicotine and glycerin were excluded from the datasets in order to simplify the data evaluation process and are not reported here.

After completion of the study (final study report issued) all raw data and processed data was copied and stored in a secured Long Term Repository (LTR). The LTR is a Write Only, Read Many network server.

Table 24 Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
1	1-Hydroxy-2-propanone / 1,2-Propenediol*	116-09-6 / 7333-03-1	Confirmed	NFDPM	1135	✓
2	Acetic acid	64-19-7	Confirmed	NFDPM	994**	×
3	Propylene glycol	57-55-6	Confirmed	NFDPM	643	✓
4	1-Monoacetin	106-61-6	Confirmed	NFDPM	409	×
5	Acetaldehyde	75-07-0	Confirmed	NFDPM & GVP	313	×
6	Methanol	67-56-1	Confirmed	NFDPM & GVP	211	×
7	Solanesol	13190-97-1	Confirmed	NFDPM	179	×
8	Isobutyraldehyde	78-84-2	Confirmed	GVP	116	×
9	Triacetin	102-76-1	Confirmed	NFDPM	112	×
10	Palmitic acid	57-10-3	Confirmed	NFDPM	105	×
11	3-(2-Hydroxymethoxy)-propane-1,2-diol	14641-24-8	Confirmed	NFDPM	100	×
12	Cembranoid degradation product (18 compounds)	N/A	Confirmed	NFDPM	93.2	×
13	Isovaleraldehyde	590-86-3	Confirmed	NFDPM & GVP	88.7	×
14	13,14-Dihydro-retinol	115797-14-3	Confirmed	NFDPM	79.1	✓
15	Linolenic acid	463-40-1	Confirmed	NFDPM	57.9	×
16	Propanal	123-38-6	Confirmed	GVP	57.4	×
17	2-Methylbutyraldehyde	96-17-3	Confirmed	GVP	54.7	×
18	Propanoic acid	79-09-4	Confirmed	NFDPM	53.2	×
19	3-Pyridinol	109-00-2	Confirmed	NFDPM	52.8	×
20	β-Nicotyrine	487-19-4	High	NFDPM	52.4	×

* semi-quantified concentration represents the sum of 2 tautomers

** concentration determined quantitatively

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
21	Pyranone	28564-83-2	Confirmed	NFDPM	51.4	✓
22	Oleic acid	112-80-1	Confirmed	NFDPM	50.2	✗
23	Furfural	98-01-1	Confirmed	NFDPM & GVP	47.4	✓
24	2-Monoacetin	100-78-7	Confirmed	NFDPM	46.8	✗
25	Linoleic acid	60-33-3	Confirmed	NFDPM	43.	✗
26	2-Furanmethanol	98-00-0	Confirmed	NFDPM & GVP	37.5	✓
27	Acetone	67-64-1	Confirmed	GVP	34.7	✗
28	2,3-Butanedione	431-03-8	Confirmed	NFDPM & GVP	34.0	✗
29	Anhydro sugar derivative	N/A	High	NFDPM	30.8	✗
30	Octadecanoic acid	57-11-4	Confirmed	NFDPM	29.4	✗
31	2-Methylfuran	534-22-5	Confirmed	GVP	28.2	✗
32	Furan	110-00-9	Confirmed	GVP	24.3	✗
33	Neophytadiene	504-96-1	Confirmed	NFDPM	23.8	✗
34	1-Linolenoylglycerol	18465-99-1	Confirmed	NFDPM	23.5	✗
35	5-Hydroxymethylfurfural	67-47-0	Confirmed	NFDPM	23.	✗
36	α-Levantenolide	30987-48-5	Medium	NFDPM	22.8	✗
37	2-Methyl-2-propenal	78-85-3	Confirmed	NFDPM & GVP	22.0	✗
38	Pentadecanoic acid	1002-84-2	Confirmed	NFDPM	18.8	✗
39	3-Chloro-1,2-propanediol	96-24-2	Confirmed	NFDPM	16.1	✓
40	4,6-Dihydroxy-20-nor-2,7-cembradien-12-one	119613-98-8†	High	NFDPM	14.6	✗

† CAS number corresponds to one of the isomeric forms for this compound

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
41	3-Methyl-pentanoic acid	105-43-1	Confirmed	NFDPM	14.5	✓
42	5-Methylfurfural	620-02-0	Confirmed	NFDPM & GVP	14.2	✓
43	1H-Pyrrole	109-97-7	Confirmed	NFDPM & GVP	14.0	✗
44	Phytoene	540-04-5	Medium	NFDPM	13.8	✗
45	Pyridine	110-86-1	Confirmed	NFDPM & GVP	13.7	✗
46	6,10,14,18,22,26-hexamethyl-5,9,13,17,21,25-Heptacosahexaen-2-one	32304-17-9†	Medium	NFDPM	13.2	✗
47	Butanoic acid	107-92-6	Confirmed	NFDPM	12.7	✗
48	1-Acetyloxy-2-propanone	592-20-1	Confirmed	NFDPM & GVP	12.2	✓
49	N-Octanoylnornicotine	38854-10-3	Confirmed	NFDPM	12.1	✗
50	5,6-Dihydropyridin-2(1H)-one	6052-73-9	Confirmed	NFDPM	11.8	✗
51	Methanethiol	74-93-1	Confirmed	GVP	11.7	✗
52	Chloromethane	74-87-3	Confirmed	GVP	11.1	✗
53	Heptacosane	593-49-7	Confirmed	NFDPM	10.2	✗
54	α-Tocopherolquinone	7559-04-8	Confirmed	NFDPM	10.	✗
55	2-Butanone	78-93-3	Confirmed	GVP	10.	✗
56	3-Hydroxy-2-butanone	513-86-0	Confirmed	NFDPM & GVP	9.43	✗
57	Arachidic acid	506-30-9	Confirmed	NFDPM	8.91	✗
58	α-Cembratriene-diol	57605-80-8†	High	NFDPM	8.49	✓
59	(9*Z*,12*Z*)-18-Hydroxy-9,12-octadecadienoic acid	4546-59-2	High	NFDPM	8.47	✗
60	2-Cyclopentene-1,4-dione	930-60-9	Confirmed	NFDPM & GVP	8.40	✓

† CAS number corresponds to one of the isomeric forms for this compound

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
61	2-Cyclopenten-1-one	930-30-3	Confirmed	NFDPM & GVP	8.20	✗
62	2H-Pyran-2-one, tetrahydro-5-hydroxy	33691-73-5	Confirmed	NFDPM	8.16	✓
63	2-Furancarboxylic acid, 3-methyl	4412-96-8	Confirmed	NFDPM	8.06	✗
64	trans-Crotonaldehyde	123-73-9	Confirmed	NFDPM & GVP	7.87	✗
65	8,11-Epoxy-2,6,12-cembratrien-4-ol	75281-94-6†	Medium	NFDPM	7.79	✗
66	Butanal	123-72-8	Confirmed	GVP	7.79	✗
67	trans-Solanone	54868-48-3	Confirmed	NFDPM & GVP	7.75	✗
68	Palmitoleic acid	373-49-9	Confirmed	NFDPM	7.46	✗
69	Isoraimonol	82458-63-7	High	NFDPM	7.42	✗
70	Not identified (GCxGC NFDPM: 10 compounds)	N/A	Not Identified	NFDPM	7.33	✗
71	Scopoletin	92-61-5	Confirmed	NFDPM	7.21	✗
72	Anatabine	581-49-7	Confirmed	NFDPM	7.15	✓
73	Behenic acid	112-85-6	Confirmed	NFDPM	6.57	✗
74	2,3-Pentanedione	600-14-6	Confirmed	GVP	6.43	✗
75	Hexadecanoic acid, ethyl ester	628-97-7	Confirmed	NFDPM	6.43	✓
76	2,5-Dimethylfuran	625-86-5	Confirmed	GVP	6.38	✗
77	Disulfide, dimethyl	624-92-0	Confirmed	GVP	6.34	✗
78	2-Methyl-3-pyridinol	1121-25-1	Confirmed	NFDPM	6.23	✗
79	5-Oxo-1-tetradecyl-3-pyrrolidinecarboxylic acid	10054-22-5	Medium	NFDPM	6.16	✗
80	α-Tocopherol	10191-41-0	Confirmed	NFDPM	5.80	✗

