



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



Казанский федеральный
УНИВЕРСИТЕТ



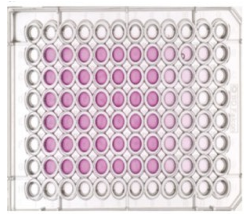
Clinical studies
(healthy volunteers)

Preclinical studies

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

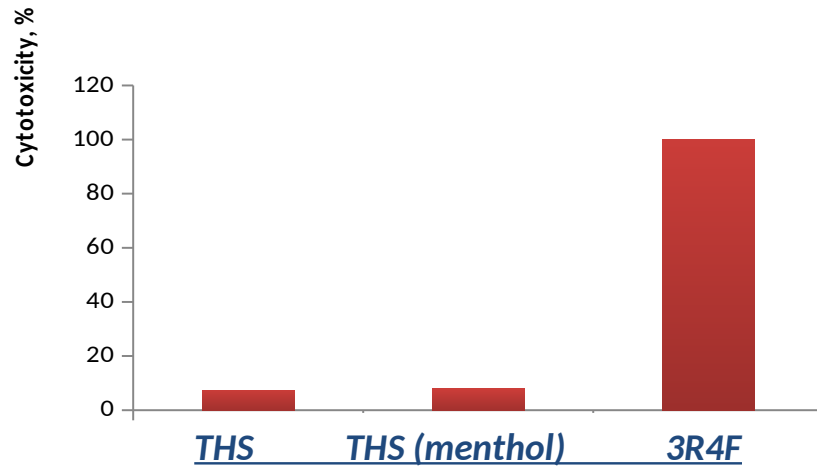
Cytotoxicity assessment

Genotoxicity assessment

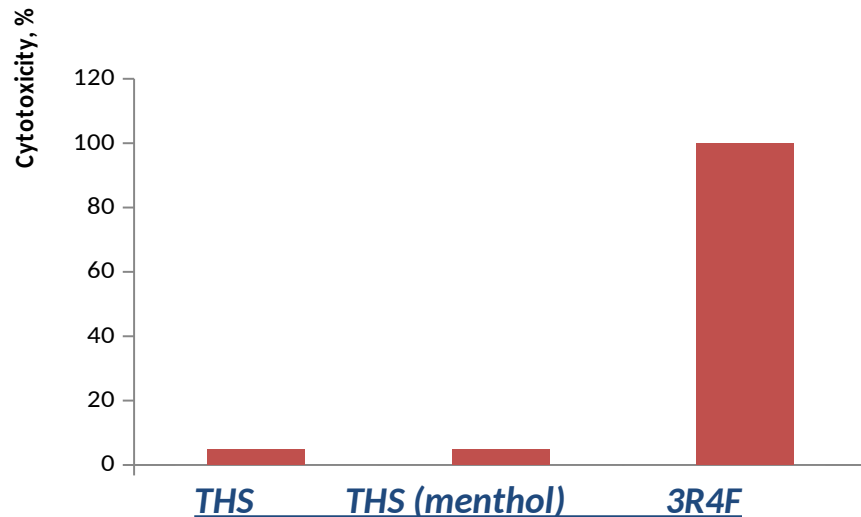


Cytotoxicity evaluation - a test for in vivo staining with neutral red (NRU-test)

Preclinical studies

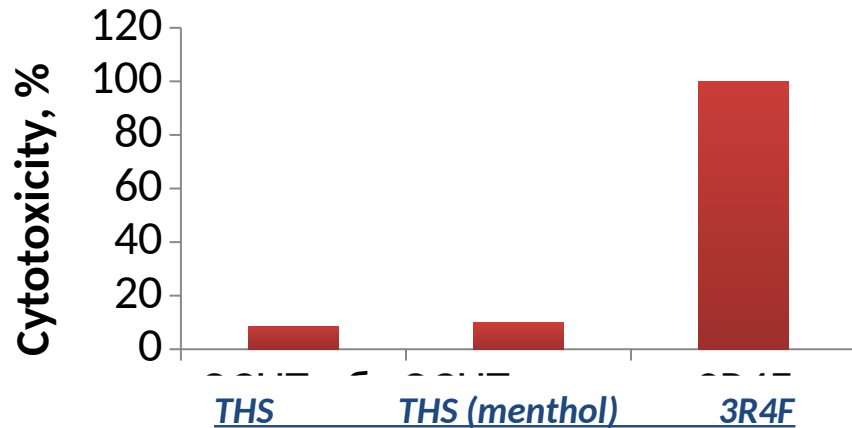
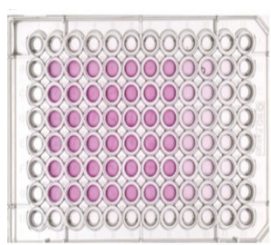


Relative cytotoxicity of the THS aerosol fractions calculated **per unit of product** (ml/unit) was **lower by 92.61% (tobacco taste) and 91.83% (menthol taste)** compared to the relative cytotoxicity of the tobacco smoke fraction of the reference 3R4F cigarette (taken as 100%).

