# Formaldehyde-Glycerol Hemiacetal
# - Absence of "Hidden" Formaldehyde in THS 2.2. Aerosols

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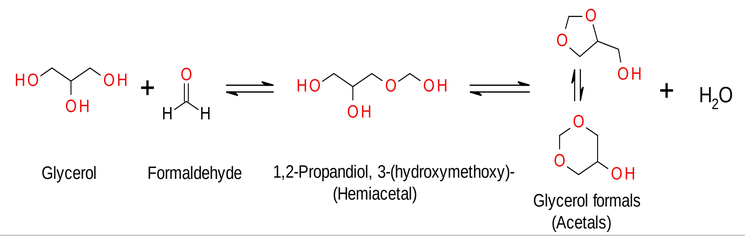
## INTRODUCTION

Reduced Risk Products ("RRPs"*) is the term used by PMI to refer to products with the potential to reduce individual risk and population harm in comparison to smoking cigarettes. We are developing a portfolio of products with the potential to reduce risk for adult smokers with a significant reduction in the levels of harmful and potentially harmful constituents (HPHCs) demonstrated in extensive and rigorous scientific studies.[1] Along with many other HPHCs, formaldehyde levels measured in the aerosol generated by our THS**2.2 heat not burn platform was found to be reduced by 90% compared to the levels measured in the smoke of reference cigarette 3R4F[2] on a per stick basis.***

In a recent correspondence in The New England Journal of Medicine from Jensen et al. the authors discussed the formation of hemiacetals from formaldehyde and humectants like propylene glycol or glycerol, associated with a potential risk of underestimating the uptake of formaldehyde by using e-cigarettes while operating under extreme energy delivery to the liquid. The formaldehyde hemiacetal (known as formaldehyde releasing agent) might represent a "hidden" formaldehyde, not covered by classical analytical approaches, releasing the formaldehyde portion after inhaling.[3]

Figure 1:
Acetalization reaction scheme for glycerol and formaldehyde




We have challenged our analytical approaches for the potential risk of underestimating formaldehyde doses due to formaldehyde hemiacetal formation. Furthermore we were interested in the potential formation of a formaldehyde hemiacetal in the aerosol of our THS2.2.

**Reduced Risk Products ("RRPs") is the term we use to refer to products with the potential to reduce individual risk and population harm in comparison to smoking cigarettes. PMI's RRPs are in various stages of development, and we are conducting extensive and rigorous scientific studies to determine whether we can support claims for such products of reduced exposure to harmful and potentially harmful constituents in smoke, and ultimately claims of reduced disease risk, when compared to smoking cigarettes. Before making any such claims, we will rigorously evaluate the full set of data from the relevant scientific studies to determine whether they substantiate reduced exposure or risk.*

***THS = Tobacco heating system*

**** internal GLP-study on an investigational THS2.2 product*

## MATERIALS & METHODS

### Custom synthesis of Formaldehyde-Glycerol Hemiacetal (FA-GLY HA)

Chemical Synthesis Laboratory:
Chemische Laboratorien, Dr. Soenke Petersen, Worms (Germany)

- Standard characterized by 1H-NMR
- Shipped on dry ice (-80°C)
- Stored in freezer

### THS2.2 aerosol / cigarette smoke samples for carbonyl analysis

Smoke/Aerosol Generation:

- Smoke of 3R4F or aerosol of THS2.2 generated using Health Canada (HC) smoking regime
- 1 accumulation, 12 puffs (THS2.2), butt length (3R4F)
- 2 micro impinger traps containing 10 mL DNPH 25mM each
- Stabilization by addition of 500 µL pyridine 15 min after aerosol collection in each impinger trap, which are then combined

Sample preparation:

- 1000 µL of stabilized aerosol extract + 500 µL of internal standards solution

### THS2.2 aerosol / cigarette smoke samples for LC-HRAM-MS analysis

Smoke/Aerosol Generation:

- Whole smoke / aerosol generated using Health Canada (HC) smoking regime
- Aerosol trapping using glass fiber filter PAD
- 2 accumulations, 12 puffs (THS2.2), 10 puffs (3R4F)
- Extracted with 10mL methanol incl. internal standards