† CAS number corresponds to one of the isomeric forms for this compound

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
81	1,2-Benzenediol	120-80-9	Confirmed	NFDPM	5.73	✗
82	Hydroquinone	123-31-9	Confirmed	NFDPM	5.71	✗
83	2-Hydroxy-3-oxo-butanal	473-80-3	High	NFDPM & GVP	5.60	✗
84	Andrograpanin	82209-74-3	Confirmed	NFDPM	5.57	✗
85	N-Cyclohexylnicotinamide	10354-56-0	Medium	NFDPM	5.56	✗
86	Lignoceric acid	557-59-5	Confirmed	NFDPM	5.47	✗
87	2(5H)-Furanone	497-23-4	Confirmed	NFDPM	5.45	✓
88	2-Methylbutanoic acid	116-53-0	Confirmed	NFDPM	5.28	✗
89	Isoprene	78-79-5	Confirmed	GVP	5.24	✗
90	Acrolein	107-02-8	Confirmed	GVP	5.20	✗
91	Not identified (GCxGC GVP: 3 compounds)	N/A	Not Identified	GVP	5.17	✗
92	3-Methyl-butanoic acid	503-74-2	Confirmed	NFDPM	5.13	✗
93	6-Methyl-3-pyridinol	1121-78-4	Confirmed	NFDPM	5.10	✗
94	1,4,7,10-Cyclotetradecatetraene, 1,7,11-trimethyl-4(1-methylethenyl)	101159-07-3	High	NFDPM	4.93	✗
95	Butyrolactone	96-48-0	Confirmed	NFDPM	4.80	✓
96	Myristic acid	544-63-8	Confirmed	NFDPM	4.62	✗
97	Stearidonic acid	20290-75-9	Confirmed	NFDPM	4.56	✗
98	Hentriacontane	630-04-6	Confirmed	NFDPM	4.54	✗
99	Benzene	71-43-2	Confirmed	GVP	4.41	✗
100	Acetamide	60-35-5	Confirmed	NFDPM	4.30	✗

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
101	3-Methylpalmitic acid	42172-35-0	Medium	NFDPM	4.27	✖
102	3-Methylfuran	930-27-8	Confirmed	GVP	4.26	✖
103	2-Cyclohexen-1-one, 2,4,4-trimethyl-3-(1,3-butadienyl)	84696-84-4	High	NFDPM	4.13	✖
104	Harmaline	304-21-2	Confirmed	NFDPM	4.07	✖
105	N-Formylnornicotine	3000-81-5	Confirmed	NFDPM	3.91	✖
106	1,3,5,7,11-Cembrapentaene	420793-93-7†	High	NFDPM	3.89	✖
107	Heptadecanoic acid	506-12-7	Confirmed	NFDPM	3.86	✖
108	Octacosanoic acid	506-48-9	Confirmed	NFDPM	3.81	✖
109	Tricosanoic acid	2433-96-7	Confirmed	NFDPM	3.80	✖
110	Farnesylacetone	762-29-8	Confirmed	NFDPM	3.80	✖
111	Toluene	108-88-3	Confirmed	GVP	3.80	✖
112	Phenol	108-95-2	Confirmed	NFDPM	3.74	✖
113	N'-Carbomethoxyanabasine	56078-09-2	High	NFDPM	3.71	✖
114	Propanoic acid, 2-oxo-, methyl ester	600-22-6	Confirmed	NFDPM & GVP	3.70	✖
115	2-Methyl-2-butene	513-35-9	Confirmed	GVP	3.60	✖
116	1-(1-Oxohexyl)-2-(3-pyridinyl)-pyrrolidine	38854-09-0†	Confirmed	NFDPM	3.52	✖
117	3-[1-(2-Furanylmethyl)-2-pyrrolidinyl]-pyridine	78210-85-2†	Confirmed	NFDPM	3.52	✖
118	Retinol	68-26-8	Confirmed	NFDPM	3.48	✖
119	Shikimic acid	138-59-0	Confirmed	NFDPM	3.41	✖
120	3-Hydroxypalmitic acid	2398-34-7	Confirmed	NFDPM	3.38	✖

† CAS number corresponds to one of the isomeric forms for this compound

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
121	Sclareolide	1216-84-8	Confirmed	NFDPM	3.31	✖
122	Methylvinylketone	78-94-4	Confirmed	GVP	3.28	✖
123	1-Hydroxy-2-butanone / 1,2-Butenediol*	5077-67-8 / 50317-11-8	Confirmed	NFDPM	3.23	✓
124	2-Methylpyrazine	109-08-0	Confirmed	NFDPM & GVP	3.20	✖
125	2-Heptadecenoic acid, (2*E*)-	2825-78-7	Confirmed	NFDPM	3.20	✖
126	Norharman	244-63-3	Confirmed	NFDPM	3.20	✖
127	cis-4-Hydroxymethyl-2-methyl-1,3-dioxolane	3674-21-3	Confirmed	NFDPM	3.18	✓
128	Nicoteine	366-18-7	Confirmed	NFDPM	3.18	✖
129	10-Nonadecenoic acid	67228-95-9	Medium	NFDPM	3.16	✖
130	2,3-Dihydrofuran	1191-99-7	Confirmed	GVP	3.14	✖
131	Cotinine	486-56-6	Confirmed	NFDPM	3.09	✖
132	2-Hydroxy-γ-butyrolactone	19444-84-9	Confirmed	NFDPM	3.09	✖
133	Methylformate	107-31-3	Confirmed	NFDPM & GVP	3.01	✖
134	5-(Hydroxymethyl)dihydro-2(3H)-furanone	10374-51-3	Confirmed	NFDPM	2.85	✖
135	1,4:3,6-Dianhydro-α-D-glucopyranose	4451-30-3	Confirmed	NFDPM	2.85	✖
136	Acetonitrile	75-05-8	Confirmed	GVP	2.85	✖
137	1-(1-Oxobutyl)-2-(3-pyridinyl)-pyrrolidine	69730-91-2†	Medium	NFDPM	2.81	✖
138	2-Pyrrolidinone	616-45-5	Confirmed	NFDPM	2.81	✖
139	2-Hydroxypyridine	72762-00-6	Confirmed	NFDPM	2.72	✖
140	t-Phytol	253686-88-3	Confirmed	NFDPM	2.70	✖

* semi-quantified concentration represents the sum of 2 tautomers

† CAS number corresponds to one of the isomeric forms for this compound

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
141	5,8,11-Eicosatriynoic acid	13488-22-7	Confirmed	NFDPM	2.70	✗
142	3-Methoxybenzidine	3365-87-5	High	NFDPM	2.67	✗
143	4-Dodecylphenol	104-43-8	Medium	NFDPM	2.60	✗
144	2-Methyltriacontane	1560-72-1	High	NFDPM	2.56	✗
145	Pentacosanoic acid	506-38-7	Confirmed	NFDPM	2.53	✗
146	1-[4-Amino-2-methyl-5-(2-methylphenyl)-1H-pyrrol-3-yl]ethanone	56463-76-4	Medium	NFDPM	2.49	✗
147	Cerotinic acid	506-46-7	Confirmed	NFDPM	2.48	✗
148	Diacetin	102-62-5	Confirmed	NFDPM	2.47	✓
149	1-Keto-α-cyperone	38043-97-9	High	NFDPM	2.45	✗
150	p-Xylene	106-42-3	Confirmed	GVP	2.39	✗
151	3-Methylhentriacontane	4981-99-1	High	NFDPM	2.39	✗
152	12-Isopropenyl-1,5,9-trimethyl-2,5,9-cyclotetradecatrien-1-ol	60026-11-1	High	NFDPM	2.37	✗
153	Acrylic acid	79-10-7	Confirmed	NFDPM	2.36	✗
154	2-Methyl-2-cyclopenten-1-one	1120-73-6	Confirmed	NFDPM & GVP	2.34	✗
155	5-Methoxy-3-(2-pyridinylmethyl)-1H-indole	101832-06-8	High	NFDPM	2.33	✗
156	2-Chloro-1,3-propanediol	497-04-1	Confirmed	NFDPM	2.32	✗
157	Vernolic acid	503-07-1	Confirmed	NFDPM	2.31	✗
158	Heneicosanoic acid	2363-71-5	Confirmed	NFDPM	2.30	✗
159	Myosmine	532-12-7	Confirmed	NFDPM	2.27	✗
160	Dihydro-α-ionone	31499-72-6	Confirmed	NFDPM	2.24	✗