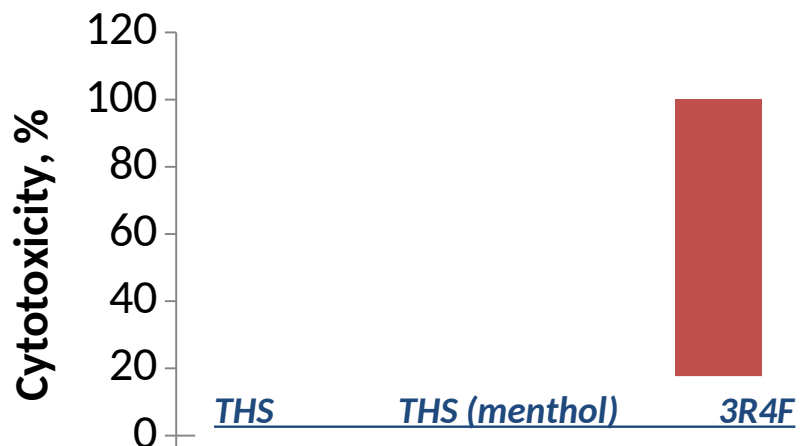


Relative cytotoxicity of the THS aerosol fractions calculated **per unit mass of nicotine** (ml/mg nicotine) was **lower by 95.29% (tobacco taste) and 95.30% (menthol taste)** compared to the relative cytotoxicity of the tobacco smoke fraction of the reference 3R4F cigarette (taken as 100%).

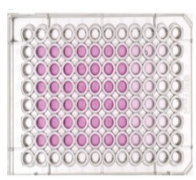
Assessing the metabolic activity of cells (MTS-test)



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%).



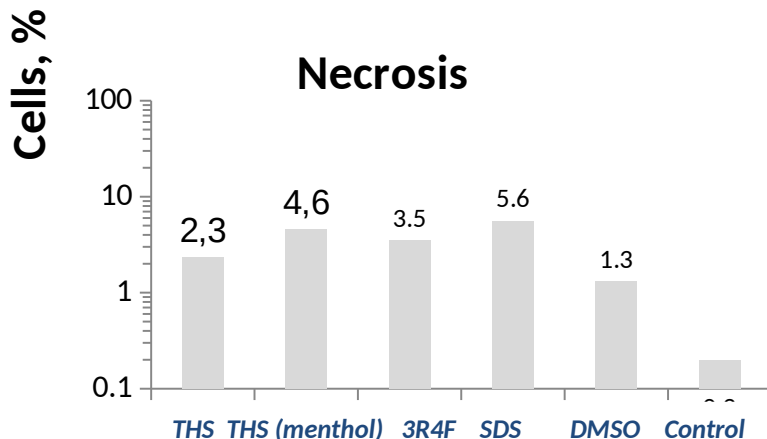
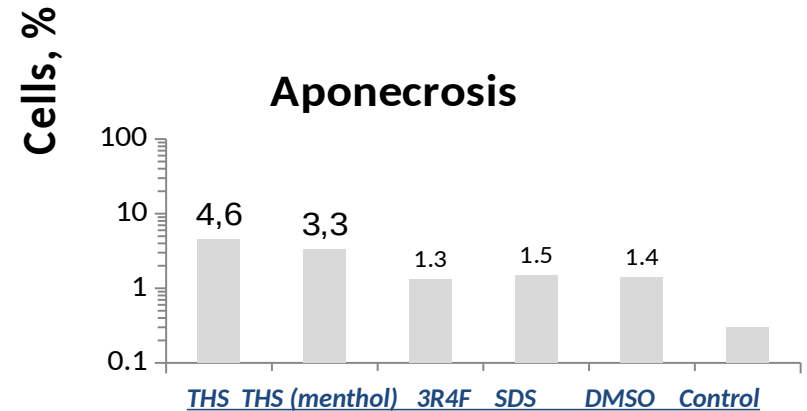
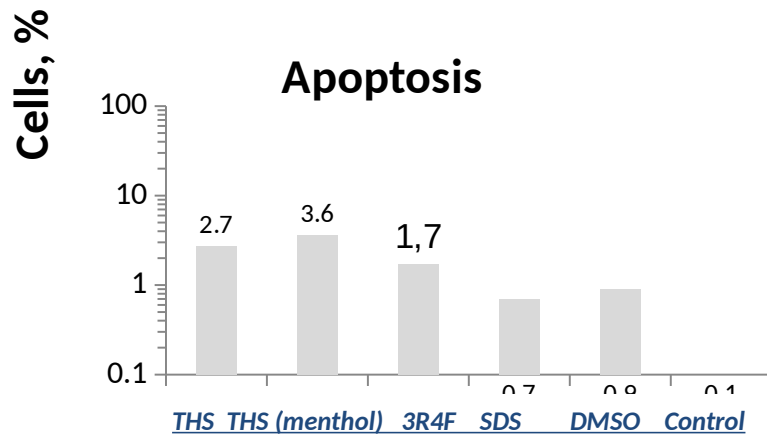
The values of the relative cytotoxicity of the THS aerosol fractions (MTS test) calculated **per unit mass of nicotine** (ml/mg nicotine) were **94.58% lower (tobacco taste) and 94.19% (menthol taste)**, compared to the relative cytotoxicity of 3R4F reference cigarette tobacco smoke fraction (taken as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



