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RESEARCH ARTICLE

Lack of respiratory and ocular effects following acute propylene glycol exposure in healthy humans

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ABSTRACT

Objective: Propylene glycol (PG) is a widely used solvent, chemical intermediate and carrier substance for foods, pharmaceutical and cosmetic products. Professional and occupational exposure to PG aerosol and vapor may occur from theatrical smoke generators and during application of deicing products to airplanes. While PG is considered to have low toxicity, the results of one study suggested that brief (1-min) exposure to PG mist elicited ocular and respiratory effects in humans. Because the high concentrations and brief exposure duration in that study were not representative of most occupational exposures, a controlled experimental exposure study was conducted to clarify or confirm the earlier findings.

Materials and methods: Ten males and 10 females were exposed to PG aerosol for 4 hrs at 20 and 100 mg/m^3 and 30 min at 200 mg/m^3. Total PG exposure concentrations (droplets plus gas phase) were 95.6, 442.4 and 871 mg/m^3 for the three conditions, respectively. Participants rode a stationary bicycle to simulate physical effort at regular intervals during exposure. Objective measures evaluated in this study included ocular irritation via eye blink task and eye photography and pulmonary function via spirometry, while subjective measures included health symptoms ratings, irritation and dryness ratings of eyes, nose, throat and mouth.

Results: Objective measures of pulmonary function and ocular irritation did not reveal any exposure-related changes. Exposure-related changes in symptom reporting were observed; however, the highest symptom ratings did not exceed "slight" on the scale.

Conclusions: The results indicate at the concentrations and acute durations tested, PG does not affect human respiratory function or produce ocular irritation.

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Propylene glycol; 1,2-propanediol; aerosol; respiratory irritation; ocular irritation

Introduction

Propylene glycol (PG) (1,2-propanediol; CAS No. 57-55-6) is a synthetic hygroscopic liquid, used in the production of polyester compounds, in deicing and antifreeze fluids and in coolants. It is also widely used as a solvent for colors and flavors in food, pharmaceutical and cosmetic products and in the paint and plastic industry. Aerosolized PG creates mist during emergency aviation and fire-fighter training, and in theatrical productions. Among the general public, the dominant exposure routes for PG are ingestion and dermal contact. In occupational settings, inhalation exposure to PG aerosol (droplets) or vapor (gas phase) occurs in operations involving heating or spraying of PG (e.g. applying deicing fluid to airplanes); moreover, inhalation exposure to PG mist can occur in theatrical productions involving generation of theatrical fog. More recently, individuals can be exposed to heated PG from the use of electronic cigarettes, in which PG is added as the matrix for nicotine and flavor compounds. Studies involving actors and singers in theatrical productions, as well as painters and personnel engaged in emergency training, have suggested that PG is a respiratory irritant, largely based on symptom reports (Burr et al., 1994; Moline et al., 2000; Varughese et al., 2005; Wieslander et al., 2001; Wieslander & Norback, 2010). However, most of these studies involved mixed exposures to multiple glycol derivatives or volatile organic compounds. Thus, the goal of this study was to evaluate the potential for respiratory and ocular effects from aerosolized PG at occupationally relevant concentrations and durations.

There have been a number of animal studies of PG inhalation using both single and repeated exposures. Among the single-exposure studies, rabbits (strain not specified) exposed to a 10% PG solution (no data on the particle size) showed an exposure-time-related increase in mucus release and degeneration of tracheal goblet cell (Konradova et al., 1978). Sprague-Dawley rats exposed to PG ranging from 14.4 to 44.9 mg/L for 4 hours exhibited minor signs that appeared to be bleeding around the eyes and nose (Werley et al., 2011), while animals exposed for 4 hours a day for 7 days to either 20.8 or 41 mg/L of PG revealed no treatment-related clinical observations (Werley et al., 2011). A 28-day repeated exposure toxicity study using Sprague-Dawley rats exposed to PG aerosol for 4 to 120 minutes /day showed minimal evidence of laryngeal squamous metaplasia in the highest exposure groups. A 90-day (6 hours/day and 5/days/week) nose-only inhalation study in Sprague-Dawley rats found that exposure to