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
161	3,4-Dimethyl-5-pentyl-2-furanundecanoic acid	57818-36-7	Medium	NFDPM	2.21	✗
162	2-Hydroxytetracosanoic acid	544-57-0	Confirmed	NFDPM	2.19	✗
163	Guaiacol	90-05-1	Confirmed	NFDPM & GVP	2.18	✗
164	N-Ethylnorcotinine	359435-41-9	Confirmed	NFDPM	2.16	✗
165	Retinal	116-31-4	Confirmed	NFDPM	2.13	✗
166	3-Oxo-α-ionol	896107-70-3	Confirmed	NFDPM	2.11	✗
167	Stigmasterol	83-48-7	Confirmed	NFDPM	2.08	✗
168	β-Damascenone	23726-93-4	Confirmed	NFDPM	2.08	✗
169	Phenylacetaldehyde	122-78-1	Confirmed	NFDPM & GVP	2.00	✓
170	2-Acetylfuran	1192-62-7	Confirmed	NFDPM & GVP	1.99	✗
171	2,5-Pyrrolidinedione	123-56-8	Confirmed	NFDPM	1.93	✗
172	Triacontanoic acid	506-50-3	Confirmed	NFDPM	1.92	✗
173	Geranylbenzoate	94-48-4	Medium	NFDPM	1.92	✗
174	3-Methyl-pyridine	108-99-6	Confirmed	NFDPM	1.92	✗
175	Furaneol	3658-77-3	Confirmed	NFDPM	1.92	✗
176	2-[2-(4-Nonylphenoxy)ethoxy]ethyldecanoate	N/A	Medium	NFDPM	1.90	✗
177	1-Butene	106-98-9	Confirmed	GVP	1.89	✗
178	Pentacosane	629-99-2	Confirmed	NFDPM	1.88	✗
179	Benzaldehyde	100-52-7	Confirmed	NFDPM & GVP	1.83	✗
180	cis-2,6-Dimethyl-4-piperidinone	13200-35-6	Confirmed	NFDPM	1.82	✗

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
181	1-(2,3,4,9-Tetrahydro-1H-β-carbolin-1-yl)acetone	69225-88-3	High	NFDPM	1.80	✗
182	3-[1-(5-Ethyl-2-furanyl)-1H-pyrrol-2-yl] pyridine	78210-88-5	Medium	NFDPM	1.79	✗
183	5-Methyl-2-pyridinol	1003-68-5	Confirmed	NFDPM	1.77	✗
184	Nonacosane	630-03-5	Confirmed	NFDPM	1.77	✗
185	3-Methyldotriacontane	20129-49-1	High	NFDPM	1.74	✗
186	Cholest-7-en-3-ol	6036-58-4	Confirmed	NFDPM	1.71	✗
187	(*Z*)-11-Eicosenoic acid	5561-99-9	Confirmed	NFDPM	1.68	✗
188	2,6-Dimethylpyrazine	108-50-9	Confirmed	NFDPM & GVP	1.68	✗
189	1,4-Naphthalenedione, 2,3-dimethyl-6-(4,8,12-trimethyltridecyl)-	68860-42-4†	Medium	NFDPM	1.67	✗
190	2-Vinylfuran	1487-18-9	Confirmed	GVP	1.62	✗
191	Campesterol	474-62-4	Confirmed	NFDPM	1.58	✗
192	cis-2-Butene	590-18-1	Confirmed	GVP	1.53	✗
193	1,3-Cyclopentadiene	542-92-7	Confirmed	GVP	1.51	✗
194	β-Sitosterol	83-46-5	Confirmed	NFDPM	1.50	✗
195	2-Methyl-2-butenal	497-03-0	Confirmed	GVP	1.49	✗
196	2,4-Dimethylcyclopent-4-ene-1,3-dione	65656-90-8	High	NFDPM & GVP	1.49	✓
197	3-Methyl-nonacosane	14167-67-0	High	NFDPM	1.48	✗
198	Ethyl linolenate	1191-41-9	Confirmed	NFDPM	1.43	✓
199	Ricinoleic acid	141-22-0	Confirmed	NFDPM	1.38	✗
200	2-Methyl-3-phenyl-pyrazine	29444-53-9	Confirmed	NFDPM	1.37	✗

† CAS number corresponds to one of the isomeric forms for this compound

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
201	Dihydrofuranone derivative	N/A	High	NFDPM	1.37	✖
202	Acrylamide	79-06-1	Confirmed	NFDPM	1.34	✖
203	2-Hydroxy-4,6-dimethylnicotinonitrile	769-28-8	Medium	NFDPM	1.32	✓
204	Glycidol	556-52-5	Confirmed	NFDPM	1.31	✓
205	3(2H)-Furanone, dihydro-2-methyl-	3188-00-9	Confirmed	NFDPM & GVP	1.30	✓
206	Nicotelline	494-04-2	Confirmed	NFDPM	1.27	✖
207	Pyridine, 3-[1-(5-propyl-2-furanyl)-1H-pyrrol-2-yl]	78210-89-6	Medium	NFDPM	1.27	✖
208	cis-2-Pentene	627-20-3	Confirmed	GVP	1.26	✖
209	2-Methyl-1-butene	563-46-2	Confirmed	GVP	1.25	✖
210	1-(6-Hydroxy-1-oxooctyl)-2-(3-pyridinyl)-pyrrolidine	77829-17-5	Confirmed	NFDPM	1.24	✖
211	(9Z,12Z,15Z)-18-Hydroxy-9,12,15-octadecatrienoic acid	51327-73-2	High	NFDPM	1.23	✖
212	2,3'-Bipyridine	581-50-0	Confirmed	NFDPM	1.23	✖
213	5-Methylcotinine	1076198-50-9	Confirmed	NFDPM	1.18	✖
214	Heneicosane	629-94-7	Confirmed	NFDPM	1.17	✖
215	cis-2-Methyl-1,3-pentadiene	1501-60-6	Confirmed	GVP	1.17	✖
216	4-(3-Hydroxy-2,6,6-trimethyl-1cyclohexen-1-yl)-3-buten-2-one	14398-34-6†	Confirmed	NFDPM	1.15	✖
217	5,6-Dimethyl-3-pyridinol	61893-00-3	Confirmed	NFDPM	1.15	✖
218	Nonadecanoic acid	646-30-0	Confirmed	NFDPM	1.15	✖
219	Styrene	100-42-5	Confirmed	GVP	1.15	✖
220	trans-2-Butene	624-64-6	Confirmed	GVP	1.14	✖

† CAS number corresponds to one of the isomeric forms for this compound

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
221	Nicotine-N-oxide	2820-55-5	Confirmed	NFDPM	1.14	✗
222	1H-Pyrrole, 1-methyl-	96-54-8	Confirmed	GVP	1.13	✗
223	Methyl-pyroglutamate	4931-66-2	Confirmed	NFDPM	1.12	✗
224	α-Acetylbutyrolactone	517-23-7	Confirmed	NFDPM	1.11	✗
225	Hexadecanoic acid, methyl ester	112-39-0	Confirmed	NFDPM	1.10	✗
226	Nonacosanoic acid	4250-38-8	Confirmed	NFDPM	1.07	✗
227	6-(Heptyloxy)-3-pyridinamine	857219-70-6	High	NFDPM	1.07	✗
228	2-Methyl-3-propyl-5,6-dihydropyrazine	15986-94-4	High	NFDPM	1.06	✗
229	N-Formyl-anatabine	77264-87-0	Confirmed	NFDPM	1.06	✗
230	2-Hydroxy-3-methyl-2-cyclopenten-1-one	80-71-7	Confirmed	NFDPM	1.06	✗
231	2,3-Dimethyl-2-cyclopenten-1-one	1121-05-7	Confirmed	NFDPM & GVP	1.05	✗
232	Ethyl linoleate	544-35-4	Confirmed	NFDPM	0.988	✓
233	Nonanoic acid	112-05-0	Confirmed	NFDPM	0.968	✗
234	Cyclopentanone	120-92-3	Confirmed	GVP	0.954	✗
235	5-Hydroxymaltol	1073-96-7	Confirmed	NFDPM	0.938	✗
236	5-Methyl-2(3H)-furanone	591-12-8	Confirmed	NFDPM & GVP	0.934	✗
237	Pentane	109-66-0	Confirmed	GVP	0.922	✗
238	cis-13-Docosenoamide	112-84-5	Confirmed	GVP	0.922	✗
239	2-Methyldotriacontane	1720-11-2	High	NFDPM	0.917	✗
240	17-Hydroxylinolenic acid	143343-97-9	Medium	NFDPM	0.905	✗