Apoptotic and necrotic cells identification

Preclinical studies

- 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**



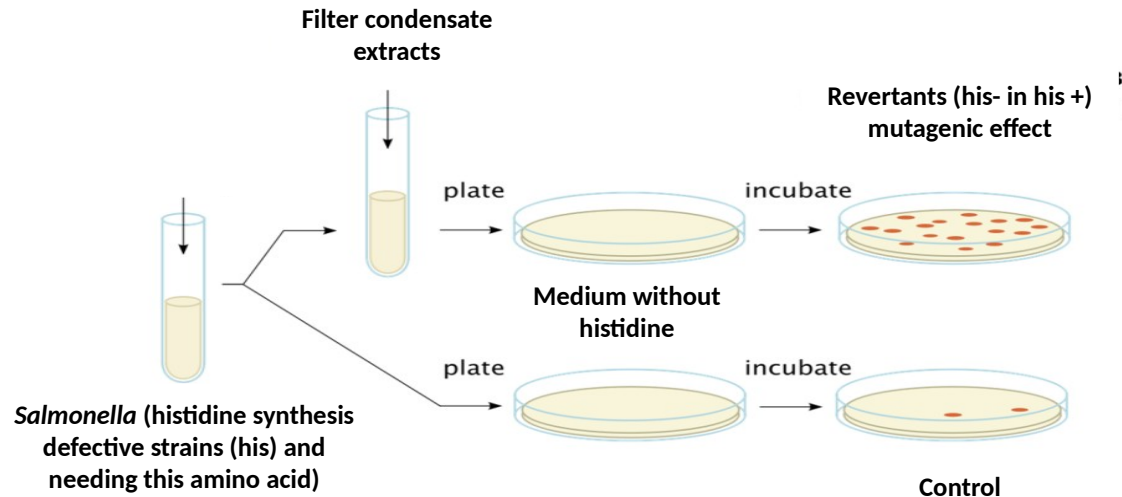
The results obtained confirm the data of the NRU-test, which demonstrated cell viability in all the studied samples in the range of 88.5-93.5% at IC10 concentrations.

Preclinical studies

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



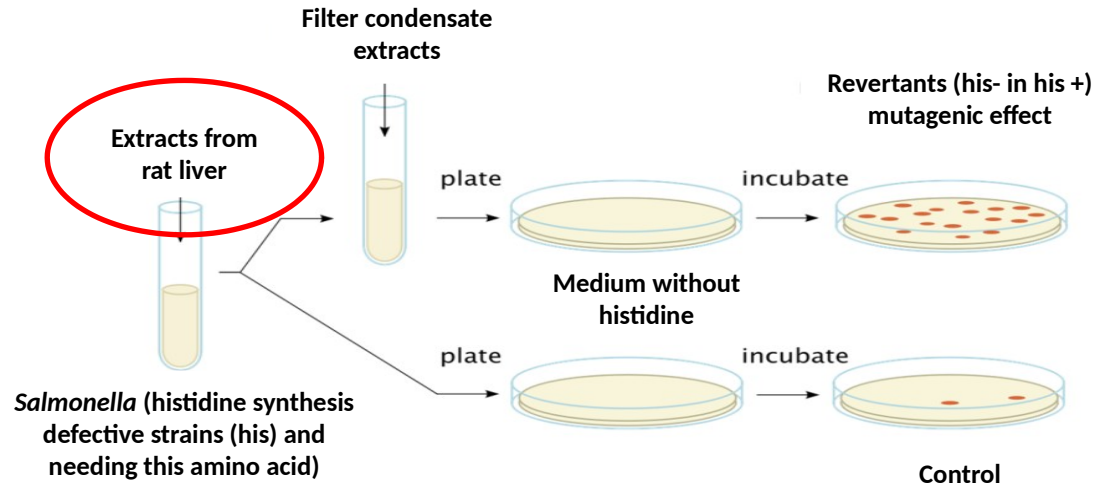
Ames test (without metabolic activation)

- **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.

Preclinical studies

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



Ames test (with metabolic activation)

THS condensate

- 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.

