A 90-day Nose-only Inhalation Study of Propylene Glycol with Selected Systems Toxicology Analysis

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Abstract: A 90-day inhalation study was performed to further characterize the biological effects resulting from exposure to a mixture containing propylene glycol (PG), a common carrier in e-vapor formulations. Aerosols of a PG:water mixture (test article) or a PG:glycerin(VG):water mixture (reference article) were generated using a capillary aerosol generator (CAG). Rats were exposed on a 5 day/week basis for 1, 3, or 6 hours to 5 mg/L TPM (Total Particulate Matter) of the test article or for 6 hours to 5 mg/L TPM of the reference article. Necropsies were performed following 90 days of exposure (primary) and following 6 weeks of recovery. Responses were compared to a concurrent filtered air control.
Food consumption and body weights were unaffected by the test or reference articles compared to the sham controls. In addition, no treatment–related alterations were observed in serum chemistry, hematology, coagulation, urinalysis or BALF cytology and clinical chemistry. Macroscopic exams at the primary and recovery necropsies as well as terminal organ weight data revealed no observations that were considered to be associated with exposure to either the test or reference article.
Following 90-day exposures, there were test and reference article-related findings of minimal hyperplasia of mucus cells noted within the nasal cavity. However, these were of minimal severity and also present in the control group, albeit at a lower incidence. Therefore, these findings were not considered adverse. The mucus cell hyperplasia incidence and severity in the high-dose test article group were similar to the 6 hour reference article group. There were no other treatment-related findings in the primary or recovery necropsies. Based on the lack of adverse findings, the NOAEC was defined as the high-dose (5 mg/L for 360 minutes) for the test article formulation.
In addition to the OECD (GLP) endpoints, exploratory (non-GLP) systems toxicology endpoints were examined in female rats. Transcriptomic analyses corrected for false discovery rate demonstrated no statistically significant differentially expressed genes in the lung or liver after 90-day exposures. An analysis of the lung and liver proteome also showed no statistically significant differentially expressed proteins. These data align with minimal respiratory tract effects observed from exposure to mixtures of PG, VG and/or water.

Methods

- Test formulation (90% PG – 0% VG – 10% water: 90-0-10); Reference formulation ((50% PG – 40% VG – 10% water: 50-40-10)
- This study was designed in general accordance with OECD Guideline 413 (2009). Seven week old SD rats were exposed on a 5 day/week basis for 1, 3, or 6 h/day to 5 mg/L TPM test article, for 6/day to 5 mg/L reference article or for 6 h to air (Sham control) for 13 weeks (primary), followed by 6 weeks of recovery.
- Exposures were conducted using a stainless-steel conventional nose-only exposure system. A liquid droplet aerosol exposure atmosphere of the test article formulation and reference article formulation were generated using a CAG, which has been previously shown to mimic e-vapor product aerosols (Werley et al., 2016). The CAG was heated to approximately 190°C for the test article formulation and approximately 280°C for the reference article formulation using a Digi-Sense Temperature controller. Aerosol samples were collected from a noseport and were analyzed for TPM, particle size, PG and carbonyls.
- Rats were bled postexposure after 4 or 12 weeks of exposure and plasma was analyzed for PG (Table 1) using an ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method in the positive electron ionization mode.
- At each sacrifice, the left lung was subjected to bronchoalveolar lavage (BAL) three times and the lavage solutions were centrifuged and the combined pellets were analyzed for total and differential cell counts. The supernatant from the first lavage was analyzed for lactate dehydrogenase, alkaline phosphatase, total protein and cytokine analyses.
- Selected tissues were collected and shipped frozen for (non-GLP) mechanistic endpoints (transcriptomic, proteomic and BALF cytokine analyses). Tissue was pulverized using a CryoPrep Impactor and total RNA was isolated using the miRNeasy mini kit (Qiagen). Gene expression was analyzed using the Affymetrix GeneChip® technology. Quantitative proteomic MS was performed using the iTRAQ 8-plex Multiplex kit (AB Sciex) (Titz et al., 2015). The BAL fluid was analyzed for cytokines using Luminex.

