Evaluation of Tobacco Heating System during 

*in vitro* and clinical studies

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14 June 2019
Aim: to study tobacco heating system manufactured by PMI in terms of its in vitro toxicity as well as the changes of possible risks of smoking-related diseases.

Study conducted in 2016-2017 in Kazan (Russia), By the Order of the Government of Russian Federation
Cytotoxicity assessment

In vitro
Cytotoxicity and genotoxicity assessment of THS and 3R4F aerosol fractions (moist condensate extracted by standard procedures by the Krasnodar Tobacco Research Institute)

Clinical studies (healthy volunteers)

Preclinical studies

Cytotoxicity assessment

Genotoxicity assessment

Clinical studies (healthy volunteers)
Cytotoxicity evaluation - a test for in vivo staining with neutral red (NRU-test)

Relative cytotoxicity of the THS aerosol fractions calculated per unit of product (ml/unit) was lower by 92.61% (tobacco taste) and 91.83% (menthol taste) compared to the relative cytotoxicity of the tobacco smoke fraction of the reference 3R4F cigarette (taken as 100%).

Relative cytotoxicity of the THS aerosol fractions calculated per unit mass of nicotine (ml/mg nicotine) was lower by 95.29% (tobacco taste) and 95.30% (menthol taste) compared to the relative cytotoxicity of the tobacco smoke fraction of the reference 3R4F cigarette (taken as 100%).
Assessing the metabolic activity of cells
(MTS-test)

Relative cytotoxicity of THS aerosol fractions in the MTS test calculated per unit of product (ml/unit) were lower by 91.48% (tobacco taste) and 89.94% (menthol taste) compared to the relative cytotoxicity of the tobacco smoke fraction of the 3R4F reference cigarettes (taken as 100%).

The values of the relative cytotoxicity of the THS aerosol fractions (MTS test) calculated per unit mass of nicotine (ml/mg nicotine) were 94.58% lower (tobacco taste) and 94.19% (menthol taste), compared to the relative cytotoxicity of 3R4F reference cigarette tobacco smoke fraction (taken as 100%).
Apoptotic and necrotic cells identification

• The values of THS aerosol fractions and conventional cigarettes tobacco smoke aerosol concentrations providing 10% of cells growth inhibition (IC10) during the NRU test, were chosen as the working concentration for further tests.

• **The number of THS sticks required to obtain the described aerosol fraction was 13.5 times (regular taste) and 12.3 times (menthol taste) more than for 3R4F**

The results obtained confirm the data of the NRU-test, which demonstrated cell viability in all the studied samples in the range of 88.5-93.5% at IC10 concentrations.
Evaluation of reverse mutations on bacteria (Ames test)

5 strains of Salmonella typhimurium:
- Salmonella typhimurium TA98
- Salmonella typhimurium TA100
- Salmonella typhimurium TA102
- Salmonella typhimurium 1535
- Salmonella typhimurium 1537

The number of revertants of the Salmonella typhimurium strain TA100, TA102, 1535 and 1537 in the presence of the studied THS and 3R4F cigarettes condensates does not exceed the number of cells in the negative control (DMSO), that indicates the absence of potential mutagenicity on these test strains.

However, for 3R4F cigarettes moist condensate, a gradual increase in the number of revertants was observed with increased dose (dose-effect is present), which suggests the possible presence of potential mutagenicity at high doses.
The number of revertants was less than 2 times higher than the number of cells in the negative control and medium.

There is no pronounced dose-effect relationship, which indicates the absence of potential mutagenicity of the investigated concentrations of THS condensate.

Significant excess of the number of revertants over the negative control (up to 2-14 times)

A pronounced dose-effect relationship in Salmonella typhimurium strains TA100, TA98, 1535, and 1537, which indicates the presence of mutagenicity for these test strains.

Mutagenicity was not detected in the Ames test with metabolic activation for THS, however was present in case of 3R4F samples.
**Evaluation of genotoxicity** - a method for assessing the induction of SOS DNA repair system on bacteria (SOS chromotest on bacteria, or Ames test)

1. Neither concentration of wet condensate of THS and 3R4F sticks led to a 2-fold excess of β-galactosidase activity compared to the medium (DMSO) in the Salmonella typhimurium TA2035/pSK1002 strain, which indicates the absence of SOS repair and, therefore, absence of DNA-damaging activity of the studied samples in the indicated concentrations.

2. There is a positive dose-effect relationship in the THS and 3R4F samples. Consequently, a possible DNA damaging effect at high concentrations could be supposed.

3. However, an 1.5-fold increase of β-galactosidase activity compared with medium (DMSO) was observed at 5 times higher concentrations of THS wet condensate than the concentrations of 3R4F wet condensate.

4. Consequently, the genotoxic effect of THS wet condensate is several times lower than that of the reference 3R4F cigarette.
Conclusions (preclinical studies)

- **Cytotoxicity**
  - The *cytotoxic effect* of the THS aerosol fractions (both normal and menthol taste) is **significantly lower** compared to reference 3R4F cigarette tobacco smoke fractions.

