Toxicity of inhaled Nicotine and Pyruvic Acid Aerosols (separately and combined) in Sprague-Dawley Rats in a 28-day OECD 412 Inhalation Study

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Introduction

Cigarette smoking can cause severe diseases such as cardiovascular disease, lung cancer, and chronic obstructive pulmonary disease. Health consequences are caused primarily by smoke constituents other than nicotine. Therefore, provision of nicotine during nicotine replacement therapies (NRTs) or by using electronic nicotine-delivery systems ("e-cigarettes") may be considered a less harmful way to facilitate smoking cessation or to reduce harm by smoking fewer tobacco cigarettes (Benowitz and Goniewicz, 2013, Franck et al., 2014). Nicotine administration *via* inhalation is an attractive approach for a NRT because it can deliver nicotine rapidly to the brain, thus better mimicking the effects of its discontinuous/episodic exposure achieved if smoking cigarettes. Aerosols of nicotine pyruvate have been proposed as improved forms of NRT for those who are not willing to stop smoking. Such aerosols have favorable pharmacodynamic properties and subjective responses (Rose et al., 2010). Along with pharmacodynamic parameters and sensory evaluations/subjective responses, limited tolerability testing and safety assessment for acute exposure has been reported by Rose et al. ((Rose et al., 2010) but the toxicity of repeated or extended use of aerosols of nicotine pyruvate has not been investigated.

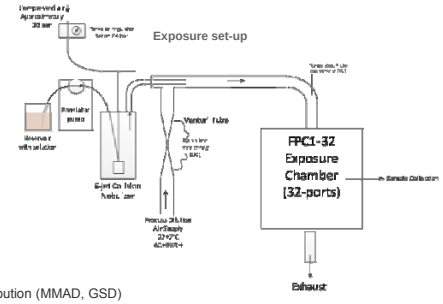
Here we report on a comprehensive repeated-dose inhalation toxicity study on aerosols of nicotine and pyruvic acid aerosols (separately and combined) in Sprague-Dawley rats in a 28-day OECD 412 (OECD TG 412, OECD, 2009) with focus on systemic toxicity and respiratory endpoints. In addition, molecular endpoints (transcriptomics and lipidomics) were assessed in an separate cohort of rats providing mechanistic insights on the classical toxicological endpoints (published elsewhere).

Exposure levels of nicotine were maximally 13.6 mg/kg – the calculated Human Equivalent Dose = 132 mg nicotine for 60-kg adult (equivalent to ≈130 conventional cigarettes smoked per day)

Study design and end points

- Sprague Dawley rats, 70 males and 70 females; 10 males and 10 females per group
- Nose-only exposure for 6hours/day, 5 days per week in a 28-day inhalation study
- Aerosols generated using Collison Nebulizers (BGI, Waltham, MA, USA)
- Nebulizer solutions are prepared in PBS and adjusted to physiological pH
- Experimental groups:

Groups		Nicotine (µg/l)	Pyruvic Acid (µg/l)	Na-pyruvate (µg/l)	Molarity (all) (µM)
Sham (fresh air)		0	0	0	0
PBS		0	0	0	0
Nicotine		50	0	0	0.308
Na-pyruvate		0	0	33.9	0.308
Nicotine + pyruvic acid LOW		18	9.8	0	0.111
Nicotine + pyruvic acid MED		25	13.6	0	0.154
Nicotine + pyruvic acid HIGH		50	27.1	0	0.308



- Test atmosphere monitoring: nicotine, pyruvate, T, RH, droplet size distribution (MMAD, GSD)
- Bio-monitoring: respiratory physiology, urinary nicotine metabolites (24 hour urine)
- Systemic end points during study: clinical observations, body weight, food consumption
- Systemic end points at the end of the study: hematology, clinical chemistry, pulmonary inflammation (cells in broncho-alveolar lavage fluid, BALF)
- Histopathology of the respiratory tract, non-respiratory tract organs

Summary and Conclusion

- The nebulizer approach was successful for generating nicotine-containing aerosols with particle (droplet) sizes similar to those from cigarette smoke in experimental setups
- All aerosols were inhaled efficiently and were well tolerated by the rats in the present 28-day inhalation study
- Systemic effects indicated low levels of toxicity for nicotine-containing aerosols in the presence or absence of pyruvic acid. Changes included slight hematological changes and induction of liver enzymes
- Mild irritation of the larynx was observed in the nicotine-containing aerosols, while the control groups revealed very mild changes probably due to particle impaction
- Exposure to nicotine-aerosols cause an increased vacuolation of hepatic tissue, vacuoles contained glycogen.