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
241	Farnesylacetic acid	6040-06-8†	Confirmed	NFDPM	0.903	✗
242	o-Toluic acid	118-90-1	Confirmed	NFDPM	0.900	✗
243	Menthol	1490-04-6	Confirmed	NFDPM & GVP	0.894	✓
244	24-Methylidenelophenol	1176-52-9	High	NFDPM	0.879	✗
245	3-Methyl-2(1H)-pyridinone	1003-56-1	Confirmed	NFDPM	0.875	✗
246	3,22,23-Trihydroxystigmastan-6-one	90524-90-6†	High	NFDPM	0.875	✗
247	Heptacosanoic acid	7138-40-1	Confirmed	NFDPM	0.874	✗
248	Crotonic acid	3724-65-0	Confirmed	NFDPM	0.866	✗
249	trans-4-hydroxymethyl-2-methyl-1,3-dioxolane	3674-22-4	Confirmed	NFDPM	0.857	✗
250	cis-4-Methyl-2-pentene	691-38-3	Confirmed	GVP	0.855	✗
251	3-Hydroxy-β-damascone	102488-09-5	High	NFDPM	0.803	✗
252	Furancarboxylic acid, methyl ester	611-13-2	Confirmed	NFDPM & GVP	0.789	✓
253	3-Furaldehyde	498-60-2	Confirmed	NFDPM & GVP	0.789	✗
254	trans-3-Penten-2-one	3102-33-8	Confirmed	GVP	0.786	✗
255	Loliolide	38274-00-9	Confirmed	NFDPM	0.782	✗
256	α-Cyperone	473-08-5	Confirmed	NFDPM	0.782	✗
257	3-(Furfuryloxy)-1,2-propanediol	20390-21-0	Medium	NFDPM	0.772	✓
258	Vitamin K1	84-80-0	Confirmed	NFDPM	0.740	✗
259	Megastigmatrienone (2 isomers)	5896-02-6	Confirmed	NFDPM	0.735	✗
260	Cholesterol	57-88-5	Confirmed	NFDPM	0.732	✗

† CAS number corresponds to one of the isomeric forms for this compound

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
261	trans-2-Pentenal	1576-87-0	Confirmed	GVP	0.718	×
262	1,3-Butadiene	106-99-0	Confirmed	GVP	0.716	×
263	3-Methyl-1-butene	563-45-1	Confirmed	GVP	0.706	×
264	Docosane	629-97-0	Confirmed	NFDPM	0.703	×
265	Dotriacontane	544-85-4	Confirmed	NFDPM	0.702	×
266	1-Methyl-1,4-cyclohexadiene	4313-57-9	Confirmed	GVP	0.698	×
267	3-Methyl-2-butanone	563-80-4	Confirmed	GVP	0.697	×
268	1-[4-(Dimethylamino)-2-butyn-1-yl]-5-methyl-2-pyrrolidinone	71970-74-6	Medium	NFDPM	0.686	×
269	4,8,13-Duvatriene-1,3-diol	7220-78-2	Confirmed	NFDPM	0.685	×
270	Limonene	138-86-3	Confirmed	GVP	0.684	×
271	3-Methyl-2-cyclopenten-1-one	2758-18-1	Confirmed	NFDPM	0.670	×
272	Thiirane	420-12-2	Confirmed	GVP	0.663	×
273	Cyclo(Pro-Leu)	5654-86-4	Confirmed	NFDPM	0.652	×
274	2-Ethylfuran	3208-16-0	Confirmed	GVP	0.647	×
275	Acetone cyanohydrin	75-86-5	Confirmed	GVP	0.647	×
276	2(3H)-Furanone, dihydro-5-(1-hydroxyethyl)	27610-27-1	Confirmed	NFDPM	0.641	×
277	16-Hydroxy-9-hexadecenoic acid	17278-80-7	Confirmed	NFDPM	0.636	×
278	Pyrrole-2-carboxamide	4551-72-8	Confirmed	NFDPM	0.624	×
279	5-Methyl-1,3-cyclopentadiene	96-38-8	Confirmed	GVP	0.593	×
280	5-Methyl-2(5H)-furanone	591-11-7	Confirmed	NFDPM	0.590	×

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
281	2-Ethylpyrazine	13925-00-3	Confirmed	NFDPM & GVP	0.586	✗
282	2,4-Dimethylfuran	3710-43-8	Confirmed	GVP	0.584	✗
283	2-Methyl-butanenitrile	18936-17-9	Confirmed	GVP	0.580	✗
284	2-Methyl-5-(prop-1-en-2-yl)-2-vinyltetrahydrofuran	54750-70-8	Confirmed	GVP	0.574	✓
285	1-Methyl-3-(1-methylethyl)-2(1H)-pyrazinone	78210-68-1	Medium	NFDPM	0.570	✗
286	Heptane	142-82-5	Confirmed	GVP	0.563	✗
287	Phytuberol	56857-64-8	Medium	NFDPM	0.562	✗
288	α-Amylcinnamyl alcohol	101-85-9	Confirmed	NFDPM	0.558	✗
289	3-Hydroxysolavetivone	62623-88-5	High	NFDPM	0.553	✗
290	trans-2-Pentene	646-04-8	Confirmed	GVP	0.552	✗
291	6-Ethyl-5,6-dihydro-2H-pyran-2-one	19895-35-3	Confirmed	NFDPM	0.550	✗
292	1-Chloro-2-propanone	78-95-5	Confirmed	GVP	0.546	✗
293	Benzylalcohol	100-51-6	Confirmed	NFDPM	0.544	✗
294	Phytone	502-69-2	Confirmed	NFDPM	0.543	✗
295	3-Pyridinebutanol, d-amino	70898-36-1	Medium	NFDPM	0.541	✗
296	Ethylvinylketone	1629-58-9	Confirmed	GVP	0.537	✗
297	Triacontane	638-68-6	Confirmed	NFDPM	0.535	✗
298	1-Methyl-1,3-cyclopentadiene	96-39-9	Confirmed	GVP	0.534	✗
299	2-Methyl-heptane	592-27-8	Confirmed	GVP	0.529	✗
300	Keto-ionone	27185-77-9	Medium	NFDPM	0.526	✗

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
301	Solerone	29393-32-6	Confirmed	NFDPM	0.525	✖
302	2-Farnesylethanol	67858-77-9†	High	NFDPM	0.522	✖
303	Pentan-2-one	107-87-9	Confirmed	GVP	0.522	✖
304	Geranyllinalool	1113-21-9	Confirmed	NFDPM	0.518	✖
305	1-Acetyloxy-2-butanone	1575-57-1	Confirmed	NFDPM	0.516	✖
306	(6E,10E,14E,18E)-2,6,10,15,19,23-Hexamethyl-1,6,10,14,18,22-tetracosahexaen-3-	97232-74-1	Medium	NFDPM	0.505	✖
307	6-Methyl-5-hepten-2-one	110-93-0	Confirmed	GVP	0.502	✖
308	p-Menthene(Cyclohexene, 1-methyl-4-(1-methylethyl)-)	1195-31-9	Confirmed	GVP	0.492	✖
309	1-Pentene	109-67-1	Confirmed	GVP	0.491	✖
310	Phenylacetic acid	103-82-2	Medium	NFDPM	0.489	✖
311	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	75581-03-2†	High	NFDPM	0.487	✖
312	4-Methyl-1-pentene	691-37-2	Confirmed	GVP	0.487	✖
313	2-Ethyl-3-pyridinol	61893-02-5	Confirmed	NFDPM	0.475	✖
314	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3propyl-	26626-89-1†	Confirmed	NFDPM	0.474	✖
315	3-Methyl-2-butenal	107-86-8	Confirmed	GVP	0.470	✖
316	Propanenitrile	107-12-0	Confirmed	GVP	0.465	✖
317	Dimethylsulfide	75-18-3	Confirmed	GVP	0.456	✖
318	Dihydromaltol	38877-21-3	Confirmed	NFDPM	0.453	✓
319	16-Hydroxy-hexadecanoic acid	506-13-8	Confirmed	NFDPM	0.447	✖
320	3-Hydroxypropionaldehyde	2134-29-4	High	NFDPM	0.445	✖

