A structure-based grouping approach for evaluating the toxicity of e-cigarette flavor ingredients: A 5-week inhalation study in A/J mice

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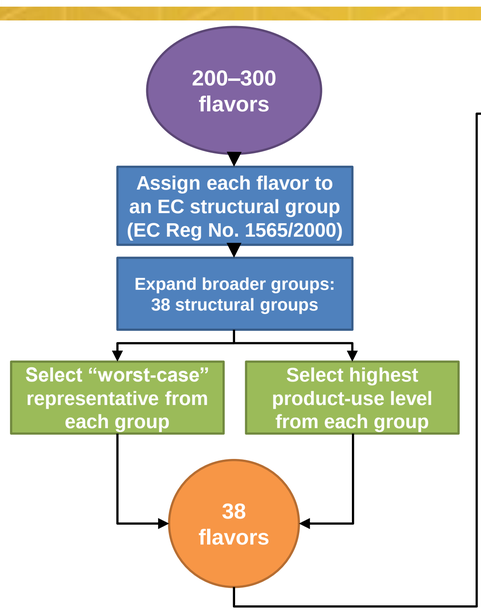
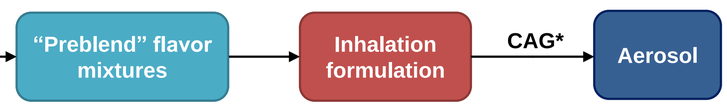
Introduction and Objective

Most flavors are generally recognized as safe (GRAS) for use in foods. However, limited toxicological information is available for evaluating the potential hazard of flavors delivered via inhalation. Because it is not feasible to test the toxicity of each compound or many combinations of formulation, we sought to evaluate flavors by using a structure-based grouping approach in a short-term inhalation study.

Structurally related flavor compounds were clustered into groups, and 38 representatives were selected—one from each structural group (Flavor Group Representative [FGR])—on the basis of known and *in silico*-predicted toxicological information. The selected FGRs were combined to create a full "toolbox" flavor mixture. This mixture was then used in a dose-range-finding study in A/J mice, with emphasis on subacute toxicity and respiratory tract irritation and inflammation to select appropriate concentrations of flavor compounds from the "toolbox" to be used in a future chronic inhalation study.

Test Item Formulation and Study Design

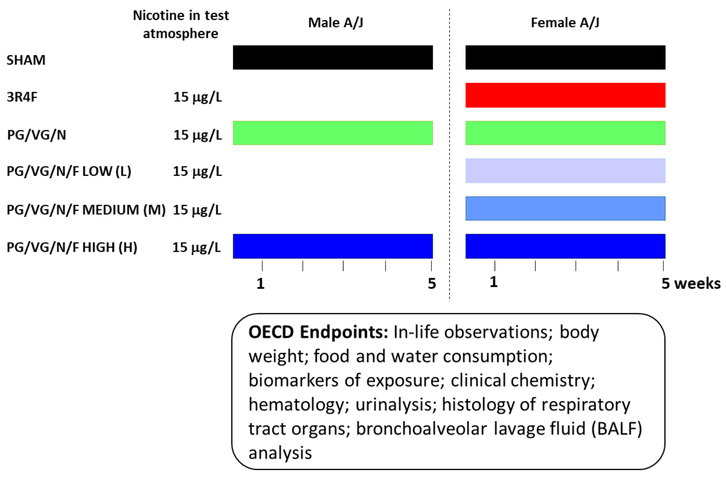




Component (g/100g)

Inhalation formulation	PG	VG	Nicotine	Water	Ethanol	Flavor
PG/VG/N	71.7	17.9	2.0	5.8	2.5	0.0
PG/VG/N/F High (H)	56.9	14.2	2.0	5.8	2.5	18.6
PG/VG/N/F Medium (M)	64.3	16.1	2.0	5.8	2.5	9.3
PG/VG/N/F Low (L)	68.0	17.0	2.0	5.8	2.5	4.6

Figure 1. *Schematic overview of the approach for flavor selection and overview of the composition of the different inhalation formulations.*
**CAG: capillary aerosol generator (Howell and Sweeney, 1998; Werley et al., 2016); PG: propylene glycol; VG: vegetable glycerol; N: nicotine.*



OECD Endpoints: In-life observations; body weight; food and water consumption; biomarkers of exposure; clinical chemistry; hematology; urinalysis; histology of respiratory tract organs; bronchoalveolar lavage fluid (BALF) analysis

A/J mice (Jackson Laboratory, Bar Harbor, ME, USA) were whole-body exposed to either fresh air (sham), aerosol from propylene glycol (PG) and vegetable glycerol (VG) with nicotine (N), aerosol from PG, VG, and N with flavors (F) at low, medium, and high concentrations, or mainstream smoke from the 3R4F reference cigarette (CS) for 6 hours per day, for 5 days per week, for 5 weeks.