Results

- The particle size distribution (mean mass median aerodynamic diameter [MMAD]) ranged from 1.7 to 2.6 µm with mean gravimetric standard deviation (GSD) of 1.55 – 1.96.
- Mean PG atmosphere concentration ranged from 4.0-4.2 mg/L in the test article groups and was 2.7 mg/L in the reference article group.
- There were no test article- or reference article-related effects on survival, body weights (Fig 1), or food consumption.
- There was test article- and reference article-related mucus cell hyperplasia noted (Table 2). Incidence of this finding was similar when the reference article group was compared to the high-dose test article group.
- There were no other test article or reference article related findings noted within survival, gross observations, organ weights or histology in the primary phase.
- No statistically significant changes in respiratory frequency were noted during Weeks 4 or 12 for any comparisons in either sex. All treatment group values were similar to filtered air control and were within 2 standard deviations of historical control baseline values. Week 4 comparisons were conducted for pooled sexes and a statistically significant decrease in tidal volume (23%) and corresponding minute volume (36%) was noted for the reference article group when compared to sham, but not when compared to the 6 hour test article group (data not shown). These decreases in the reference article group were considered a treatment effect, however, this effect reversed by Week 12. When the Week 12 comparisons were conducted for pooled sexes, a statistically significant decrease in minute volume was noted for the 3 hour mid dose test article group when compared to sham and the 1 hour low dose test article group, but not when compared to the 6 hour high dose test article group (Fig 2). There was no dose-related trend.
- There were no notable differences in hematology, coagulation, blood carboxyhemoglobin, serum chemistry, urinalysis, and chemistry. No differences in liver and lung weights or BALF cytology and chemistry were observed. BALF cytokines showed some difference but these differences were not dose related (Fig 3).
- Lung and liver transcriptomic and proteomic analyses showed no statistically significant changes compared to the sham (Figs 4 and 5).

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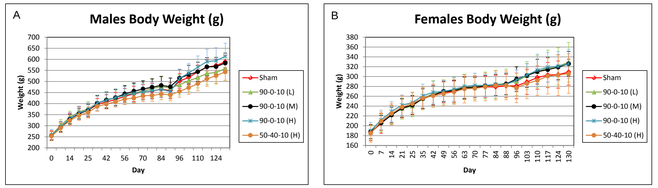

Figure 1 Body weight changes in sham and test article exposed male (A) and female (B) rats (mean ± SD, N = 5-30). Body weights were unaffected by test article administration.

Table 1. Plasma PG concentrations at weeks 4 and 12 (mean ± SD, N = 5)

	Plasma PG Concentration (ng/mL)			
	Week 4		Week 12	
Group	Male	Female	Male	Female
Sham	BLQ (<1.0)	BLQ (<1.0)	BLQ (<1.0)	BLQ (<1.0)
Test: 90-0-10 (1hr)	82,450 ± 26,071	98,820 ± 26,403	86,720 ± 16,565	94,120 ± 17,097
Test: 90-0-10 (3hr)	178,400 ± 16,441	202,600 ± 24,141	208,200 ± 26,715	212,000 ± 40,466
Test: 90-0-10 (6hr)	243,200 ± 29,786	280,800 ± 33,922	273,800 ± 26,527	269,200 ± 43,649
Reference: 50-40-10 (6hr)	116,860 ± 18,454	130,800 ± 25,917	88,240 ± 23,153	106,080 ± 20,099

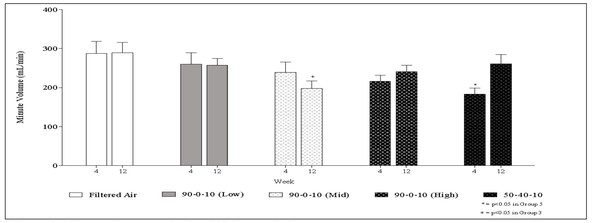

Figure 2 Minute volume at weeks 4 and 12 from pooled sexes

Table 2. Histopathology findings in Nasal Section II at Study Week 12 Primary Necropsy: Incidence and (Severity[1])

	Male					Female				
Treatment	Sham (6hr)	90-0-10 (1hr)	90-0-10 (3hr)	90-0-10 (6hr)	50-40-10 (6hr)	Sham (6hr)	90-0-10 (1hr)	90-0-10 (3hr)	90-0-10 (6hr)	50-40-10 (6hr)
Nasal Section II[2]	10	10	9	10	10	10	10	10	10	10
Hyperplasia, Mucus cell	5 (1)	1 (1)	3 (1)	8 (1)	9 (1)	1 (1)	2 (1)	4 (1)	8 (1)	8 (1)

[1]Severity Grades: 1 (Minimal), 2 (Mild), 3 (Moderate), 4 (Marked), 5 (Severe)
[2] Number of tissues examined from each group

Table 3. Summary of selected endpoints including liver and lung weights and BAL fluid analysis. (mean ± SD, N = 5-10)