† CAS number corresponds to one of the isomeric forms for this compound

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
321	4-Methyl-2-pentanone	108-10-1	Confirmed	GVP	0.445	×
322	2-Methyloctacosane	1560-98-1	High	NFDPM	0.445	×
323	Methylacrylate	96-33-3	Confirmed	GVP	0.438	×
324	3-Hexene-2,5-dione	4436-75-3	Confirmed	GVP	0.437	×
325	2-Cyclohexen-1-one	930-68-7	Confirmed	NFDPM & GVP	0.436	×
326	13'-Hydroxy-γ-tocopherol	1215088-63-3	High	NFDPM	0.430	×
327	Squalene	111-02-4	Confirmed	NFDPM	0.429	×
328	2-Methyl-propanoic acid	79-31-2	Confirmed	NFDPM	0.425	×
329	12-Oxo-phytodienoic acid	67204-66-4	Medium	NFDPM	0.422	×
330	[1-Methyl-3-oxo-2-pentylidenecyclopentyl]acetic acid, (2*E*)-	958790-53-9	High	NFDPM	0.418	×
331	Tricosanal	72934-02-2	Confirmed	NFDPM	0.417	×
332	Stigmasta-5,7,22,25-tetraen-3-ol	119386-11-7	High	NFDPM	0.409	×
333	Higher molecular weight derivative of Farnesylacetone (C22)	N/A	High	NFDPM	0.408	×
334	2-Methyl-pyridine	109-06-8	Confirmed	NFDPM & GVP	0.407	×
335	β-Damascone	23726-91-2	Confirmed	NFDPM	0.402	×
336	2-Methylpentane	107-83-5	Confirmed	GVP	0.401	×
337	Higher molecular weight derivative of Farnesylacetone (C26)	N/A	High	NFDPM	0.401	×
338	2,3-Dimethylfuran	14920-89-9	Confirmed	GVP	0.394	×
339	2-Hydroxycerotic acid	14176-13-7	Medium	NFDPM	0.387	×
340	1-Ethyl-9H-pyrido[3,4-ß]indole	20127-61-1	Confirmed	NFDPM	0.378	×

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
341	2-Methylbutane	78-78-4	Confirmed	GVP	0.378	×
342	Glutarimide	1121-89-7	Confirmed	NFDPM	0.370	×
343	Plastoquinone 3	1168-52-1	High	NFDPM	0.365	×
344	3,4-Hexanedione	4437-51-8	Confirmed	GVP	0.362	×
345	2-Hydroxy-2-cyclopenten-1-one / 1,2-Cyclopentanedione*	10493-98-8 / 3008-40-0	Confirmed	NFDPM	0.358	×
346	Scoparone	120-08-1	Confirmed	NFDPM	0.357	×
347	Cyclopentene	142-29-0	Confirmed	GVP	0.355	×
348	Ethylbenzene-	100-41-4	Confirmed	GVP	0.354	×
349	2,3-Dihydro-1,4-dioxin	543-75-9	Confirmed	GVP	0.346	×
350	Dihydro-4-hydroxy-2(3H)-furanone	7331-52-4	Confirmed	NFDPM	0.340	×
351	Cyclohexene, 3-(2-propenyl)- or Cyclohexene, 3-(1-methylethyl)-	15232-95-8 or 3983-08-2	Medium	GVP	0.333	×
352	trans-1,3-Pentadiene	2004-70-8	Confirmed	GVP	0.330	×
353	Pentan-3-one	96-22-0	Confirmed	GVP	0.328	×
354	Pyrazine	290-37-9	Confirmed	NFDPM & GVP	0.318	×
355	2-Methyl-2-propenoic acid	79-41-4	Confirmed	NFDPM	0.311	×
356	Cyclohexylphenylacetic acid	3894-09-5	Confirmed	NFDPM	0.309	×
357	Pentanal	110-62-3	Confirmed	GVP	0.308	×
358	3-Oxo-7,8-dihydro-a-ionol	60047-19-0	Confirmed	NFDPM	0.307	×
359	2,3-Dimethylpyrazine	5910-89-4	Confirmed	NFDPM & GVP	0.307	×
360	Geranylacetone	3796-70-1	Confirmed	NFDPM	0.294	×

* semi-quantified concentration represents the sum of 2 tautomers

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
361	3(2H)-Furanone	3511-31-7	Medium	NFDPM	0.294	✗
362	2-Oxo-propionamide	631-66-3	Confirmed	NFDPM	0.291	✗
363	trans-2,6-Octadiene, 2,6-dimethyl-	2609-23-6	Confirmed	GVP	0.288	✗
364	Glycidylacetate	6387-89-9	Confirmed	NFDPM	0.282	✗
365	Cyclo(Phe-Pro)	3705-26-8	Confirmed	NFDPM	0.280	✗
366	Harman	486-84-0	Confirmed	NFDPM	0.278	✗
367	6,10,14,18-Tetramethyl-5,9,13,17-nonadecatetraen-2-one	6809-52-5	Confirmed	NFDPM	0.278	✗
368	Squalene derivative (C28)	N/A	Medium	NFDPM	0.277	✗
369	Norsolanadione	60619-46-7	High	NFDPM	0.275	✗
370	2-Cyclopenten-1-one, dimethyl- (configurational isomer 1)	N/A	High	NFDPM & GVP	0.273	✗
371	Higher molecular weight derivative of Farnesylacetone (C30)	N/A	High	NFDPM	0.268	✗
372	Norcotinine	5980-06-3	Confirmed	NFDPM	0.263	✗
373	3-Hydroxy-4-methylbenzoic acid	586-30-1	High	NFDPM	0.260	✗
374	1H-Pyrrole, 1-ethyl-	617-92-5	Confirmed	GVP	0.258	✗
375	Benzene, 2-(1,3-butadienyl)-1,3,5-trimethyl-	5732-00-3	Confirmed	NFDPM	0.255	✗
376	Isofucosterol	481-14-1	High	NFDPM	0.255	✗
377	Octadecanoic acid, ethyl ester	111-61-5	Confirmed	NFDPM	0.253	✓
378	1,2-Propadiene	463-49-0	Confirmed	GVP	0.249	✗
379	Steroid derivative	N/A	High	NFDPM	0.247	✗
380	trans-3-Methyl-2-pentene	616-12-6	Confirmed	GVP	0.246	✗

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
381	Higher molecular weight derivative of α-Longipinene or α-Neoclovene	N/A	High	NFDPM	0.242	✗
382	4-Ethenyl-2,6-dimethoxy-phenol	28343-22-8	Confirmed	NFDPM	0.241	✗
383	3-Hexanone	589-38-8	Confirmed	GVP	0.240	✗
384	N-Acetylanatabine	91565-91-2	Confirmed	NFDPM	0.239	✗
385	p-Cresol	106-44-5	Confirmed	NFDPM	0.236	✗
386	Formic acid	64-18-6	Confirmed	NFDPM	0.233	✗
387	2-Ethyl-1-butene	760-21-4	Confirmed	GVP	0.233	✗
388	Adenine	73-24-5	Confirmed	NFDPM	0.231	✗
389	Caprolactone	502-44-3	Confirmed	NFDPM	0.229	✗
390	2-Heptanone	110-43-0	Confirmed	GVP	0.229	✗
391	Octacosane	630-02-4	Confirmed	NFDPM	0.228	✗
392	2-Acetylpyrrole	1072-83-9	Confirmed	NFDPM	0.225	✗
393	N-Acetylanabasine	3350-86-5	Confirmed	NFDPM	0.221	✗
394	Norfuraneol	19322-27-1	Confirmed	NFDPM	0.219	✗
395	2-Heptanone, 6-methyl-	928-68-7	Confirmed	GVP	0.215	✗
396	Benzoic acid, 2-hydroxy-4-methyl	50-85-1	Medium	NFDPM	0.214	✗
397	N-acetyl-4(H)-pyridine	67402-83-9	High	NFDPM	0.213	✓
398	Butyl-1H-imidazole	50790-93-7	Confirmed	NFDPM	0.213	✗
399	4-Pentenal	2100-17-6	Confirmed	GVP	0.213	✗
400	Phenol, 4-ethenyl-	2628-17-3	Confirmed	NFDPM	0.206	✗

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
401	Methylpropionate	554-12-1	Confirmed	GVP	0.199	✗
402	2,7,11-Trimethyl-1,6,10-dodecatriene	502723-87-7	High	NFDPM	0.198	✗
403	2-Acetyl-2-hydroxy-g-butyrolactone	135366-64-2	Confirmed	NFDPM	0.197	✗
404	Labdanediol	10267-21-7†	High	NFDPM	0.196	✓
405	Eicosane	112-95-8	Confirmed	NFDPM	0.194	✗
406	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	21835-01-8	Confirmed	NFDPM	0.194	✗
407	Nicotinamide	98-92-0	Confirmed	NFDPM	0.192	✗
408	Dimethyltrisulfide	3658-80-8	Confirmed	GVP	0.192	✗
409	Hydrogen sulfide	7783-06-4	Confirmed	GVP	0.190	✗
410	2,5-Hexanedione	110-13-4	Confirmed	NFDPM	0.190	✗
411	2-Methyl-3-pentanone	565-69-5	Confirmed	GVP	0.189	✗
412	2,3-Dimethyl-1-butene	563-78-0	Confirmed	GVP	0.188	✗
413	Pentadecanal	2765-11-9	Confirmed	NFDPM	0.183	✗
414	trans-2-Methyl-1,3-pentadiene	926-54-5	Confirmed	GVP	0.182	✗
415	1-(4-Methylphenyl)-ethanone	122-00-9	Confirmed	NFDPM	0.182	✗
416	cis-3-Methyl-2-pentene	922-62-3	Confirmed	GVP	0.179	✗
417	3'-Hydroxycotinine	34834-67-8	Confirmed	NFDPM	0.178	✗
418	4-Vinylguaiacol	7786-61-0	Confirmed	NFDPM	0.177	✗
419	2,2,6-Trimethyl-1-(3-methylbuta-1,3-dienyl)-7-oxabicyclo[4.1.0]heptan-3-ol	1427305-74-5	Medium	NFDPM	0.177	✗
420	Maltol	118-71-8	Confirmed	NFDPM	0.175	✗

