Comprehensive Evaluation of the Ploom® System's Subchronic Inhalation Toxicity Potential in Rats

DJ Smart[1], TR Moir-Savitz[2], AM Easley[2], J Zhang[3], TB Langston[3], SJ Harbo[1]

[1] JT International SA, Rue Kazem-Radjavi 8, CH-1202 Geneva, Switzerland; [2] AmplifyBio, 1425 NE Plain City-Georgesville Rd, West Jefferson, OH 43162, United States;

[3] Altria Client Services LLC, Richmond, VA, United States

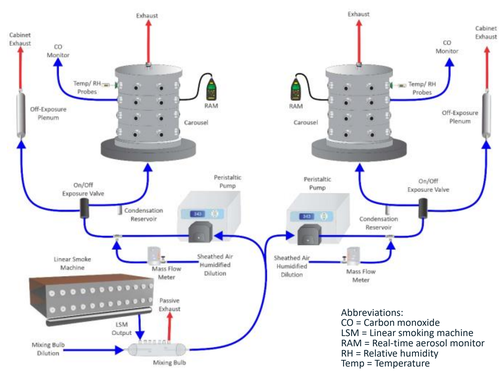
INTRODUCTION

- The Ploom® system—a heated tobacco product (HTP) consisting of heated tobacco sticks (HTS) and a battery-powered tobacco heating device—has undergone comprehensive testing to assess its reduced-risk potential versus combustible cigarettes.
- As part of the nonclinical toxicity evaluation, two inhalation toxicology studies were conducted: an initial 14-day range-finding study to define the exposure conditions for a 21 CFR Part 58 (Good Laboratory Practice)-compliant 90-day subchronic study. Note, cigarette smoke was not assessed concurrently because its effects are well-documented.

METHODS

- In the 14-day range-finding study, male (M) and female (F) Sprague Dawley rats were exposed nose-only to Ploom® system-generated aerosol from tobacco (R8) or menthol (MX3) HTS varieties with a target nicotine concentration of 70 µg/L for either 1, 2, 4, or 6 h per day or filtered air (FA) for 6 h per day for 14 consecutive days (**Figure 1**). Multiple in-life endpoints were assessed, including clinical observations, body weight/food consumption measurements, respiratory parameters, clinical pathology, and blood exposure markers (nicotine, cotinine, and carboxyhemoglobin [COHb]). Upon termination on day 15, bronchoalveolar lavages (BAL) were performed as well as organ weighing and tissue processing for histopathological evaluation.
- In the 90-day study, target nicotine concentrations were 15, 23, and 50 µg/L based on the range-finding data, and exposures occurred exclusively for 6 h per day for 90 consecutive days, with a 41-day recovery cohort. Endpoints were predicated on OECD Test Guideline 413 and included those from the 14-day study and toxicokinetics, urinalysis, and a panel of genotoxicity parameters (micronucleus [MN], Pig-a, comet assays). In both inhalation studies, animals exposed to the aerosols were compared to the FA controls.
- Aerosol particle size parameters, namely mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD), were derived using a cascade impactor and probit analysis.

Figure 1. Schematic of R8 and MX3 aerosol exposure system design.



Abbreviations:
CO = Carbon monoxide
LSM = Linear smoking machine
RAM = Real-time aerosol monitor
RH = Relative humidity
Temp = Temperature

RESULTS: 14-d RANGE-FINDER

- Aerosol nicotine levels were confirmed at 74.5 µg/L (R8) and 72.9 µg/L (MX3), while MMAD and GSD were 0.8-0.9 µm and 1.5, respectively, for both aerosols.
- No deaths and low, transient clinical observations, such as partially closed eyes, rough coat and tremors (4/6-h exposure groups) were recorded.
- Minimal body weight decrease (≤ -11%) relative to FA and no statistically significant changes in respiratory parameters (rate, tidal and minute volumes) were observed.
- No R8/MX3 aerosol-related findings in hematology, clinical chemistry, BAL fluid chemistry or cytology, or COHb levels were recorded.
- Evidence of a stress response, demonstrated by changes in reticulocytes (↑-F), neutrophils (↑-F), lymphocytes (↓-F), alanine aminotransferase (↑-M) and alkaline phosphatase (↑-F) and specific organ weights (**Figure 2**), as well as squamous metaplasia of the larynx (epiglottis) with and without hyperkeratosis after ≥1-h of exposure were observed.

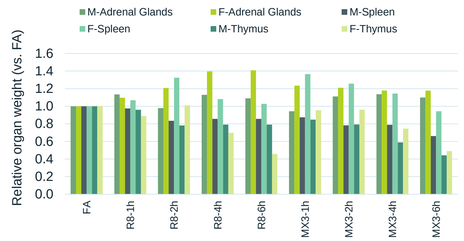


Figure 2. Stress-related, exposure duration-dependent changes in weights of adrenal glands, spleen and thymus of M and F rats.