Care and use of the mice was in accordance with the National Advisory Committee for Laboratory Animal Research Guidelines (2004). All animal experiments were approved by the Institutional Animal Care and Use Committee (P15055).

Figure 2. *Schematic overview of study design and endpoints.*

Aerosol Exposure and Uptake

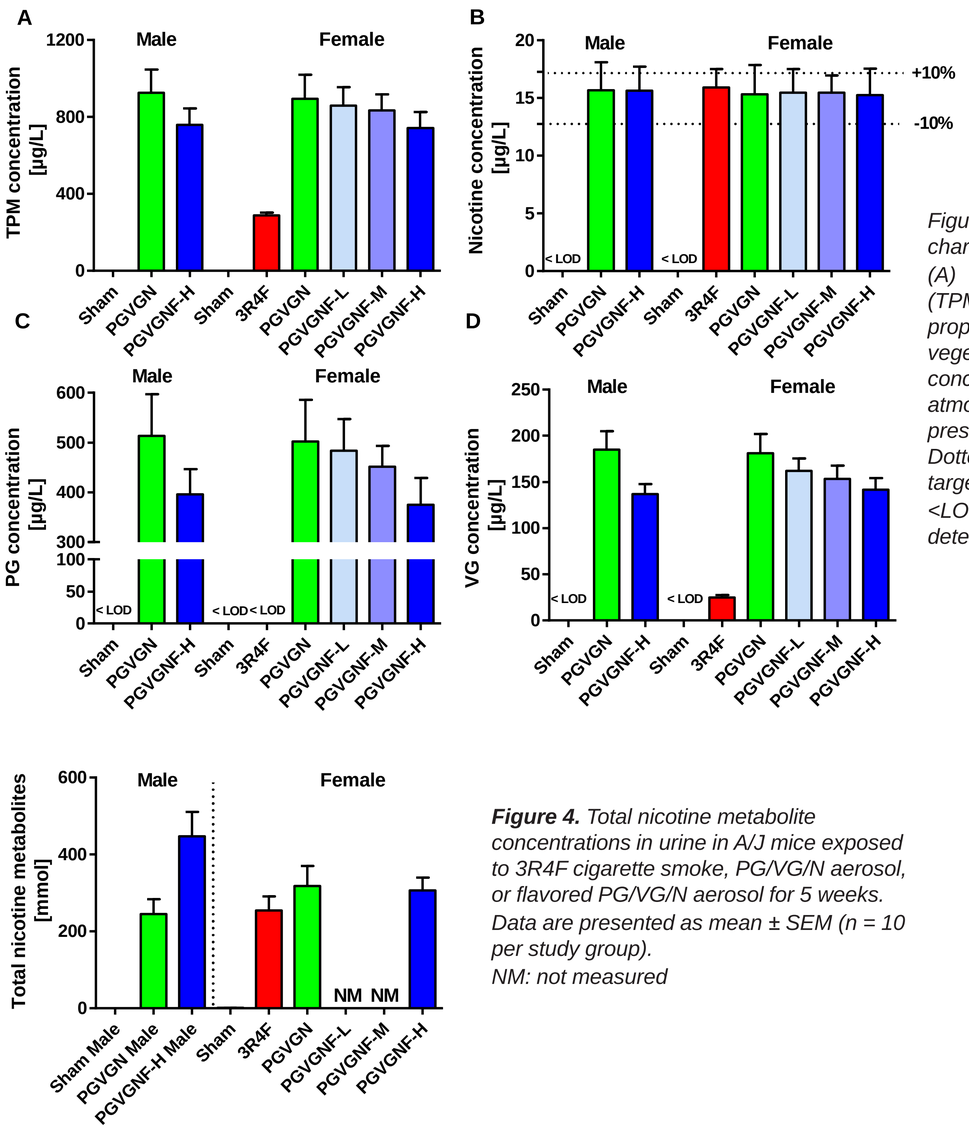


Figure 3. Test atmosphere characterization.
(A) Total particulate matter (TPM), (B) nicotine, (C) propylene glycol (PG), and (D) vegetable glycerol (VG) concentrations in the test atmosphere. Data are presented as mean ± SD. Dotted lines indicate ±10% of target nicotine concentration.
<LOD: Below the limit of detection

Figure 4. *Total nicotine metabolite concentrations in urine in A/J mice exposed to 3R4F cigarette smoke, PG/VG/N aerosol, or flavored PG/VG/N aerosol for 5 weeks. Data are presented as mean ± SEM (n = 10 per study group).*
NM: not measured

