Comparison of E-Vapor Carrier Mixtures to Air Control and Cigarette Exposure Using 90-day Inhalation Studies in Rats

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Abstract

Propylene glycol (PG), glycerin and water are used as the carrier in e-vapor product formulations. OECD 413 compliant (+BALF analysis) 90-day nose-only inhalation studies were performed where Sprague Dawley rats were exposed (160 min/day, 7days/week) to aerosols generated from three different carrier mixtures of PG, glycerin and water. Biological responses were compared to the concurrent filtered air (Control). In addition, histopathological findings in respiratory tract were also compared to literature values from Sprague Dawley rats exposed to mainstream (2R4F) cigarette smoke. To acknowledge procedural differences in histopathology evaluations among studies, for each change noted, a "histopathology response" was determined by multiplying the severity of each change by its incidence. The most severe histopathology response from each change, from either sex, was added together to obtain an overall histopathology response for all changes noted in the nose, larynx and lung. The e-vapor and 2R4F groups were then compared at "Cumulative Daily Exposure" (CDE; exposure time multiplied by concentration [mg/L]). Compared to the control groups, the e-vapor carrier groups showed no differences in body mass, clinical chemistry, hematology, organ weights or urinalysis, and histopathological findings (incidence and severity). In the bronchoalveolar lavage (BAL) parameters measured after 13 weeks of exposure, e-vapor groups shows a slight but non-statistically significant increase in lactate dehydrogenase (LDH) in males but not females. In histopathology response, the e-vapor carrier groups displayed a flat dose-response in the nasal and laryngeal findings similar to the air control. At comparable CDE, the average nasal and laryngeal response to cigarette smoke was 4 and 8 times greater, respectively, than the average response from the e-vapor group. The lung histopathology response for the e-vapor carrier group showed no response with increasing CDE. At one-third the CDE cigarette smoke showed a lung histopathology response over 15 times greater than the e-vapor group. In conclusion, there was no significant differences in biological endpoints between e-vapor carrier and filtered air control groups. In contrast, e-vapor carrier exposures showed substantially reduced histopathological responses in all regions of the respiratory tract compared to mainstream cigarette smoke exposure.

Methods

- Three OECD 413 90-day inhalation studies comparing different e-vapor product carrier (also called vehicle) mixtures (Table 1) were conducted using 5-7 week old Sprague Dawley rats. The study endpoints included pre-study and weekly in-life observations and sample collections at 28, 90 and 132 days (interim, primary and recovery necropsy groups, respectively) to measure treatment-related effects and their potential for reversibility.
  - Aerosols were generated using a capillary aerosol generator previously shown (Werley et al. 2016) to mimic E-vapor product aerosols and animals were exposed at a target concentration of 1 mg/L of total aerosol.
- A data mining approach was used to compare the histopathology results from the current studies to similar designed OECD 90-day inhalation studies using the same strain of rats (Gaworski et al., 2011, Coggins et al., 2011a-g).
  - For the nose, larynx, and lung, the maximum histopathology score for any change noted, regardless of sex, resulted in the "histopathology response". This "response" was obtained by multiplying the severity score (0=normal, 1=minimal, 2=mild, 3=moderate, 4=marked, and 5=severe), by the incidence (%).
  - The ordinate was defined as the Cumulative Daily Exposure (expressed as mg total particulate matter or aerosol/liter, multiplied by the exposure time in minutes).

Table 1. E-vapor product carrier mixtures: target levels (%).

Study	Propylene Glycol	Glycerin	Water	Ethanol
1	51	34	15	0
2	58	39	0	3
3	34	51	15	0