RESULTS: 90-d SUBCHRONIC EXPOSURE

- Aerosol nicotine concentrations were confirmed at 13.9, 21.6, 47.6 µg/L (R8) and 15.6, 23.2, 48.3 µg/L (MX3). MMAD and GSD were 0.90-0.94 µm and 1.46-1.48, respectively, for all aerosols.
- Four deaths (M-R8-L, M-R8-H, F-R8-H, F-MX3-H) and low, transient clinical observations such as partially closed eyes, rough coat and tremors were observed.
- Exposed males gained weight across the 90 days, however, at a slower rate than FA controls (**Figure 3**). In contrast, exposed females gained weight like FA controls, while all recovery rats gained weight at a similar rate.

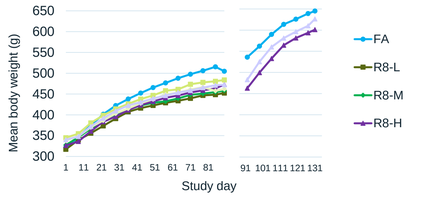


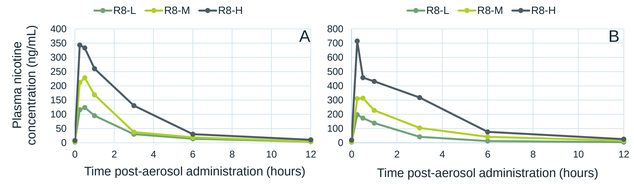
Figure 3. Male body weight evolution across the 90 days of exposure and through the recovery period.

- Stress-related changes in some hematology and clinical chemistry parameters, as well as organ weights, were again observed. However, BAL fluid chemistry and cytology, respiratory parameters and COHb levels were generally comparable to FA controls.
- R8/MX3 aerosol was negative in MN and Pig-a assays (peripheral blood) and negative in comet assay (male and female nasal, liver and lung tissue), except for the liver in females where the results were equivocal.

RESULTS: 90-d CONTINUED

- Plasma nicotine levels were generally exposure-related in rats exposed to aerosols at day 6, and females demonstrated higher C_{max} and AUC (**Figure 4**).

Figure 4. Toxicokinetic profile of nicotine in males (A) and females (B) exposed to R8 aerosol.



- Squamous metaplasia of moderate severity (w/ and w/o hyperkeratosis) at the epiglottis were observed at mid to high concentrations (**Figure 5**) but resolved after the recovery period. No other organs exhibited aerosol-related histopathological changes.

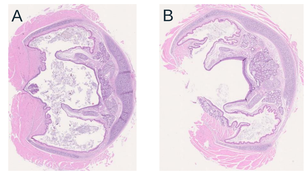


Figure 5. Representative images of FA- (A) and R8 aerosol-exposed larynxes (B).

SUMMARY & CONCLUSION

- In the 14-d range-finder, respirable R8 and MX3 aerosols containing nicotine concentrations of 74.5 and 72.9 µg/L for 1-6 h/day were well tolerated.
- However, a generalized stress response was observed, as well as the presence of squamous metaplasia at the epiglottis.
- In the 90-d study, respirable aerosols containing nicotine (R8: 13.9, 21.6, 47.6 µg/L and MX3: 15.6, 23.2, 48.3 µg/L) for 6 h/day induced a similar stress response and laryngeal effects in a concentration-dependent manner, however, they were considered nonadverse and not indicative of significant human risk (Everds *et al*., 2013 and Osimitz *et al*., 2007)
- Micronucleus, Pig-a and comet assay (nose, lung in males and females; liver in males) were negative, while the biological relevance of the female liver comet assay findings is unclear.
- Overall, these findings demonstrate markedly reduced toxic potential versus the effects induced by cigarette smoke as demonstrated in the literature, e.g. highly suppressed body weight gain, elevated COHb levels, respiratory tract lesions and lung inflammation, while similar to other HTPs (Wong *et al*., 2016 and Oviedo *et al*., 2016).

REFERENCES & ACKNOWLEDGEMENTS

- Everds *et al*., 2013. Toxicologic Pathology, 41(4), 560-614; Osimitz *et al*., 2007. Toxicology & Applied Pharmacology, 225(3), 229-237. Oviedo *et al*., 2016. Regulatory Toxicology & Pharmacology, 81, Sup 2: S93-S122; Wong *et al*., 2016. Regulatory Toxicology & Pharmacology, 81, Sup 2: S59-S81.
- The authors thank K.M. Lee (ex-Altria) for contributions to study design.

Visit www.jt-science.com

JT SCIENCE