† CAS number corresponds to one of the isomeric forms for this compound

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
421	3,4-Dimethyl-2,5-furandione	766-39-2	Confirmed	NFDPM	0.174	✗
422	3-Methyl-2(5H)-furanone	22122-36-7	Confirmed	NFDPM	0.174	✗
423	2H-Pyrrol-2-one, 4-ethyl-1,5-dihydro-3-methyl	766-45-0	High	NFDPM	0.173	✗
424	2,2-Diisopropyl-1,3-dioxolan-4-yl-methanol	470-43-9	High	NFDPM	0.171	✗
425	α-Ionol	25312-34-9	Confirmed	GVP	0.169	✗
426	10-Heneicosene	95008-11-0	Confirmed	NFDPM	0.167	✗
427	2-Methylhydroquinone	95-71-6	Confirmed	NFDPM	0.166	✗
428	1-Heptene	592-76-7	Confirmed	GVP	0.165	✗
429	Isobutyronitrile	78-82-0	Confirmed	GVP	0.165	✗
430	5-Methyl-2-furanmethanol	3857-25-8	Confirmed	NFDPM	0.164	✓
431	5-Isopropyl-2,4-imidazolidinedione	16935-34-5	Confirmed	NFDPM	0.163	✗
432	Pantolactone	79-50-5	Confirmed	NFDPM	0.163	✗
433	cis-2,6-Octadiene, 2,6-dimethyl-	2492-22-0	Confirmed	GVP	0.160	✗
434	1-Methylcyclopentene	693-89-0	Confirmed	GVP	0.160	✗
435	Isocrotonic acid	503-64-0	Confirmed	NFDPM	0.159	✗
436	2,5-Dihydro-3,5-dimethyl-2-furanone	5584-69-0	High	NFDPM	0.158	✗
437	9-Eicosyne	71899-38-2	High	NFDPM	0.158	✗
438	2-Methylphenol	95-48-7	Confirmed	NFDPM	0.158	✗
439	Butyl-hydroxytoluene	128-37-0	Confirmed	NFDPM	0.155	✓
440	c-Phytol	854039-21-7	Confirmed	NFDPM	0.154	✗

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
441	2-Vinyl-5-methylfuran	10504-13-9	Confirmed	GVP	0.153	×
442	trans-4-Methyl-2-pentene	674-76-0	Confirmed	GVP	0.151	×
443	cis-3-Hexene	7642-09-3	Confirmed	GVP	0.150	×
444	Cyclohexene	110-83-8	Confirmed	GVP	0.149	×
445	Diacetin monopropanoate	36600-62-1	Confirmed	NFDPM	0.149	×
446	1-Docosanol	661-19-8	Confirmed	NFDPM	0.149	×
447	4-Vinylpyridine	100-43-6	Confirmed	NFDPM	0.148	×
448	5-Cyanonicotine	42459-12-1	Confirmed	NFDPM	0.146	×
449	Thiophene	110-02-1	Confirmed	GVP	0.145	×
450	4-Vinylcatechol	6053-02-7	Confirmed	NFDPM	0.140	×
451	9-Nonadecene	31035-07-1	High	NFDPM	0.137	×
452	4-Ethylguaiacol	2785-89-9	Confirmed	NFDPM	0.137	×
453	cis-Verbenol	18881-04-4	Confirmed	NFDPM	0.137	×
454	1-Hexadecanol	36653-82-4	Confirmed	NFDPM	0.136	×
455	2-Cyclopenten-1-one, dimethyl- (configurational isomer 2)	N/A	High	GVP	0.135	×
456	trans-Caryophyllene	87-44-5	Confirmed	NFDPM	0.135	×
457	7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-)-	72777-88-9	Medium	NFDPM	0.135	×
458	1-Methylcyclohexene	591-49-1	Confirmed	GVP	0.134	×
459	Phenylethyl alcohol	60-12-8	Confirmed	NFDPM	0.133	×
460	Chloroethane	75-00-3	Confirmed	GVP	0.131	×

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
461	Cyclobutanone	1191-95-3	Confirmed	GVP	0.130	✗
462	Linolenic acid, methyl ester	301-00-8	Confirmed	NFDPM	0.130	✗
463	trans-2-Hexene	13269-52-8	Confirmed	GVP	0.130	✗
464	Butanenitrile, 2-methylene	1647-11-6	Confirmed	GVP	0.130	✗
465	2,4-Dimethyl pyridine	108-47-4	Confirmed	NFDPM	0.129	✗
466	3-Acetoxypyridine	17747-43-2	Confirmed	NFDPM	0.128	✗
467	Tridecane, 2,6,10-trimethyl-	3891-99-4	High	NFDPM	0.126	✗
468	2-Methyl-2-hexene	2738-19-4	Confirmed	GVP	0.126	✗
469	3,5-Dimethylcyclopentene	7459-71-4	High	GVP	0.126	✗
470	2-Methylcyclopentanone	1120-72-5	Confirmed	GVP	0.125	✗
471	3-(4,8,12-Trimethyltridecyl)furan	54869-11-3	Confirmed	NFDPM	0.125	✗
472	N-Nitrosoanatabine	887407-16-1	Confirmed	NFDPM	0.125	✗
473	4-Ethylcatechol	1124-39-6	Confirmed	NFDPM	0.124	✗
474	Hexacosane	630-01-3	Confirmed	NFDPM	0.123	✗
475	Bulnesol	22451-73-6	High	NFDPM	0.122	✗
476	Nonane	111-84-2	Confirmed	GVP	0.121	✗
477	Eicosane, 2-methyl-	1560-84-5	Confirmed	NFDPM	0.121	✓
478	2-Furancarbonitrile	617-90-3	Confirmed	GVP	0.120	✗
479	2-Methyl-1,4-pentadiene	763-30-4	Confirmed	GVP	0.119	✗
480	1,2-Cyclohexanedione	765-87-7	Confirmed	NFDPM	0.119	✓

Table 24 (continued) Summarized Results for THSR

#	Proposed Compound Name	CAS Number	Identification Confidence	Aerosol Fraction	Concentration THSR [µg/item]	THSR > 3R4F by NTDS
481	trans,trans-2,4-Hexadiene	5194-51-4	Confirmed	GVP	0.119	×
482	2-Tritriacontanone	75207-55-5	High	NFDPM	0.118	×
483	1,3-Cyclohexadiene	592-57-4	Confirmed	GVP	0.115	×
484	Dococosanal	57402-36-5	Confirmed	NFDPM	0.114	×
485	Sinapyl alcohol	537-33-7	Confirmed	NFDPM	0.114	×
486	Squalene oxide	7200-26-2	Confirmed	NFDPM	0.113	×
487	Octane, 3,3-dimethyl-	4110-44-5	Confirmed	GVP	0.111	×
488	N-Furfurylpyrrole	1438-94-4	Confirmed	NFDPM	0.108	×
489	3-Methyltritriacontane	14167-69-2	High	NFDPM	0.108	×
490	Octane	111-65-9	Confirmed	GVP	0.108	×
491	o-Cymene	527-84-4	Confirmed	GVP	0.106	×
492	9-Methyladenine	700-00-5	Confirmed	NFDPM	0.106	×
493	2-Methylnonadecane	1560-86-7	Confirmed	NFDPM	0.106	×
494	2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl-, (R)-	599-04-2	Confirmed	NFDPM	0.105	×
495	Nonadecane	629-92-5	Confirmed	NFDPM	0.105	×
496	2-Ethylpyridine	100-71-0	Confirmed	GVP	0.101	×
497	3-Methylcinnamic acid	3029-79-6	Medium	NFDPM	0.101	×
498	2,5-Dimethyl-3(2H)-furanone	14400-67-0	Confirmed	NFDPM	0.100	×

6 DISCUSSION

In contrast to quantitative analysis, where chemical constituents (compounds) of interest are targeted to the exclusion of all others, a non-targeted approach considers the indiscriminate determination of all compounds relevant to a specific chemical space. A portfolio of 7 complementary methods was used to evaluate the chemical space related to tobacco-derived aerosols, based upon two-dimensional gas chromatography with mass spectrometry and liquid chromatography with high resolution mass spectrometry. These methods are semi-quantitative in nature, whereby concentrations of a large number of compounds are estimated versus a restricted number of reference standards of known concentration. These methods can be applied to both non-targeted differential screening (NTDS) and NTS investigations.