3R4F cigarettes 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

Preclinical studies

Evaluation of genotoxicity - a method for assessing the induction of SOS DNA repair system on bacteria (SOS chromotest on bacteria, or Ames test)

- SOS chromotest has high specificity due to the absence of false-positive results.
- Moreover, SOS chromotest can supplement the Ames test for mutagenesis to detect carcinogens that show mutagenicity only when activated in a liquid medium; detection of toxic compounds for bacteria, which are identified in the Ames test as mutagens only on the *S. typhimurium* TA 102 strain, as well as for the determination of antimetabolite inhibitors of DNA synthesis.

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)

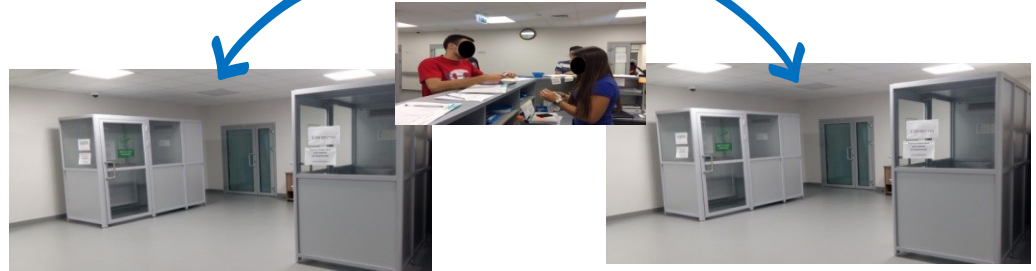


5 days

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), S-phenyl mercapturic (SPMA), 3-hydroxy-1-methylpropyl mercapturic acid (HMPMA), 4-(methylnitrozamine)-1-(3-pyridyl)-1-butanol (NNAL), N-nitrosornicotine (NNN), nicotine, cotinine, nornicotine, trans-3'-hydroxycotinine, nicotine and cotinine glucuronides, anabazine

Blood:

Carboxyhaemoglobine, cotinine, trans-3-hydroxycotinine

2. Safety assessment

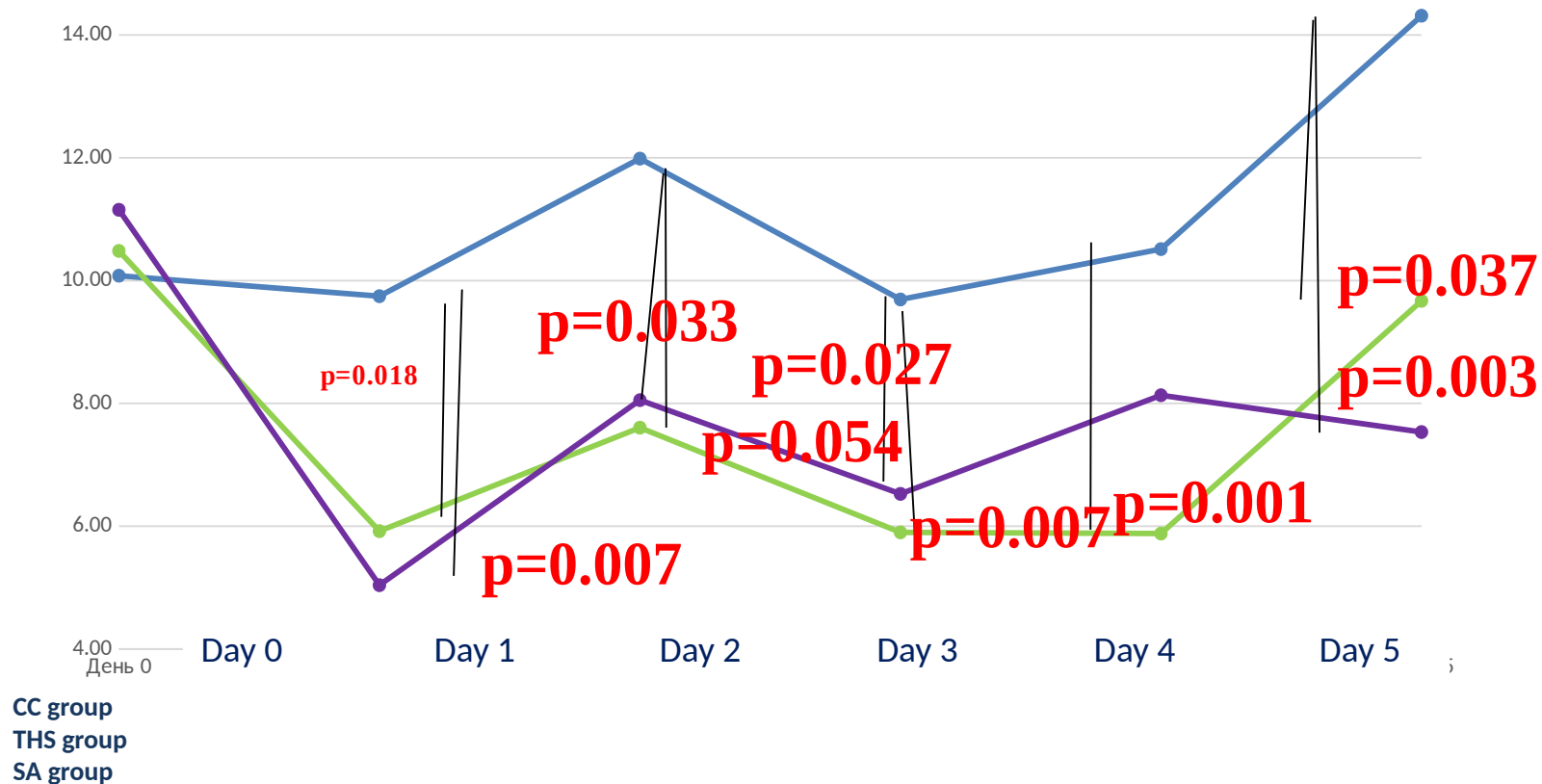
Total blood count, urinalysis, blood biochemistry, ECG, spirometry, general physician assessment incl. vital signs evaluation (blood pressure, heart rate, body T), AE/SAE monitoring