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PG resulted in histologic changes in the nasal mucosa suggestive of irritation (Suber et al., 1989). The changes included a significant increase in the number of goblet cells or an increase in the mucin con-tent of the existing goblet cells in the nasal passages of the animals that received 1000 and 2200 mg/ m3 PG exposures. The No Observed Adverse Effect Level for this finding was 160 mg/m3. Based on this study, the Netherlands MAC was set at 50 mg/m3 as an 8-hour time weighted average (TWA) concentration applied to the sum of the concentration of PG existing as a vapor and an aerosol. Taken together, however, all of these minor clinical findings could be attributable to the strongly hygroscopic nature of PG and the adaptive response of the mucosa and airway epithelium to dehydration effects. In contrast to the data from the animal literature, there are very few studies on human inhalation exposure to PG aerosol and, to date, no controlled human exposure studies of PG alone are available. The National Institute of Occupational Safety and Health (NIOSH) conducted a study (Burr et al., 1994) evaluating symptom reports among actors in Broadway productions who were exposed to a variety of glycol compounds in addition to PG, including ethylene glycol, 1,3 butylene glycol, diethylene glycol and trie-thylene glycol. Personal breathing zone and area samples combined with a questionnaire on irritant effects were obtained from personnel engaged in productions with and without theatrical smoke. Although there was a significant increase in the reports of irritant symptoms such as runny and stuffy nose among performers exposed to theatrical smoke, no direct conclusions regarding PG could be made due to the simultaneous exposures to multiple glycol compounds.

Moline and colleagues (Moline et al., 2000) also evaluated clinically relevant effects of exposure to a mixture of glycol compounds, including butylene, diethylene, triethylene and propylene glycol among a group of 439 performers. Results revealed no significant gross structural changes to the vocal fold tissue, functional vocal fold vibration patterns, auditory perceptual voice quality or pulmonary function from pre-versus post-exposure. Actors with exposure to elevated or peak levels of glycols reported more health symptoms compared to those with lower exposures. Given the mixed nature of the studied exposures, the peak concentrations associated with symptoms could not be attributed to PG alone.

In a similar study, Varughese and colleagues evaluated symptoms and respiratory function among employees exposed to theatrical fogs (Varughese et al., 2005). They found increased symptom reports and statistically significant reduced pulmonary function (forced vital capacity) among workers with exposure to theatrical fog relative to unexposed controls. However, the difference in the percent predicted forced vital capacity (FVC) between the comparison group and the exposed cohort was less than 5%, which is not con-sidered to be a clinically relevant decrease (Gardner, 1988). As well, the percent predicted forced expiratory volume at 1 minute (FEV1) for the group with the highest exposure did not differ from the group with no exposure. Finally, the mixed nature of the exposures (uncharacterized in this study) preclude any conclusions regarding the relationship between symptoms, pulmonary function and PG alone.

Wieslander & Norback (2010) evaluated the effects of exposure to a water-based paint that included PG, other gly-col compounds and volatile organic compounds. Ocular symptoms, tear film stability, na-sal patency and biomarkers in nasal lavage were obtained from house painters and unex-posed controls. The authors concluded that the increase in eosinophilic cationic protein (ECP) obtained from na-sal lav-age was indicative of airway irritation. However, as was true for the theatrical studies, it is impossible to attribute any findings and symptom reports to PG, due to the mixed exposures to different components emitted from the water-based paints.

In the sole study of ocular and respiratory exposure to PG mist alone, Wieslander and colleagues conducted an observational study of aircraft workers engaged in aviation emergency training (Wieslander et al., 2001). PG exposure occurred in a flight simulator using a commercial PG solu-tion for smoke generation. Twenty-two individuals experi-enced a 1-minute exposure to total PG concentrations ranging from 176–851 mg/m3. Before and after exposure, symptoms were recorded and pulmonary function, nasal patency and ocular blink rate were measured. The authors observed a slight, significant increase in reported ocular and throat symptoms. They also reported an increase in blink latency post-exposure, interpreted as a surrogate measure of irritant-induced, tear film stability and a small but signifi-cant decrease of FEV1/FVC. Based on their interpretation of the changes observed, they concluded that PG elicited adverse respiratory effects.

Of the studies reviewed, only the Wieslander et al. (2001) study was capable of associating any observed effects to PG alone. However, the varying exposure concentrations over only a 1-minute exposure duration rendered this study less useful for understanding whether PG elicits respiratory effects, particularly for individuals who are occupationally exposed for longer periods albeit at lower concentrations. Additionally, exposure to PG droplets in the respirable range may have effects that differ from PG in gas phase on respiratory outcomes. However, the study only analyzed total PG concentrations during the 1-minute exposures, but did not separately measure either the concentration of drop-lets or droplet size. Accordingly, to address these shortcom-ings and evaluate the acute effects from exposure to PG mist at occupationally relevant concentrations and durations, we conducted a controlled exposure chamber study in which individuals were exposed to each of three concentrations of respirable PG droplets and gas phase and measured both subjective symptoms and measures of ocular irritation and pulmonary function.