Male

Endpoint	Necropsy	Sham (6hr)	90-0-10 (1hr)	90-0-10 (3hr)	90-0-10 (6hr)	50-40-10 (6hr)
Lung Weight (g)	Primary	1.48 ± 0.07	1.47 ± 0.17	1.43 ± 0.11	1.46 ± 0.12	1.49 ± 0.13
	Recovery	1.76 ± 0.18	1.64 ± 0.16	1.73 ± 0.11	1.83 ± 0.11	1.59 ± 0.16
Liver Weight (g)	Primary	9.39 ± 1.1	10.38 ± 1.7	10.13 ± 0.89	9.36 ± 1.22	9.49 ± 0.73
	Recovery	12.96 ± 1.04	11.93 ± 1.36	12.81 ± 1.16	13.47 ± 1.39	12.19 ± 1.1
BALF LDH (U/L)	Primary	106 ± 47.6	124 ± 56.3	94 ± 44.1	103 ± 45	96 ± 24.8
	Recovery	101 ± 30.1	117 ± 43	109 ± 64.5	80 ± 30	112 ± 41.2
BALF ALP (U/L)	Primary	51 ± 20.6	50 ± 18.9	47 ± 17.9	50 ± 13.1	60 ± 18.5
	Recovery	39 ± 6.4	38 ± 15.9	32 ± 7.1	48 ± 18.9	41 ± 15.8
BALF Prot (mg/dL)	Primary	15.2 ± 8.96	12.7 ± 4.82	10.3 ± 4.32	10.1 ± 3.59	9.7 ± 2.23
	Recovery	10.2 ± 3.11	12.1 ± 4.55	11.4 ± 5.01	9.9 ± 2.63	11.6 ± 4.62
Cell Count (x10⁶)	Primary	6.63 ± 2.0	7.06 ± 3.71	4.71 ± 1.66	6.29 ± 2.28	6.38 ± 4.56
	Recovery	10.27 ± 5.95	11.51 ± 1.85	9.65 ± 3.89	14.11 ± 8.35	8.61 ± 2.77
AVM %	Primary	99.25 ± 0.59	98.3 ± 1.7	98.94 ± 0.92	97 ± 4.3	98.85 ± 0.47
	Recovery	92.8 ± 7.68	93.1 ± 3.96	93 ± 5.14	78.83 ± 38.8	96.6 ± 2.43
Neu %	Primary	0.2 ± 0.48	0.75 ± 1.1	0.56 ± 0.53	2.5 ± 4.3	0.65 ± 0.58
	Recovery	5.5 ± 6.64	5.1 ± 4.02	5.6 ± 5.4	3.83 ± 6.82	2.4 ± 2.13
Lym %	Primary	0.55 ± 0.5	0.95 ± 0.93	0.5 ± 0.9	0.5 ± 0.47	0.45 ± 0.44
	Recovery	1.6 ± 0.49	1.8 ± 0.91	1.4 ± 1.1	1.08 ± 0.08	1.0 ± 0.5
Eos %	Primary	0	0	0	0	0.05 ± 0.16
	Recovery	0.1 ± 0.22	0	0	0	0
Baso %	Primary	0	0	0	0	0
	Recovery	0	0	0	0	0