3. Questionnaires

+ VAS for cough assessment, Likert scale for cough severity, frequency and amount of sputum



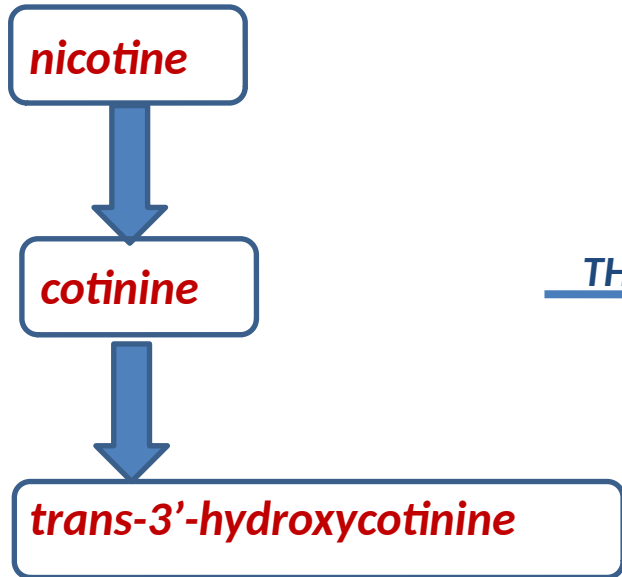
Assessment of difference between CoHb concentration in different groups