Sample Preparation:

- 200µL extracted aerosol sample + 800µL methanol

### FA-GLY HA by GCXGC-TOFMS

2-Dimensional gas chromatography (GCXGC):

- Agilent 7890A + $LN_2$ Modulator + secondary oven
- Carrier: helium, 1mL/min
- Injection: Cool-on-column, 0.1µL
- Pre-column: 2m SLB-IL60
- Column 1: 30m DB-FFAP
- Column 2: 1.9m VF-624ms

| | primary oven | secondary oven |
|---|---|---|
| initial | 35°C (2min) | 55°C (2min) |
| rate | 5°C/min | 4.6°C/min |
| final | 250°C (23min) | 285°C (16min) |

Mass Spectrometry (TOFMS):

- LECO Pegasus 4D
- Ionization: EI, 70eV
- Scan range: 29-700 Da
- Data acquisition rate: 200 spectra/s

### Determination of carbonyls in aerosol samples by UPLC-MS/MS

Liquid chromatography (LC):

- LC-pump: Waters Acquity UPLC I-Class
- Column: Waters Acquity BEH C18 (50mmx2.1mm, 1.7µm)+guard column
- LC-Gradient:

| Time [min] | A [%] 10mM NH4AC in water:ACN 65:35 v:v | B [%] Acetonitrile |
|---|---|---|
| 0 | 100 | 0 |
| 2.0 | 77 | 23 |
| 3.7 | 77 | 23 |
| 3.8 | 0 | 100 |
| 4.8 | 0 | 100 |
| 5.0 | 100 | 0 |

- Flow: 500µL/min, Inj.Vol: 1µL
- Post-column flow split: 1:5

Mass Spectrometry (MS/MS):

- Waters Xevo TQ-S
- Ionization: ESI(-)
- MRM: 208.9->163.1

### FA-GLY HA and THS2.2 aerosol by LC/DI-HRAM-MS

Liquid chromatography (LC):

- LC-pump: Thermo Accela 1250
- Column: Hypersil Gold™ (150mmx2.1mm, 1.9µm) + guard column
- LC-Gradient:

| Time [min] | A [%] 10mM NH4AC in water | B [%] 1mM NH4AC in methanol |
|---|---|---|
| 0 | 85 | 15 |
| 7.0 | 10 | 90 |
| 12.8 | 0 | 100 |
| 14.5 | 0 | 100 |
| 15.0 | 85 | 15 |
| 16.0 | 85 | 15 |

- Flow: 400µL/min, Inj.Vol: 1.5µL

Direct Infusion (DI):

- 5µL/min

Mass Spectrometry (HRAM-MS):

- Thermo QExactive™
- Ionization (LC): ESI(+)
- Ionization (DI): ESI(+), APCI(+), ESI(-)
- Full scan: 80 – 800 Da, resolution: 70000
- dd-MS2 (TopN): stepped NCE, resolution: 17500

### FA-GLY HA in THS2.2 aerosol by 1H-NMR

- Bruker AVIII HD with Cryo-Probe
- Magnet: 600MHz

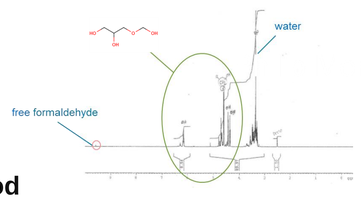
## RESULTS

### FA-GLY HA 'hide' formaldehyde for classical carbonyl analysis?

#### Custom synthesis of Formaldehyde- Glycerol Hemiacetal (FA-GLY HA)

Content analysis of synthesized reference compound performed by 1H-NMR:

- FA-GLY HA: ~30%
- Water: ~70%
- Free formaldehyde: <1%




#### Analysis of synthesized reference compound with classical carbonyl method

Experimental procedure

- 10.9mg of synthesized FA-GLY HA standard was prepared in 5mL DNPH solution
- Diluted 1:10 in DNPH, 10min. derivatization time, then addition of 250µL pyridine for stabilizing
- Addition of internal standard and analysis by LC-MS/MS
- Concentration of FA-GLY HA in measurement solution: 64µg/mL, corresponding to 15.7µg/mL formaldehyde