Results

1. Body weight

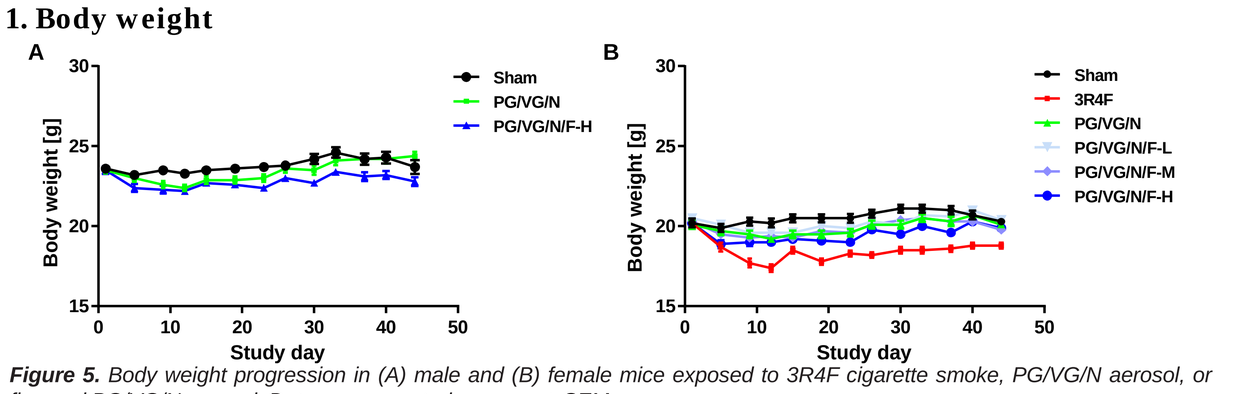


Figure 5. *Body weight progression in (A) male and (B) female mice exposed to 3R4F cigarette smoke, PG/VG/N aerosol, or flavored PG/VG/N aerosol. Data are presented as mean ± SEM.*

2. Histopathology of the nose and larynx (prominent findings only)

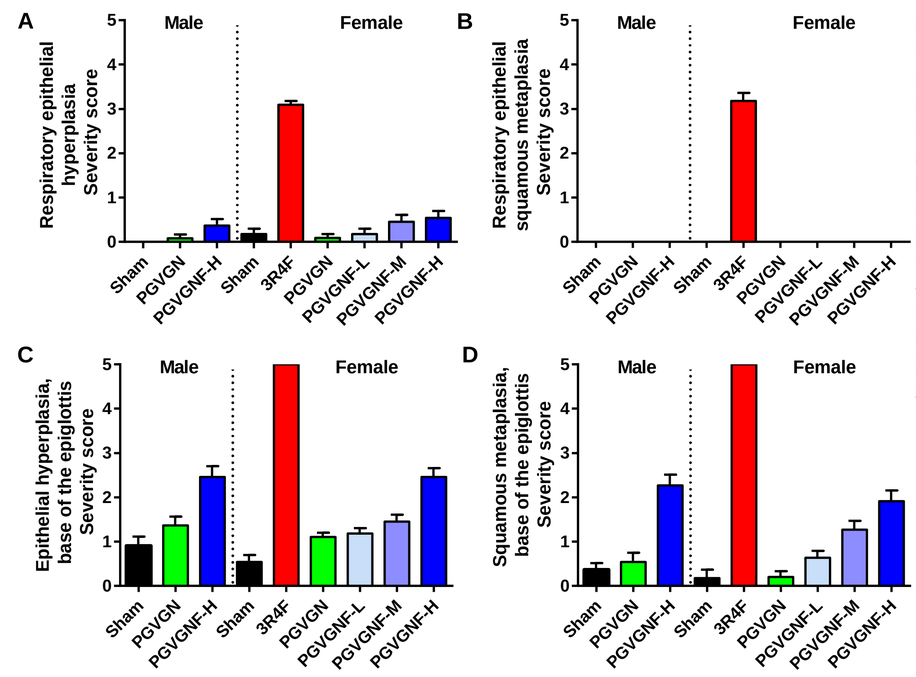


Figure 6. *Histopathological findings in the nose and larynx. (A) Respiratory epithelial hyperplasia and (B) respiratory epithelial squamous metaplasia in the nose and (C) epithelial hyperplasia and (D) squamous metaplasia at the base of the epiglottis in the larynx in mice exposed to 3R4F cigarette smoke, PG/VG/N aerosol, or flavored PG/VG/N aerosol for 5 weeks. Data are presented as mean ± SEM (n = 10 per study group).*