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

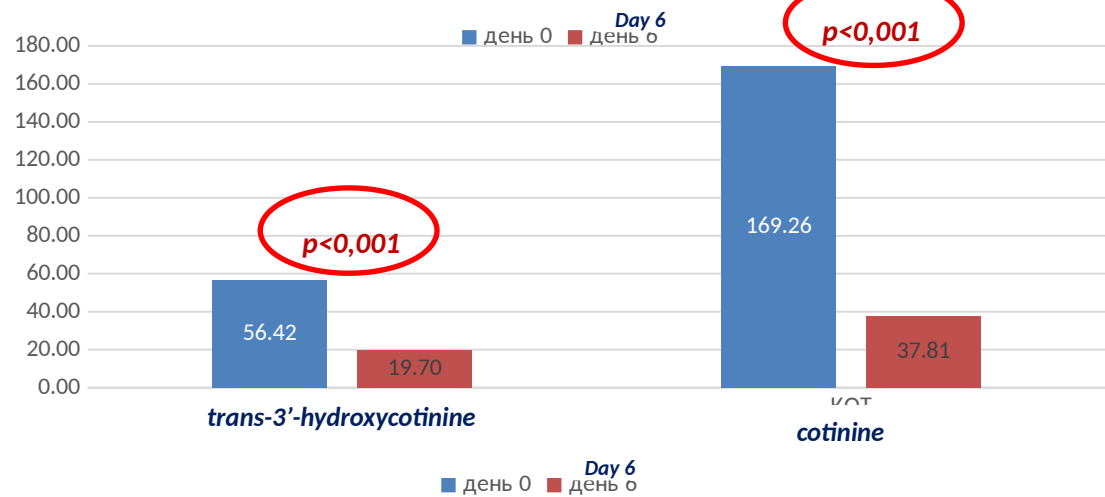
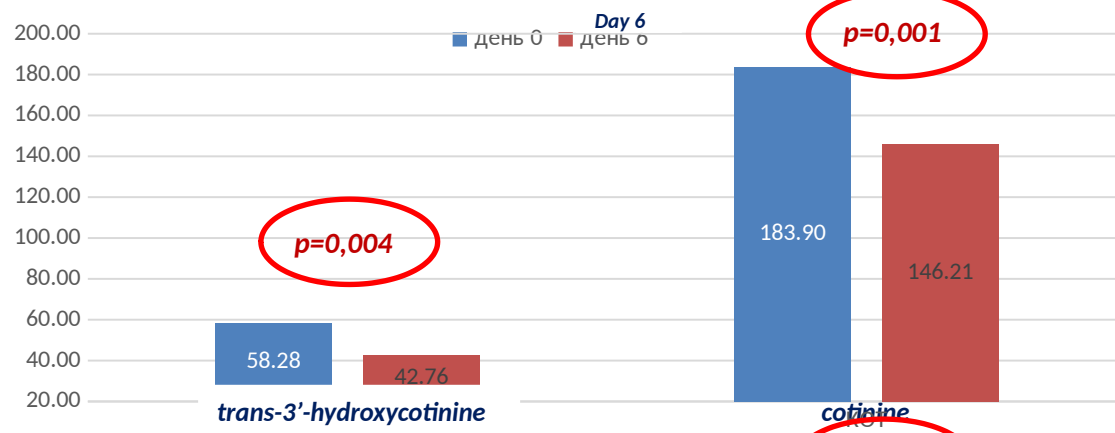
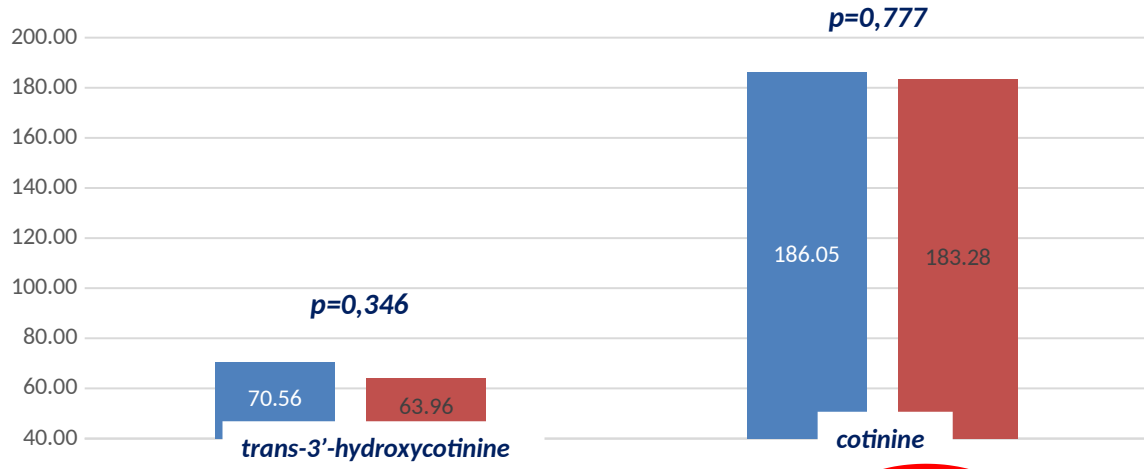
Plasma nicotine metabolites: Day 0, Day 6 (CC, THS, SC groups)

Conventional cigarettes (CC)