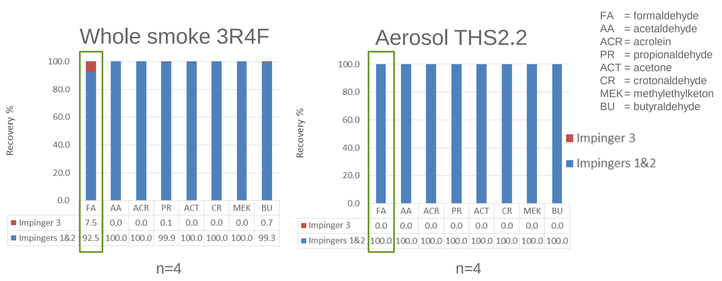
- **Formaldehyde analyzed: 17.7µg/mL (~113% of theoretical)**

### FA-GLY HA trapping efficiency using standard carbonyl method

#### Trapping efficiency by sequential impinger trapping

Experimental approach

- Aerosol of THS2.2 and mainstream smoke of the 3R4F were generated under Health Canada smoking protocol
- 3 Impinger traps, each containing 10mL of DNPH trapping solution including a set of stable isotope labelled internal standards
- Combined first 2 impingers were analyzed and compared to 3rd impinger carbonyl results



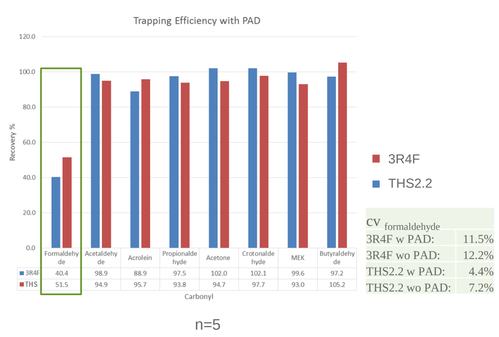

- **Trapping efficiency formaldehyde (2 impinger traps)**
  **3R4F: 92.5%**
  **THS2.2: 100%**

### Formaldehyde associated with particle phase?

#### Formaldehyde analysis w/wo PAD

Experimental approach

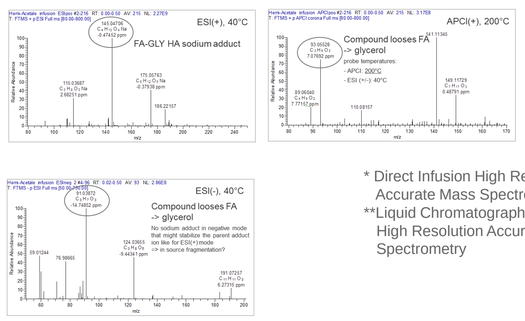
- Introduction of glass fiber filter PAD in front of 2 impinger traps
- Comparison to results using 2 impingers without PAD




- **FA associated to particle phase**
  **3R4F: ~ 60%**
  **THS2.2: ~ 48%**

### FA-GLY HA in aerosol of THS2.2 or smoke of 3R4F by LC/DI-HRAM-MS

#### FA-GLY HA reference standard DI-HRAM-MS*

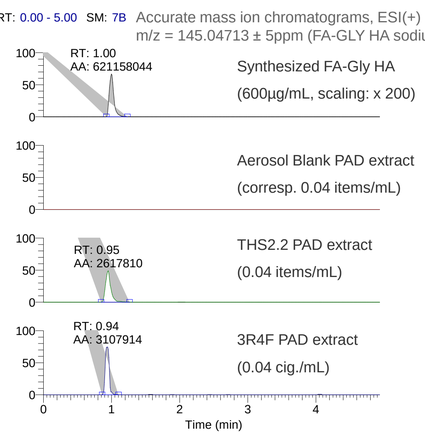



* Direct Infusion High Resolution Accurate Mass Spectrometry
**Liquid Chromatography coupled to High Resolution Accurate Mass Spectrometry