In order to accelerate the highly time consuming data evaluation process, an automated workflow is used to select appropriate quantifier ions from the mass spectra for each identified chemical constituent. In addition to the estimation of semi-quantified abundances using a limited number of internal standards, designed to be representative for a large range of diverse chemical constituents, it is recognized that this automated ion selection process is also associated with an inherent degree of variability. To assess this variability, a comparison of semi-quantified concentrations for analytes determined in this investigation versus quantitatively determined analytes within the PMI 54 list of HPHCs was performed. It demonstrated that the methodology was able to semi-quantify within a ±4-fold deviation from the true concentration.

When making comparisons of semi-quantified values between different non-targeted investigations, it is therefore possible to observe concentration differences in the region of 16-fold, in extreme cases (4-fold under-estimate in one study versus 4-fold over-estimate in another study). However, the ratio of abundances between samples (e.g. THS 2.2 versus 3R4F) for each constituent will remain consistent between investigations, since the selection of quantifier ions is made once and is applied to all samples/products analyzed within the same study.

It is important to note that the concentrations previously reported for the four compounds identified by PMI (in the NTDS investigation) as being of toxicological concern were fully quantitative and therefore are not subject to the same level of variability associated with semi-quantification.

In the previously conducted NTDS study which compared THS 2.2 aerosol with 3R4F smoke [1], a statistical approach was used to identify only those compounds present in THS 2.2 aerosol that were significantly higher in abundance than in the smoke of the reference cigarette 3R4F. This study was used to detect whether there were any new hazards associated with using THS 2.2 compared to cigarettes. In this context, for THS 2.2 regular aerosol, a total of 53 compounds were identified as being higher in abundance in the aerosol compared to 3R4F smoke.

In this study 'P1 Characterization' a NTS approach has been applied to the same product (THS 2.2 regular). All chemical constituents identified present at a concentration of ≥100 ng/stick have been reported. PMI used 100 ng/stick as a general reporting cut-off limit, which enabled an estimated 99.8% of the total aerosol mass to be determined.

The objective of this study was to fully characterize the aerosol of THS 2.2. To meet the objective it was important to understand the aerosol fraction in which chemical constituents were present. Accordingly, a combination of CFP and impingers for aerosol trapping were used, in order to make separate evaluations for the composition of the particulate and gas vapor phase. Meanwhile, a cold trap system was used to collect whole aerosol for LC-HRAM-MS analysis in the NTDS study. These differences in the trapping approach between the two studies for LC-HRAM-MS, and additionally improvements to the compound identification process (including confirmation with reference standards), have resulted in a small number of differences for the identified compounds between these two studies, as presented in **Table 25**. The compounds listed in **Table 25** have been evaluated from a toxicological perspective and, as such, this investigation has not revealed any compounds that present a 'new hazard' when compared with cigarette smoke.

For NTDS using GCxGC-TOFMS, a number of compounds reported as being higher in THS 2.2 than in 3R4F are not reported in this study since their levels in THS 2.2 aerosol were below the reporting limit of 100 ng/stick applied for this study.

A total of 529 compounds, excluding water, nicotine and glycerin, were identified as being present in the aerosol of THS 2.2 at a concentration of 100 ng/stick or greater. Over 80% of all compounds identified were confirmed by reference standard. Of these, 529 compounds, 363 (68.6%) were exclusively found in the particulate phase (NFDPM), 127 (24.0%) were exclusively found in the gas vapor phase and 39 (7.4%) compounds were found to be partitioned between both particulate and gas vapor phases. 81.3% of the total mass determined by NTS was present in the particulate phase. Furthermore, all 529

compounds were also identified in 3R4F reference cigarette smoke. The compounds that were previously determined by NTDS to be present at greater concentrations in THS 2.2 than in the smoke of 3R4F are indicated in **Table 24**.

Table 25 Evaluation of Reporting Consistency with Compounds Identified by NTDS using LC-HRAM-MS

Compound Identification NTDS	Compound Identification NTS for P1 Characterization	Comment
Lanost-8-en-3-ol, 24-methylene-, (3ß)	1,4-Naphthalenedione, 2,3-dimethyl-6-(4,8,12trimethyltridecyl)-, (R*,R*)	Compound identified by NTDS is considered to be an adduct of the reported compound
12,14-Labdadiene-7,8-diol, (8a,12E)	a-Cembratriene-diol	Cembranoid degradation product confirmed by analysis of thermally treated duvatriene standard
Isolinderanolide	13,14-Dihydro-retinol	Compound identified by NTDS is considered to be an adduct of the reported compound
Ethyl 2,4-dioxohexanoate	3-(Furfuryloxy)-1,2-propanediol	Revised proposal using an improved process for the evaluation of fragmentation spectra
Benzoic acid, 2,5-dihydroxy-methyl	Not detected	Below limit of detection due to separate analysis of NFDPM and GVP fractions
Ergosterol	Not detected	Below limit of detection due to separate analysis of NFDPM and GVP fractions
Isoquinoline, 3-methyl	Anatabine	Compound identified by NTDS is considered to be derived from the reported compound
Pyridoxin	2-Hydroxy-4,6-dimethylnicotinonitrile	Compound identified by NTDS is considered to be derived from the reported compound

The unique THS 2.2 compounds identified previously (NTDS study) were below the 100 ng/stick threshold.

Before full completion of this investigation, an estimate for the number of compounds present at a concentration ≥ 100 ng/stick in THS 2.2 aerosol was communicated. This estimate of ~750 compounds has shown to be an overestimate, with a reduced number of 529 demonstrated as being present. The use of additional standards to confirm compounds has increased the number of overlapping identifications between LC-HRAM-MS and GCxGC-TOFMS methods, thereby reducing the overall number of compounds identified.

As illustrated in **Figure 1**, the total mass of TPM delivered by THS 2.2 is 56.18 mg/stick, with 5.25 mg of the NFDPM portion indicated to be of unknown composition (‘others’). The total mass of NFDPM estimated by NTS in this study was 5.20 mg, representing 99.0% of this previously unknown portion. Of the 402 compounds identified as being present in NFDPM, 74.1% were confirmed, 14.7% were identified with high certainty, 8.7% were

identified with medium certainty and 2.5% were not identified (unknown). However, when the distribution is considered in terms of mass contribution to NFDPM (excluding glycerin), the amount of constituents identified with medium certainty or assigned as unknown was equivalent to 2.1% of the estimated total, and the amount of constituents either confirmed or identified with high certainty was 97.9% of the estimated total mass (see **Figure 3**). Over 80% of the determined NFDPM mass for THS 2.2 aerosol is represented by 34 chemical constituents (see **Figure 4**).

Since 97.9% (5.09 mg) of the NFDPM mass (excluding glycerin) estimated by NTS is either confirmed or identified with high certainty, this currently leaves 0.16 mg (0.3%) of the total collected aerosol mass (TPM) as uncharacterized, or characterized with a low degree of certainty. Therefore, at this point in time, it can be considered that 99.7% of THS 2.2 TPM (total 56.18 mg) is of known composition. To the best of our knowledge, this is the first time that such a comprehensive in-depth chemical characterization of the aerosol composition of a heat-not burn product has been reported. It represents several years of effort by PMI R&D scientists in the field of analytical method development and advanced structural identification techniques, which have been applied to the aerosol of THS 2.2.