Participants

Twenty participants (10 males/10 females) were recruited to participate using a research database of previously tested participants and online advertisements. Females were

included as required by the institutional review board. Sample size was determined from a power analysis based on the variance associated with pulmonary function testing in prior research. Informed consent was obtained from all participants using a form that was approved by Schulman Associates Institutional Review Board (Blue Ash, OH). During the screening session, participants completed a 19-item medical history questionnaire regarding medications, surgeries and illnesses and underwent pulmonary function testing to exclude the presence of any underlying respiratory disorders. Exclusion criteria included history of smoking or vaping, contact lens use or required corrective lenses use during the testing, history of respiratory disease including asthma and COPD, and pulmonary function test (PFT) results outside of the normal predicted value for each participants' height, weight, and ethnicity. Screening PFT results were reviewed and inclusion was approved by an occupational medicine physician. Additionally, female participants were required to have a negative pregnancy test prior to each exposure. Of the 62 screened participants, 20 participants (10 males, 10 female) met all inclusion criteria (mean age of 36 and 29.8, respectively). The relatively high screen failure was in part due to many participants' inability to perform PFT that met the required criteria for an acceptable test.

Equipment

Aerosol generator

The aerosol generator was a customized version of the VC-AERO Model of smoke generators manufactured by Concept Engineering/Smoke Systems Limited (Berkshire, UK) capable of using neat PG to generate an aerosol mist. It was customized to deliver droplets in aerosol in the respirable range (2–5 micrometers), in order to evaluate the effect of this type of exposure on pulmonary function. PG (USP – 99.9%) was obtained from ChemWorld (Roswell, GA). Although the purity of the PG was extremely high, and analysis revealed no additional compounds following heating the PG to 250 °C, the existence of one or more degradation products cannot be ruled out.

Aerosol analysis via opacity meter

To monitor the concentration of liquid droplets generated by the customized VC-AERO, the DSL-460 Double Pass Opacity/Particulate Monitor manufactured by Dynoptic Systems Limited (Northamptonshire, UK) was used. This unit contains a transmitter and receiver to determine increases in droplet density in mg/m^3 by measuring disturbances in open path light transmission caused by suspended droplets passing through the light path. The instrument was specifically calibrated for pure PG droplets. The DSL-460 was connected to the VC-AERO generator to trigger the onset/offset of PG droplet generation based on detected amounts of PG droplets that act as criteria to achieve target concentrations. Additionally, the DSL-460 was attached to a desktop computer to log data accumulated during exposures.

Total PG analysis via sorbent tubes

While our primary interest was to study the effects of respirable droplets of PG on respiratory and ocular irritation, we recognized that gas-phase PG would also be produced during the exposures. Accordingly, to measure total PG aerosol concentration (both the droplet phase monitored by the DSL-460 and the gas-phase concentration), air samples during each exposure condition were collected on XAD-7 sorbent tubes (SKC; Eighty Four, PA). The Gilian BDX II abatement air sampling pump (Sensodyne; St. Petersburg, FL, USA) was used to pull air through the sorbent tubes. Samples were analyzed following solvent extraction via GC/MS at Cornerstone Analytics Laboratories in Maryville, TN (USA).

Procedure

We employed a within-subjects design, in which each subject participated in each condition and served as their own pre–post exposure control. Participants had exposure to three target droplet concentrations of PG: 20, 100 and 200 mg/m^3. For the 4-hour session with 20 and 100 mg/m3 concentrations, the concentration order was counterbalanced across the participants. The final condition was 200 mg/m^3 concentration for 30 minutes. Each exposure was followed by a minimum of five days of recovery time to avoid carryover effects. Participants were blinded to conditions and were deceived that one condition at random would contain no PG, but only water vapor. At the end of the study, participants were debriefed.

Figures 1 and 2 depict the sequence of events for both the 30 minute and 4-hour exposure condition. Baseline measurements of the health symptoms questionnaire, spirometry, eye-blink and eye photography were collected. During exposure, participants made ratings using the health symptoms questionnaire, location diagrams and odor quality every 10 minutes for the first 30 minutes of exposure and then every 30 minutes for the remainder of the 4-hour session. At the halfway point for the 4-hour exposure, participants left the room for mid-point measurements. The process was repeated for the second half of the exposure session. Additionally, participants were instructed to ride a stationary bike for 10 minutes every 30-minute epoch of exposure. This was done to simulate physical activity during exposure such as might occur in occupational settings and that would result in increased exposure of the respiratory tract to PG. In the 4-hour exposure conditions, they rode the bike for 8 times in total and, in the 30-minute exposure condition, they rode the bike for the middle 10 minutes only.