- Mean exposure concentrations for each study were within 2% of target exposure concentration. The mean mass median aerodynamic diameters ranged from 0.8 – 1.2 microns with mean geometric standard deviations of 1.69 – 1.81. In each study, there were no effects on survival, body weights (Figure 1), body weight gain (by interval or cumulative), or food consumption. There was no clinical observations (prior to dosing and post-dosing observations) and no ophthalmic lesions, indicative of toxicity in any of the studies.
- After 90 days of exposure, no changes in the measured respiratory parameters (respiratory frequency (RR), tidal volume (TV), or minute volume (MV)) were noted in study 1 or 3. In study 2, a statistically significant increase in TV (31.6%) and MV (31.1%) were noted, but without concurrent change in RR. Study 2 pooled group means were considered to be artificially elevated due to a higher than expected tidal volume for a single animal, and therefore not considered to be treatment related.
- No differences in hematology, serum chemistry, urinalysis, or organ weights (absolute or relative to brain weight) were found. After 90-days of exposure, no significant differences in histopathology were found (Table 2) between air control and vehicle exposed in any of the three studies.
- Only one difference in bronchoalveolar lavage fluid (BALF) chemistry or cytology was found, a difference LDH in males, but not females, only in study 2.

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Results

Table 2. Summary of selected histopathology findings in the respiratory tract after 90 days of exposure.

Organ/Observation	Sex	Incidence[a], Mean Severity[b]					
		Study 1		Study 2		Study 3	
		Air Control	Carrier	Air Control	Carrier	Air Control	Carrier
Nasal Cavity Level 1							
Unremarkable	M	10, NA	10, NA	10, NA	9, NA	10, NA	10, NA
	F	10, NA	10, NA	10, NA	10, NA	10, NA	10, NA
Squamous epithelium hyperplasia	M	ND	ND	ND	1, 1.00	ND	ND
	F	ND	ND	ND	ND	ND	ND
Nasal Cavity Level 2							
Unremarkable	M	ND	1, NA	3, NA	3, NA	2, NA	3, NA
	F	4, NA	5, NA	4, NA	4, NA	6, NA	7, NA
Mixed cell infiltrate or inflammation	M	7, 1.00	6, 1.00	7, 1.00	7, 1.00	ND	4, 1.00
	F	2, 1.00	2, 1.00	5, 1.00	6, 1.00	2, 1.00	ND
Mucous cell hyperplasia	M	3, 1.00	4, 1.00	1, 1.00	2, 1.00	7, 1.00	6, 1.17
	F	2, 1.00	1, 1.00	2, 1.00	1, 1.00	3, 1.00	2, 1.00
Transitional epithelium hyperplasia	M	10, 1.00	8, 1.00	7, 1.00	7, 1.00	3, 1.00	2, 1.50
	F	5, 1.00	5, 1.00	6, 1.00	6, 1.00	ND	ND
Nasal Cavity Level 3							
Unremarkable	M	10, NA	9, NA	10, NA	10, NA	9, NA	10, NA
	F	10, NA	10, NA	10, NA	10, NA	10, NA	9, NA
Mixed cell infiltrate	M	ND	1, 2.00	ND	ND	1, 1.00	ND
	F	ND	ND	ND	ND	ND	ND
Mononuclear cell infiltrate	M	ND	ND	ND	ND	ND	ND
	F	ND	ND	ND	ND	ND	1, 1.00
Nasal Cavity Level 4							
Unremarkable	M	10, NA	10, NA	10, NA	10, NA	10, NA	10, NA
	F	10, NA	10, NA	10, NA	10, NA	10, NA	9, NA
Mononuclear cell infiltrate	M	ND	ND	ND	ND	ND	ND
	F	ND	ND	ND	ND	ND	1, 1.00
Nasal Cavity Level 5							
Unremarkable	M	10, NA	10, NA	10, NA	10, NA	10, NA	9, NA
	F	10, NA	10, NA	10, NA	10, NA	8, NA	8, NA
Nasal Cavity Level 6							
Unremarkable	M	10, NA	10, NA	10, NA	10, NA	10, NA	8, NA
	F	10, NA	10, NA	10, NA	10, NA	9, NA	10, NA
Pharynx							
Unremarkable	M	10, NA	10, NA	10, NA	10, NA	10, NA	10, NA
	F	10, NA	10, NA	10, NA	10, NA	10, NA	10, NA
Larynx							
Unremarkable	M	10, NA	9, NA	6, NA	8, NA	1, NA	ND
	F	10, NA	10, NA	9, NA	7, NA	3, NA	1, NA
Luminal exudate	M	ND	1, 1.00	2, 1.00	2, 1.00	ND	ND
	F	ND	ND	1, 1.00	3, 1.00	ND	ND
Glandular dilatation	M	ND	ND	ND	ND	8, 1.00	6, 1.00
	F	ND	ND	ND	ND	4, 1.00	5, 1.00
Mononuclear cell infiltrate	M	ND	ND	2, 1.00	ND	7, 1.14	7, 1.00
	F	ND	ND	ND	ND	4, 1.00	6, 1.00
Trachea							
Unremarkable	M	10, NA	10, NA	10, NA	10, NA	9, NA	10, NA
	F	10, NA	10, NA	10, NA	10, NA	9, NA	7, NA
Mononuclear cell infiltrate							
	M	ND	ND	ND	ND	1, 1.00	ND
	F	ND	ND	ND	ND	1, 1.00	3, 1.00
Lung							
Unremarkable	M	10, NA	10, NA	10, NA	10, NA	3, NA	3, NA
	F	10, NA	10, NA	10, NA	10, NA	5, NA	3, NA
Alveolar macrophages	M	ND	ND	ND	ND	4, 1.00	5, 1.00
	F	ND	ND	ND	ND	5, 1.00	4, 1.00