- In conclusion, these results suggest that exposure to high concentrations of nicotine (alone or in combination with pyruvic acid) did not result in substantial toxicity and further suggest an alteration in metabolism, as seen in the liver

Results

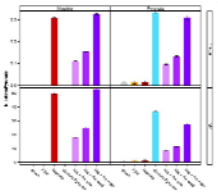
Test atmosphere characterization

Target concentrations were met

Particle (droplet) size: equally respirable aerosols:

- MMAD: 1.4 - 2.0 µm, GSD: 1.7 – 2.2

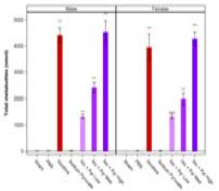
Concentrations of Nicotine and Pyruvate in the test atmospheres. Means ±SD



Bio-monitoring

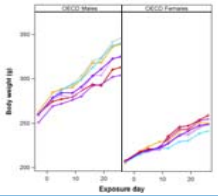
Nicotine form nebulized aerosol is taken up as evidenced by the amount of 5 nicotine metabolites recovered in 24-h urine

Nicotine metabolites in 24-hour urine. Means ± SEM; ***p ≤ 0.001



Systemic effects

Reduced **body weight** gain in nicotine-treated male rats



Systemic effects (cont'd)

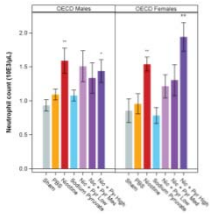
Organ weight (relative to body weight): nicotine concentration-dependent changes in

- Liver – higher than controls
- Spleen – lower than controls

Hematology: nicotine concentration-dependent changes in:

- Mean erythrocyte volume (females only) – higher
- Neutrophils – higher
- Lymphocytes - lower

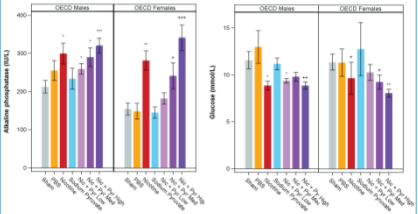
Neutrophils in blood. Means ±SEM; *p≤0.05, r**p ≤ 0.01, ***p ≤ 0.001 elative to sham



Clinical Chemistry: nicotine concentration-dependent changes in:

- Alkaline phosphatase and alanine aminotransferase - higher
- Total cholesterol and glucose - lower

Blood alkaline phosphatase activity and glucose concentration. Means ±SEM; *p≤0.05, r**p ≤ 0.01, ***p ≤ 0.001 relative to control

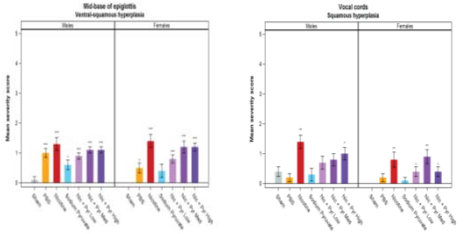


Histopathology – Respiratory tract

Minimal to mild epithelia changes in nicotine-exposed rats

- Nose: low level hyperplasia of the respiratory epithelium, nose level 1
- Larynx: minimal to mild hyperplasia of squamous epithelium, minimal squamous metaplasia, increased epithelia thickness at vocal cords
- Lung; no noteworthy findings, no obvious lung inflammation

Laryngeal histopathological changes. Means ±SEM; *p≤0.05, r**p ≤ 0.01, ***p ≤ 0.001 relative to control

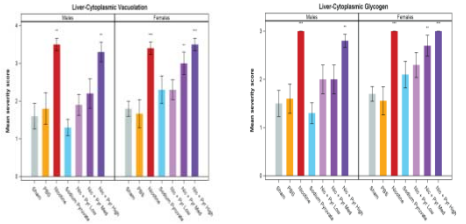


Histopathology – Non- respiratory tract organs

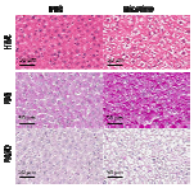
Liver effects: nicotine concentration-dependent

- Cytoplasmic vacuolation,
- Vacuoles contains PAS-positive material, likely to be consist of glycogen granules

Liver vacuolation. Means ±SEM; *p≤0.05, r**p ≤ 0.01, ***p ≤ 0.001 relative to control



Liver histopathology, vacuolation of hepatocytes; glycogen content confirmed by staining with PAS and PAS-diastase



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