3. Lung inflammation

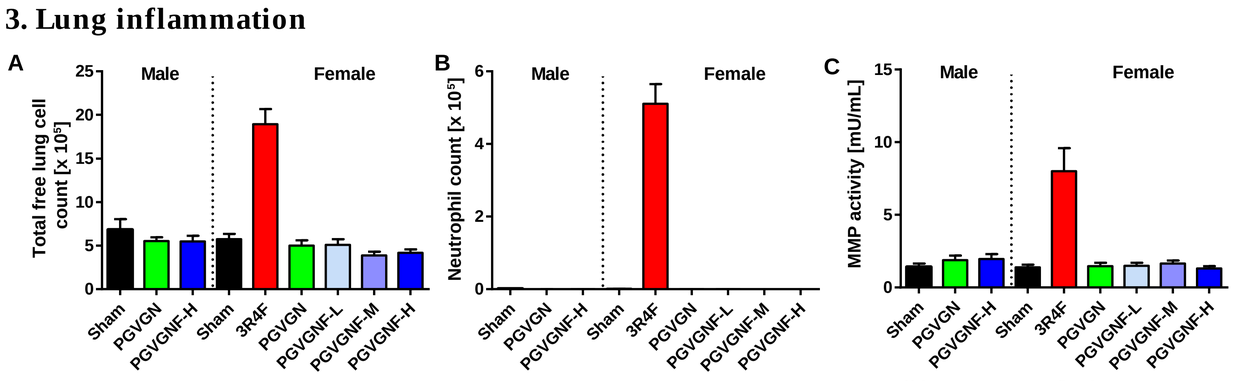


Figure 7. *(A) Total free lung cell and (B) neutrophil counts and (C) matrix metalloproteinase (MMP) activity in BALF in mice exposed to 3R4F cigarette smoke, PG/VG/N aerosol, or flavored PG/VG/N aerosol for 5 weeks. Data are presented as mean ± SEM (n = 10 per study group).*

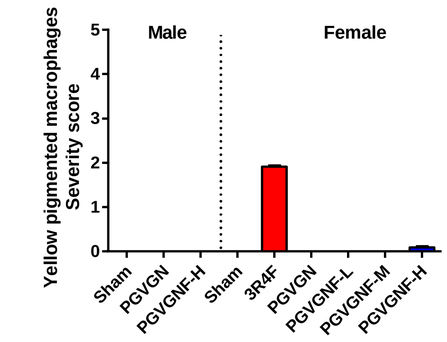
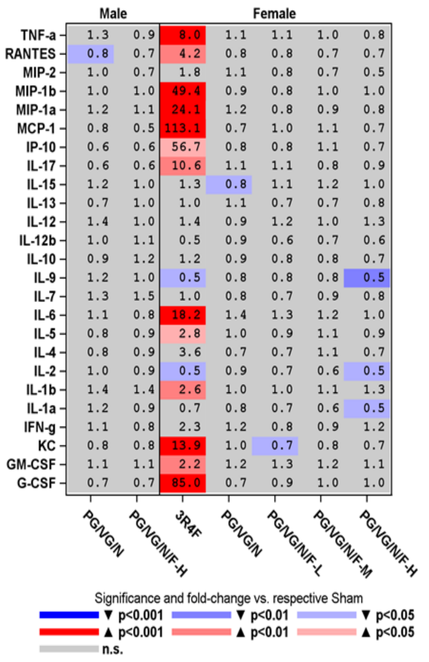


Figure 8. *(Left) Extent of lung infiltration by yellow pigmented macrophages expressed as severity score in the lungs; (Right) Fold changes in the levels of inflammatory mediators in BALF in A/J mice exposed to 3R4F cigarette smoke, PG/VG/N aerosol, or flavored PG/VG/N aerosol for 5 weeks relative to sham (n = 10 per study group).*



Summary

The aerosols were well tolerated by the mice, without signs of severe acute toxicity post-exposure. Exposure to the flavored aerosols, even at the highest flavor concentration, did not cause lung inflammation, as evidenced by the lack of immune cell infiltrates in bronchoalveolar lavage fluid and histopathological findings. In contrast, exposure to CS resulted in lung inflammation and also moderate to severe adaptive changes in nasal and laryngeal epithelia. Most of the latter changes were absent in mice exposed to flavored aerosol from e-vapor products, and, when present, they were significantly less severe than in the CS-exposed mice.

The tested flavor concentrations did not result in severe subacute toxicity or respiratory tract irritation/inflammation and were considered suitable for use in future chronic inhalation studies in A/J mice.

References

Howell, T.M., and Sweeney, W.R. (1998). Aerosol and a method and apparatus for generating an aerosol (Patent WO1997042993A3).

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