- **Genotoxicity**
  - According to the results of the SOS-chromotest, the *genotoxicity* and *mutagenicity* of the aerosol fractions of two THS types (with/without menthol) are **significantly lower** compared with 3R4F reference cigarette tobacco smoke fractions;

- **Mutagenicity** *was not revealed in the Ames test* without metabolic activation on Salmonella typhimurium strains TA98, TA100, TA102, 1535 and 1537 for THS, but a dose-dependent effect was observed for 3R4F;

- Mutagenicity was not revealed for THS in the Ames test with metabolic activation, however, it occurred in case of 3R4F samples.
Clinical study involved healthy volunteers (60 subjects)

- Smoking abstinence - psychologist
- THS or conventional

1. Analysis of biomarkers of exposure
   - Urine: Monohydroxy butenyl mercapturic acid (MHBMA), 3-hydroxypropyl mercapturic acid (HPMA), 2-cyanoethyl mercapturic acid (CEMA), 2-phenyl mercapturic (SPMA), 3-hydroxy-1-methylpropyl mercapturic acid (HMPMA), 4-(methylnitroamine)-1-(3-pyridyl)-1-butanol (NNAL), N-nitrosonornicotine (NNN), nicotine, cotinine, nornicotine, trans-3'-hydroxycotinine, nicotine and cotinine glucuronides, anabazone
   - Blood: Carboxyhaemoglobin, cotinine, trans-3'-hydroxycotinine

2. Safety assessment
   - Total blood count, urinalysis, blood biochemistry, ECG, spirometry, general physician assessment incl. vital signs evaluation (blood pressure, heart rate, body T), AE/SAE monitoring

3. Questionnaires
   - + VAS for cough assessment, Likert scale for cough severity, frequency and amount of sputum
Assessment of difference between CoHb concentration in different groups

Statistically significant difference in COHb blood concentration was observed at all days of study (Day 1 - Day 5) between THS/SA groups comparing with CC group.
Plasma nicotine metabolites:
Day 0, Day 6
(CC, THS, SC groups)

Conventional cigarettes (CC)

nicotine

cotinine

trans-3'-hydroxycotinine

THS

Smoking abstinence

p=0.777

p=0.346

p=0.004

p<0.001

p<0.001
**Mercapturic acids**

### Conventional cigarettes

<table>
<thead>
<tr>
<th>Mercapturic acid</th>
<th>Substance-precursor</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-2-cyanoethyl-mercapturic acid (CEMA)</td>
<td>Acrolein</td>
</tr>
<tr>
<td>3-hydroxy-1-methylpropyl-mercapturic acid (HMPMA)</td>
<td>Crotonaldehyde</td>
</tr>
<tr>
<td>S-(3-hydroxypropyl) mercapturic acid (HPMA)</td>
<td>Acrolein</td>
</tr>
<tr>
<td>Monohydroxybutenylmercapturic acid (MHBMA)</td>
<td>1,3-butadien</td>
</tr>
<tr>
<td>S-phenylmercapturic acid (SPMA)</td>
<td>Benzole</td>
</tr>
</tbody>
</table>

### THS

- **p<0.001**
- **p=0.001**
- **p<0.001**

### Smoking Abstinence

- **p=0.006**
- **p=0.001**
- **p=0.001**
Mercapturic acids content in CC/THS/SA groups

HMPMA (Day 0 and Day 5)
3-hydroxy-1-methylpropyl-mercapturic acid

HPMA (Day 0 and Day 5)
S-(3-hydroxypropyl) mercapturic acid
Healthy volunteers study (conclusions)

- **Clinical and laboratory assessment** (general examination and vital signs, blood count, urinalysis, blood biochemistry, ECG, spirometry, AE/SAE monitoring) proved the safety of THS.

- THS use resulted in tendency to:
  - Decreasing the severity (statistically significant difference on Day 6 comparing with initial data) and frequency of cough (statistically significant changes on Day 5 and Day 6 comparing with initial data).
  - Decreasing the amount of sputum (statistically significant difference on Day 6 comparing with initial data).
Healthy volunteers study (conclusions)

• Switching to THS for 5 days leads to decrease of blood COHb level, which was compatible with smoking cessation for 5 days.

• Switching to THS leads to statistically significant decrease of concentration of all studied mercapturic acids in urine by Day 5, which was comparable with smoking cessation group data.

• On Day 5 mercapturic acids levels in THS group did not differ significantly from SC group, however, were significantly lower than CC group data. So, the minimum content of harmful/potentially harmful constituents in THS could be proposed.
General conclusions

• Both in vitro and clinical studies indicate reduced toxicity and reduced exposure to toxic substances usually found in cigarette smoke between THS and cigarettes

• Study results are published:
Thank you for your attention