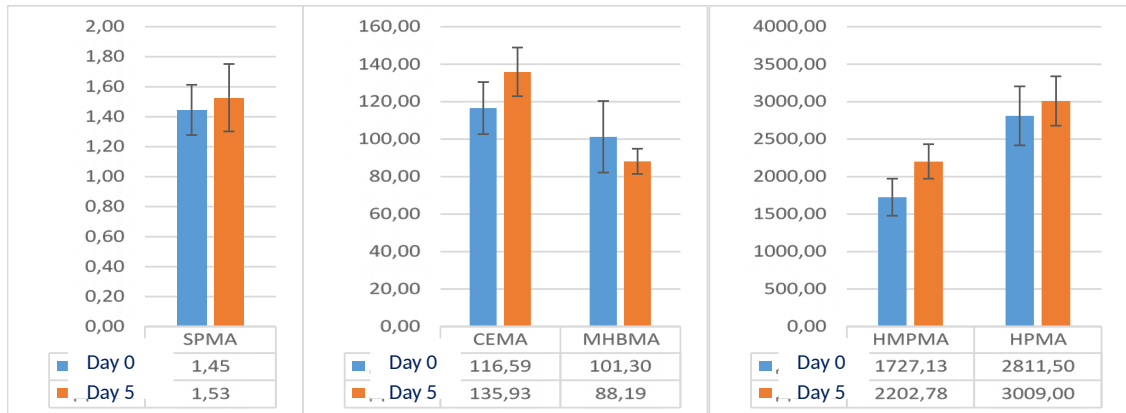
THS

Smoking abstinence



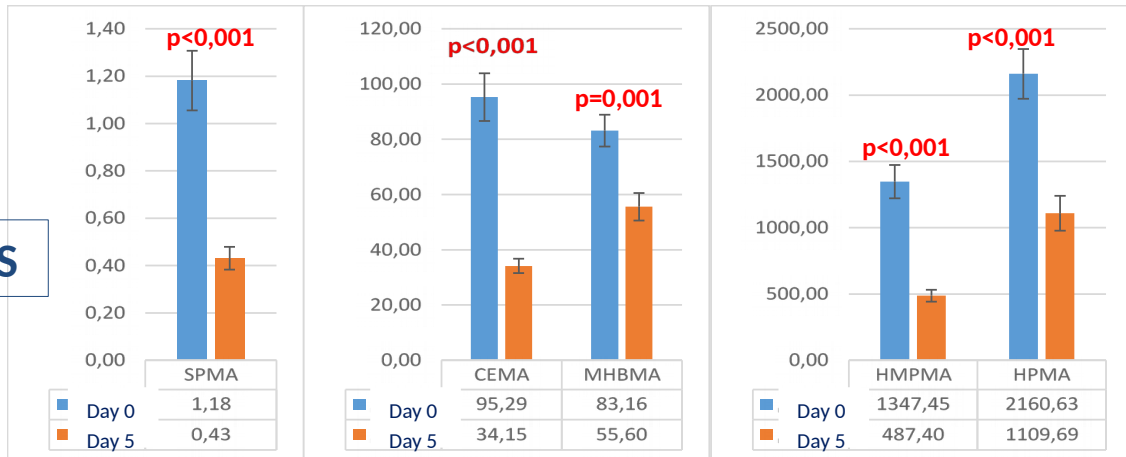
Mercapturic acids

Conventional cigarettes

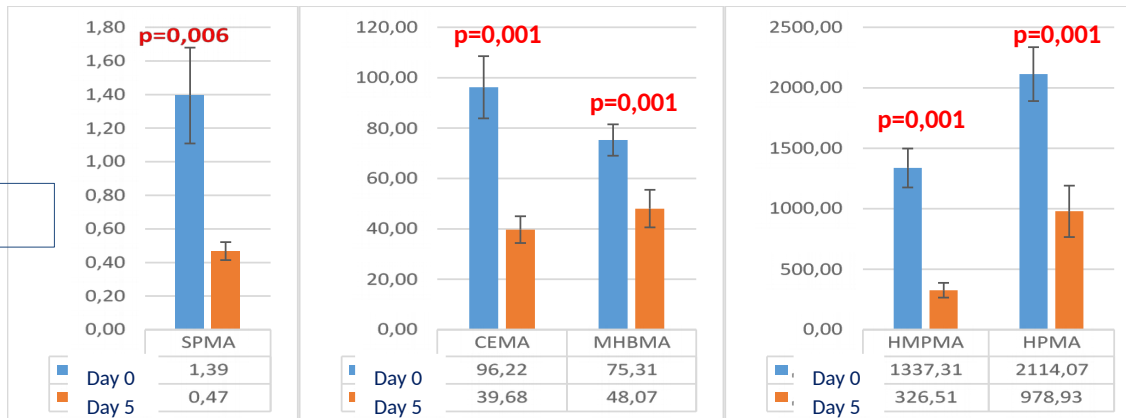


Mercapturic acid	Substance-precursor
S-2-cyanoethyl-mercapturic acid (CEMA)	Acrolein
3-hydroxy-1-methylpropyl-mercapturic acid (HMPMA)	Crotonaldehyde
S-(3-hydroxypropyl) mercapturic acid (HPMA)	Acrolein
Monohydroxybutenylmercapturic acid (MHBMA)	1,3-butadien
S-phenylmercapturic acid (SPMA)	Benzole