During the 4-hour long exposure for the 20 mg/m^3 and 100 mg/m^3 mist concentrations, midpoint measurements were recorded after the first two hours. Participants exited the chamber and measurements were taken in the following order: spirometry, eye photography, eye-blink task and questionnaires (health symptoms and location diagrams). They were allowed to take a bathroom break as needed and permitted to drink a maximum of 8 ounces of water and then

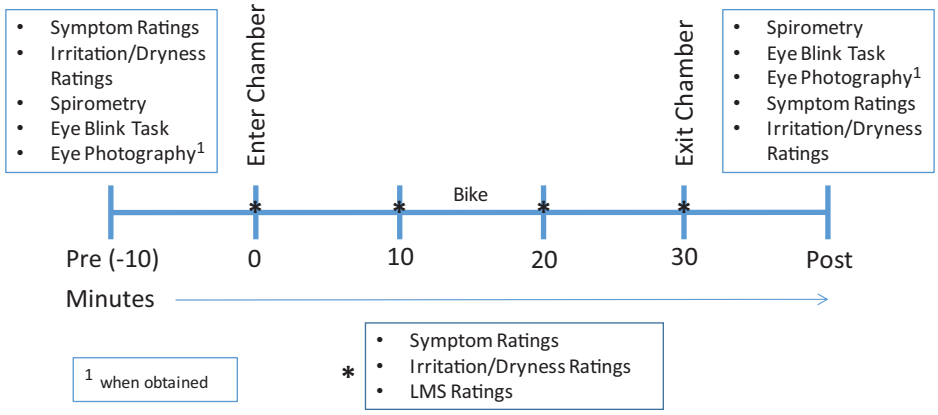

Figure 1. High PG concentration timeline.

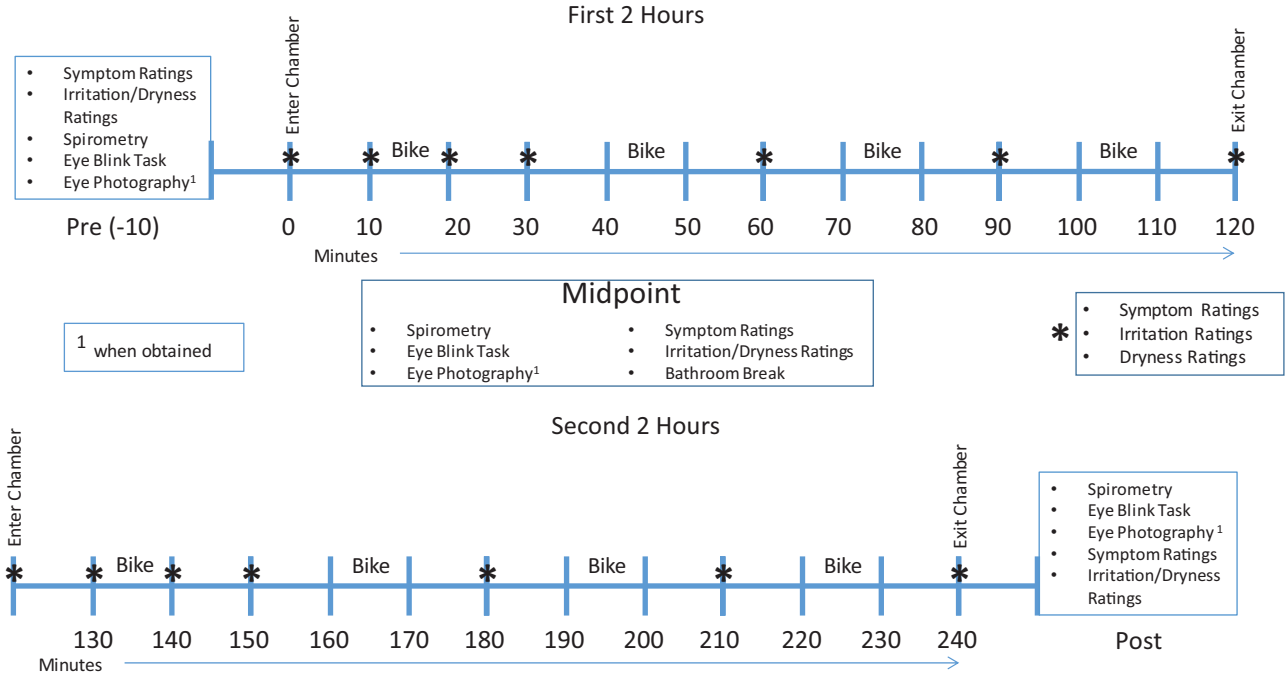

Figure 2. Low and medium PG concentration timeline.

returned to the chamber for the second 2-hour session. Measurements were performed in the same order at the end of the session as at the midpoint.