Female

Endpoint	Necropsy	Sham (6hr)	90-0-10 (1hr)	90-0-10 (3hr)	90-0-10 (6hr)	50-40-10 (6hr)
Lung Weight (g)	Primary	1.17 ± 0.1	1.15 ± 0.13	1.17 ± 0.07	1.19 ± 0.16	1.23 ± 0.16
	Recovery	1.23 ± 0.07	1.25 ± 0.17	1.20 ± 0.06	1.24 ± 0.07	1.24 ± 0.1
Liver Weight (g)	Primary	6.81 ± 0.68	6.64 ± 0.79	6.66 ± 0.54	6.80 ± 1.01	6.92 ± 1.06
	Recovery	7.28 ± 0.46	7.60 ± 1.96	7.59 ± 0.76	8.37 ± 1.18	7.66 ± 1.31
BALF LDH (U/L)	Primary	94 ± 26	120 ± 71.7	128 ± 79.7	133 ± 45.8	119 ± 50.1
	Recovery	103 ± 44.6	114 ± 62.4	95 ± 57.6	105 ± 27.1	102 ± 35.2
BALF ALP (U/L)	Primary	57 ± 11.7	48 ± 14.3	55 ± 13.3	51 ± 9.5	55 ± 14.2
	Recovery	51 ± 9.4	52 ± 11	46 ± 11.3	42 ± 5.4	53 ± 17.1
BALF Prot (mg/dL)	Primary	11.5 ± 2.74	13.1 ± 5.78	14.9 ± 6.55	15.1 ± 5.55	22.3 ± 26.85
	Recovery	10.9 ± 3.63	13.3 ± 4.83	9.5 ± 4.22	15.3 ± 1.76	13.3 ± 3.92
Cell Count (x10⁶)	Primary	4.35 ± 1.7	3.52 ± 2.35	4.13 ± 2.26	5.46 ± 3.29	4.93 ± 2.27
	Recovery	5.72 ± 2.13	5.71 ± 2.53	6.88 ± 4.13	7.51 ± 3.42	3.8 ± 1.89
AVM %	Primary	98.85 ± 1.29	98.2 ± 1.48	98.35 ± 0.63	98.95 ± 1.12	98.7 ± 0.59
	Recovery	97 ± 3.06	96.9 ± 3.19	93.52 ± 5.64	96.4 ± 2.58	96.7 ± 1.82
Neu %	Primary	0.6 ± 0.84	0.9 ± 1.37	0.4 ± 0.21	0.4 ± 0.46	0.4 ± 0.52
	Recovery	0.3 ± 0.45	1.10 ± 2.19	1.6 ± 2.82	0.9 ± 1.25	1.2 ± 1.3
Lym %	Primary	0.5 ± 0.53	0.9 ± 0.57	1.2 ± 0.79	0.65 ± 0.91	0.9 ± 0.52
	Recovery	2.7 ± 2.95	2.0 ± 1.23	4.88 ± 3.0	2.6 ± 2.46	2.1 ± 1.08
Eos %	Primary	0.05 ± 0.16	0	0.05 ± 0.16	0	0
	Recovery	0	0	0	0.1 ± 0.22	0
Baso %	Primary	0	0	0	0	0
	Recovery	0	0	0	0	0

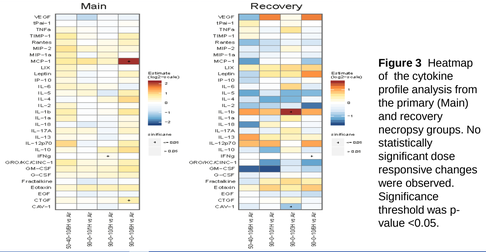

Figure 3 Heatmap of the cytokine profile analysis from the primary (Main) and recovery necropsy groups. No statistically significant dose responsive changes were observed. Significance threshold was p-value <0.05.

Lung Gene Expression

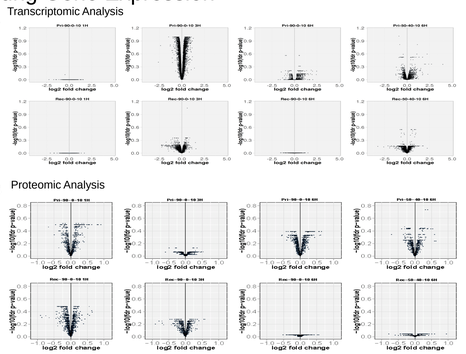

Figure 4 Volcano plots of lung transcriptomic (A) and proteomic (B) analyses from primary (upper) and recovery (lower) necropsies. No statistically significant differential expression was observed. Significance threshold was fdr-adjusted p-value < 0.05, corresponding to a –log10 fdr threshold of 1.3.

Liver Gene Expression

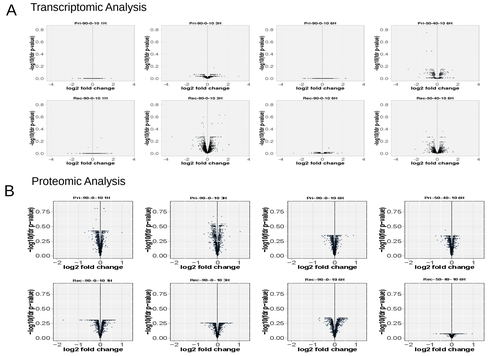

Figure 5 Volcano plots of liver transcriptomic (A) and proteomic (B) analyses from primary (upper) and recovery (lower) necropsies. No statistically significant differential expression was observed. Significance threshold was fdr-adjusted p-value < 0.05, corresponding to a –log10 fdr threshold of 1.3.

Observations and Conclusions

- There was test article- and reference article-related mucus cell hyperplasia noted in nasal section II that was not considered adverse.
- No other test article or reference article related findings were noted within survival, gross observations, organ weights or histology in the primary phase of this study.
- No statistically significant differential gene expression was observed in lung and liver. These "omics" data align with minimal respiratory tract effects observed from exposure to mixtures of propylene glycol, glycerin and/or water (Werley et al., 2011; Phillips et al., 2017).

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