THS



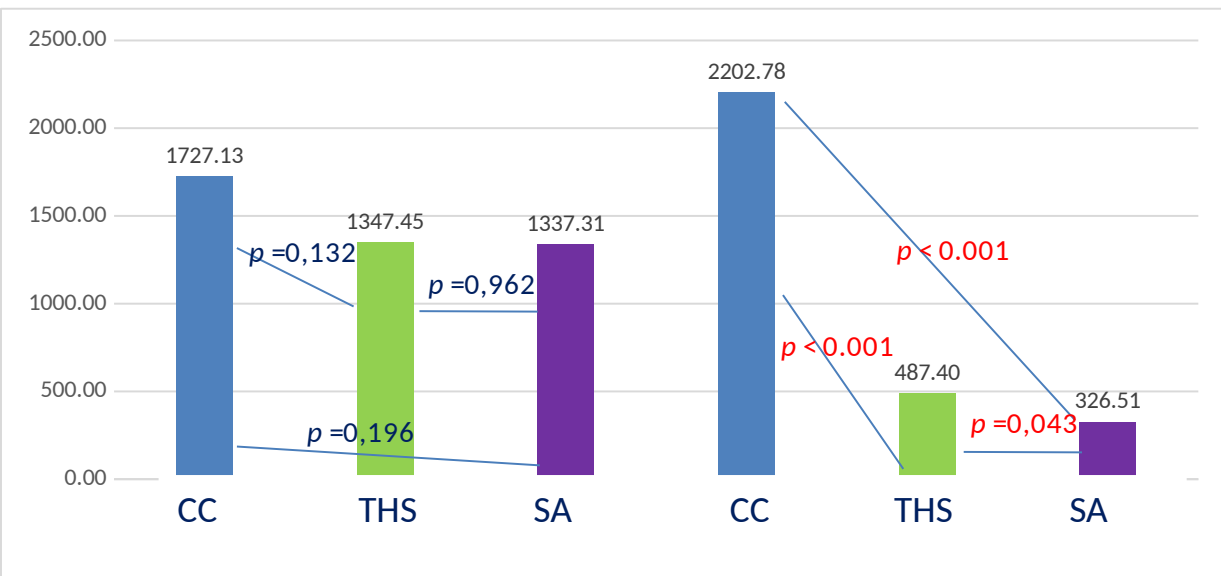
Smoking Abstinence



Mercapturic acids content in CC/THS/SA groups

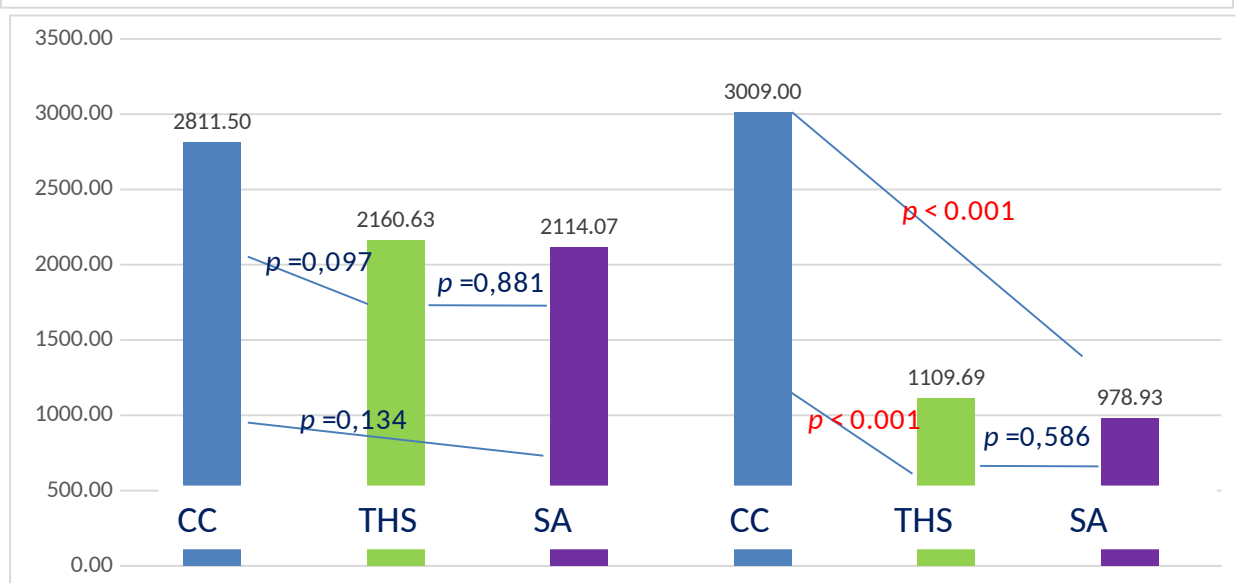
HMPMA (Day 0 and Day 5)

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:
 - LC-MS METHOD DEVELOPMENT FOR SIMULTANEOUS DETERMINATION OF TRANS-3'-HYDROXYCOTININE AND THREE MERCAPTURIC ACIDS IN URINE. Lopukhov L.V., Laikov A.V., Romanova V.A., Gatina D.Z., Lopukhov V.L., Abdulkhakov S.R., Salafutdinov I.I., Grigoryeva T.V., Zaitseva T.A., Medvedeva S.N., Gnuchikh E.V. BioNanoScience. 2018. T. 8. № 3. C. 924-929.
 - SCREENING ASSESSMENT OF RENAL FUNCTION STATUS IN HEALTHY SMOKING VOLUNTEERS. Abdulkhakov, S.R., Arkhipov, E.V., Faizullin, R.I. et al. BioNanoSci. (2019) 9: 510. <https://doi.org/10.1007/s12668-019-0602-3>

Thank you for your attention