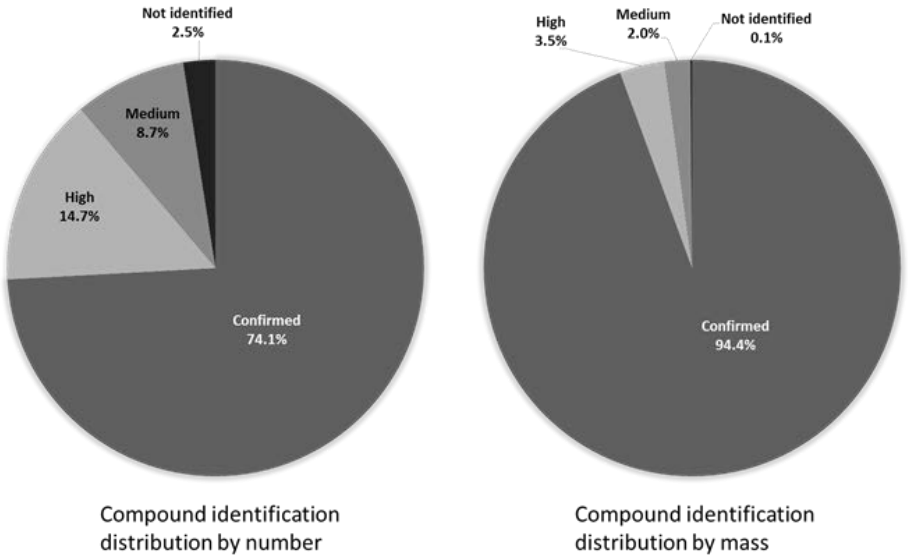

Figure 3: Compound identification status distribution expressed in terms of absolute numbers (left) and in terms of total NFDPM mass collected (right) (excluding glycerin)

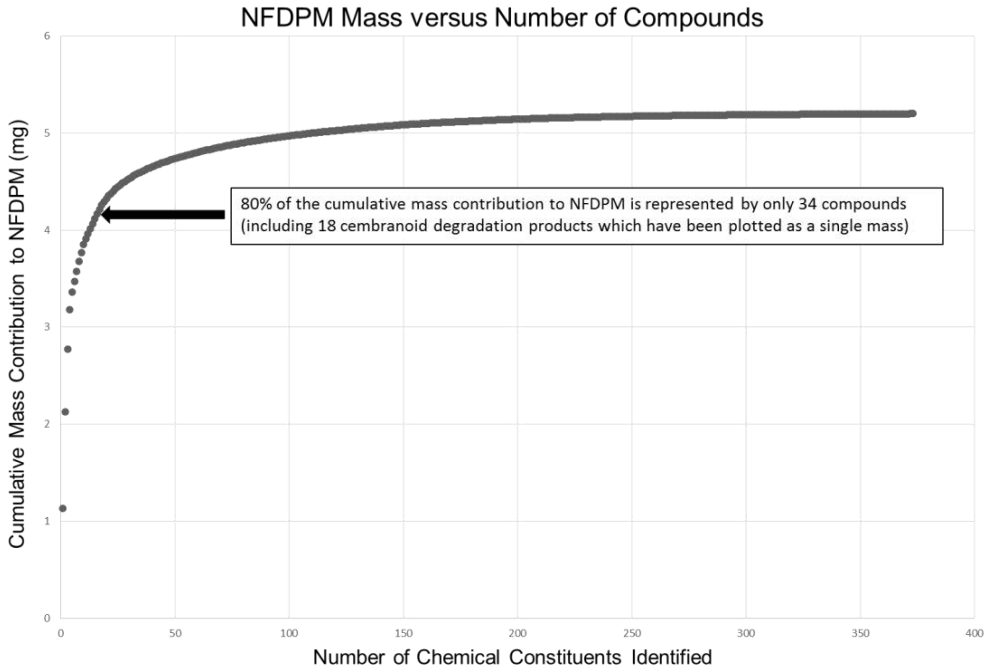

Figure 4: Cumulative mass of individual chemical constituents contributing to THS 2.2 NFDPM (excluding glycerin) ranked from highest (left) to lowest (right) individual mass contribution

7 CONCLUSIONS

The present study provides data that are complementary with the prior NTDS aerosol investigation and with other aerosol characterization studies previously performed.

The major findings of this study are highlighted below:

- 529 chemical constituents, excluding water, nicotine and glycerin, were identified as being present in the aerosol of THS 2.2 at a concentration of 100 ng/stick or greater
- These 529 chemical constituents were also present in the smoke of the 3R4F reference cigarette
- Over 80% of all compounds identified were confirmed by reference standard
- 363 (68.6%) compounds were exclusively found in the particulate phase (NFDPM), 127 (24.0%) were exclusively found in the gas vapor phase and 39 (7.4%) compounds were found to be partitioned between both particulate and gas vapor phases
- 81.3% of the total mass determined by NTS was present in the particulate phase, and the remaining 18.7% in GVP
- The total mass of NFDPM (excluding glycerin) estimated by NTS for THS 2.2 aerosol in this investigation was 5.20 mg, representing 99.0% of the previously unknown portion (5.25 mg)
- 97.9% of the total mass of NFDPM (excluding glycerin) estimated by NTS (5.09 mg) was identified with high confidence or confirmed by reference standard
- 99.7% of the TPM generated by THS 2.2 aerosol is of known composition
- There are no compounds identified in this study that would be considered as a ‘new hazard’ when compared with cigarette smoke

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(b) (4)

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(b) (4)

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(b) (4)

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(b) (4)

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9 ABBREVIATIONS

AGC	:	Automatic Gain Control
APCI	:	Atmospheric Pressure Chemical Ionization
API	:	Atmospheric Pressure Ionization
BP	:	Boiling Point
CAS	:	Chemical Abstracts Service
CASI	:	Computer-Assisted Structure Identification
°C	:	Degree Centigrade
CFP	:	Cambridge Filter Pad
CID	:	Collision Induced Dissociation
csv	:	Comma Separated Variable
1D	:	First Dimension
2D	:	Second Dimension
Da	:	Dalton
DCM	:	Dichloromethane
dd	:	Data Dependent
DMF	:	Dimethylformamide
2DRelRT	:	Second Dimension Relative Retention Time
EDMS	:	Electronic Document Management System
eV	:	Electron Volt
FDA	:	Food and Drug Administration
FTMS	:	Fourier Transform Mass Spectrometry
GC×GC-TOFMS	:	2-Dimensional Gas Chromatography with Time-of-Flight Mass Spectrometry
GC-HR-MS	:	Gas Chromatography with High Resolution Mass Spectrometry
GVP	:	Gas Vapor Phase
HC	:	Health Canada Intense
HILIC	:	Hydrophilic Interaction Liquid Chromatography
HMDB	:	Human Metabolome Database
HPHC	:	Harmful or Potentially Harmful Constituent
ISO	:	International Organization for Standardization

ISTD	:	Internal Standard
IT	:	Ion Inject Time
LC	:	Liquid Chromatography
LC-HRAM-MS	:	Liquid Chromatography with high resolution Accurate Mass Spectrometry
LTR	:	Long Term Repository
min	:	Minute
MF	:	Match Factor
µg	:	Microgram
µL	:	Microliter
mL	:	Milliliter
mm	:	Millimeter
MRTP(A)	:	Modified Risk Tobacco Product (Application)
MS	:	Mass Spectrometry
ms	:	Millisecond
MS^2 or MS/MS	:	First Order Mass Spectrometric Fragmentation
MW	:	Molecular Weight
m/z	:	Mass to Charge Ratio
n	:	Number
NFDPM	:	Nicotine Free Dry Particulate Matter
NIH	:	National Institute of Health
NIST	:	National Institute of Standards and Technology
NLM	:	National Library of Medicine
N/A	:	Not Applicable
NTS	:	Non-targeted Screening
NTDS	:	Non-targeted Differential Screening
P1	:	Platform 1
PDIMS	:	Product Development Information Management System
PDSP	:	Programmable Dual Syringe Pump
PMI	:	Philip Morris International
PMMTB	:	Philip Morris Manufacturing and Technology Bologna

ppm	:	Parts per Million
%	:	Percent
QSPR	:	Quantitative Structure-Property Relationship
3R4F	:	Reference Cigarette from the University of Kentucky (US)
RH	:	Relative Humidity
RI	:	Retention Index
RIM	:	Retention Index Marker
RP	:	Reversed Phase (Chromatography)
rpm	:	Revolutions per Minute
RRP	:	Reduced Risk Product
RSD	:	Relative Standard Deviation
s	:	Second
SIS	:	Specialized Information Service
SME	:	Subject Matter Expert
S/N	:	Signal-to-Noise Ratio
THD	:	Tobacco Heating Device
THS	:	Tobacco Heating System
THSR	:	Tobacco Heating System using Regular HeatSticks
TPM	:	Total Particulate Matter
UCSD	:	Unique Compound Spectra Database
UHPLC	:	Ultra High Performance Liquid Chromatography
v	:	Volume
WS	:	Working Solution
WKI	:	Work Instruction

10 REVIEW AND APPROVAL

This document has been reviewed and approved by:

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11 DOCUMENT VERSION HISTORY

Version No.	Description of Change	Justification
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