NA, mean severity not applicable; ND, observation not detected
a Incidence presented as number of rats affected
b Mean severity = sum of the severities ÷ number affected (minimal = 1; mild = 2; moderate = 3; severe = 4)

Figure 1. Body weight changes in sham and vehicle exposed male and female rats in three 90-day inhalation studies (mean ± SD, N=5-20) with e-vapor product carrier formulations.

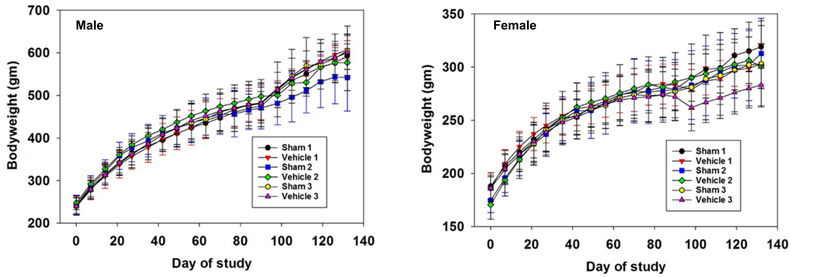
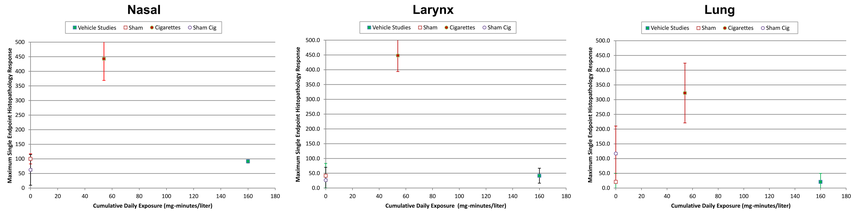


Figure 2. Mean (SD) maximal nasal, larynx and lung histopathology response as a function of CDE for e-vapor product carrier (filled square), sham response from e-vapor product carrier (open square), and mean (SD) maximal mainstream tobacco smoke control cigarette and sham exposed groups (filled and open circles, respectively). Small SD values maybe within the symbol.



Conclusions

- No effects on survival, clinical observations, body weight, food consumption, ophthalmic examinations, respiratory measurements, standard clinical chemistry parameters (hematology, serum clinical chemistry, and urinalysis) or organ weights were considered to be e-vapor vehicle related when compared to sham.
- Changes in BALF chemistry or cytology were limited to a difference LDH in males, but not females, only in study 2.
- No differences in respiratory tract or other organ histopathology were considered to be e-vapor vehicle related when compared to sham.
- Compared to the histopathology from exposure to mainstream cigarette smoke, the histopathology response due to exposure to e-vapor vehicles is substantially reduced.

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