DI-ESI(+): Sodium adduct of FA-GLY HA
DI-APCI(+): Thermal instability => FA loss
DI-ESI(-): FA-GLY HA probably not stable without adduct => FA loss

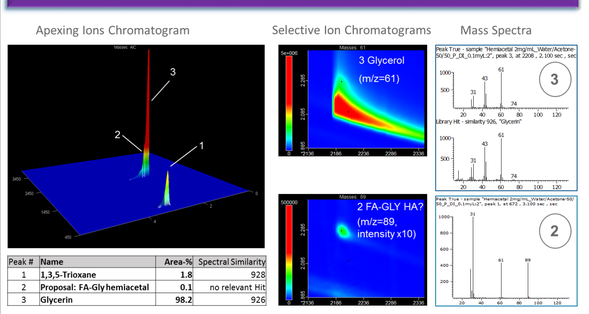
- FA-GLY HA well ionized by ESI(+) as sodium adduct
- APCI(+) and ESI(-) leads to parent-ion fragmentation, not suited

#### FA-GLY HA in aerosol or smoke samples LC-HRAM-MS**




- FA-GLY HA probably found in 3R4F and THS2.2 (further proof needed)
- Method development might be complicated and challenging

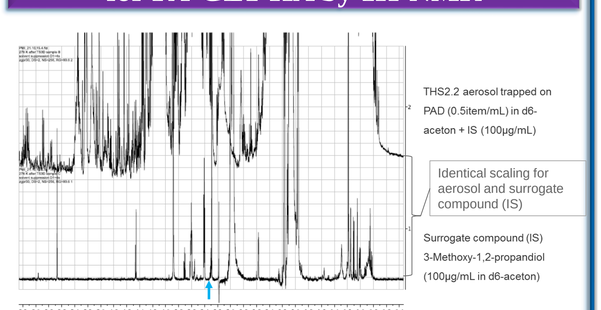
### FA-GLY HA by GCxGC-TOF




| Peak # | Name | Area-% | Spectral Similarity |
|---|---|---|---|
| 1 | 1,3,5-Trioxane | 1.8 | 928 |
| 2 | Proposal: FA-Gly hemiacetal | 0.1 | no relevant Hit |
| 3 | Glycerin | 98.2 | 926 |

- FA-GLY HA analysis by GC methods not possible => thermo-degradation of hemiacetal (one major product: glycerol)

### THS2.2 aerosol analysis for FA-GLY HA by 1H-NMR




- FA-GLY HA analysis by 1H-NMR in THS2.2 aerosol not possible due to complexity of matrix

## CONCLUSIONS

- We could demonstrate that there is no underestimation of formaldehyde (i.e. hidden formaldehyde) in the aerosol generated by THS 2.2 when appropriate analytical methods are used
- Our reported formaldehyde yields in the aerosol generated by THS 2.2 represent the total formaldehyde present in the aerosol (free formaldehyde as well as formaldehyde associated with formaldehyde-glycerol hemiacetal)
- The standard analytical method used for carbonyls (measured as DNPH derivatives with LC-MS/MS) (re-)covers formaldehyde from the unstable hemiacetal
- A significant portion of formaldehyde (e.g. as hemiacetal) is associated with the particle phase of aerosol or smoke
- Evidence of the presence of formaldehyde-glycerol hemiacetal in cigarette smoke as well as in THS 2.2 aerosol, shows that the hemiacetal is not unique for heat-not burn products or extreme heated e-liquids

## REFERENCES

1. https://www.pmiscience.com
2. University of Kentucky (www.3r4f.com), (http://www2.ca.uky.edu/refcig/3R4F%20Preliminary%20Analysis.pdf)
3. Hidden Formaldehyde in E-Cigarette Aerosols, Jensen et al., N Engl J Med, January 22, 2015

- Glycerol acetals, kinetic study of the reaction between glycerol and formaldehyde, I. Agirre et al., BIOMASS AND BIOENERGY 35, 2011

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