Objective measures

Spirometry

The EasyOne Plus Frontline Spirometer (ndd Medical Technologies, Andover, MA) was used to measure respiratory function at screening and pre- and post-exposures. Participants were instructed to inhale as much air as possible and exhale forcefully into the device for six seconds. We collected two "good effort" trials and a minimum of four trials for a successful measurement, with "good effort" meaning the participant met the criteria of exhaling for a full 6 seconds as determined by the device to capture the endpoints. A maximum of eight trials were performed during each measurement to avoid alterations due to fatigue effects. We used NHANES III reference standards (Hankinson et al., 1999) to generate percent predicted normal spirometric values for forced expiratory volume (FEV_1) at one second, forced vital capacity (FVC), and their ratio (FEV_1/FVC) based on age, height, gender and race.

Eye blink

To measure ocular irritation participants were video recorded while staring at a fixation point and naturally blinked. Participants were instructed to close their eyes, say "One, two, three" aloud and then open their eyes until they naturally blinked. Participants repeated this three times during each recording. Videos were recorded using a Canon Vixia HF R400 video camera and edited and analyzed using Windows Movie Maker. The dependent variable was the duration between eye opening and first eyeblink in seconds.

Eye photography

The bulbar region of the scleral conjunctiva was chosen for the assessment of hyperemia as in prior studies (Opiekun et al., 2003). This region coats the anterior portion of the eyeball, is highly vascularized and readily observable. A Nikon D-810 digital SLR camera with a 105 mm MicroNikkor lens (Nikon Corp., Tokyo, Japan) attached to a Tiffen 52 mm close-up lens (Tiffen Corp. Hauppauge, NY), all mounted on a Bogen camera stand (Bogen Corp., Ramsey, NJ) was used to capture pre/mid/post photographs of eyes to document the presence of ocular hyperemia (Opiekun et al., 2003). Lighting was achieved using a Polaroid Macro Ring Flash (Polaroid Corp. Minnetonka, MN) set to one-quarter power. Participants placed their chin and forehead on a headrest. They were instructed to open one eye as wide as possible, look up and gently pull down the skin directly underneath the eye. The order of pictures captured was consistently the right eye followed by the left eye. Since the lens focal length was permanently set to the closest focal distance of 0.314 m, the distance of the lens to the conjunctival surface was adjusted slightly by sliding the camera on a track attached to the stand until the conjunctival vessels were in focus.

Subjective measures

To examine any interactions between personality and subjective health ratings, the Positive and Negative Affect Schedule (PANAS) and the Eysenk Personality Inventory were administered once at the first visit. Negative affectivity includes a range of negative mood states including anger, disgust, guilt and fearfulness, which could potentially affect subjective ratings. Participants responded to 20-items using a 5-point category scale with the following labels: (1) Not at all, (2) Slightly, (3) Moderately, (4) Very, (5) Extremely. The Eysenk Personality Questionnaire – Short Version (EPQ-R) measured the following personality traits: psychoticism, extroversion, neuroticism and lying. Participants responded to 48 items using yes/no responses to further investigate specific personality traits in relation to subjective ratings.

Participants made other subjective ratings before, during and after exposure sessions as shown in Figures 1 and 2. Measurements included the health symptoms questionnaire and location diagrams for rating the intensity and location of perceived irritation and dryness. The health symptoms questionnaire consisted of 24 items on the same 5-point category scale as above. Health items included nose, throat, eye and miscellaneous symptoms as shown in Table 1. The location diagram (Figure 3) displayed a sagittal view of the aerodigestive tract with six labeled locations: (1) tip of the nose, (2) sinus area, (3) back of sinus/upper throat, (4) lower throat, (5) mouth/oral area and (6) eyes. Two location diagram questionnaires were administered, one specifically for irritation and the other for dryness. All ratings used the same 5-point scale. Participants made ratings on these measures at every time point noted in Figures 1 and 2.

Table 1. Symptoms organized by category.

Eye symptoms
Sore eyes
Itching in the eyes
Dry eyes
Gritty eyes
Eye redness
Swollen eyelids
Nose symptoms
Nasal catarrh (buildup of mucus)
Nasal itch
Sneezing
Nasal obstruction
Throat symptoms
Throat dryness
Sore throat
Irritating cough
Difficulties in breathing
Skin
Facial itching
Facial rash
Itching on the hands
Rashes on the hands
Eczema
Miscellaneous
Sensation of catching a cold
Headache
Nausea
Fatigue
Thirsty

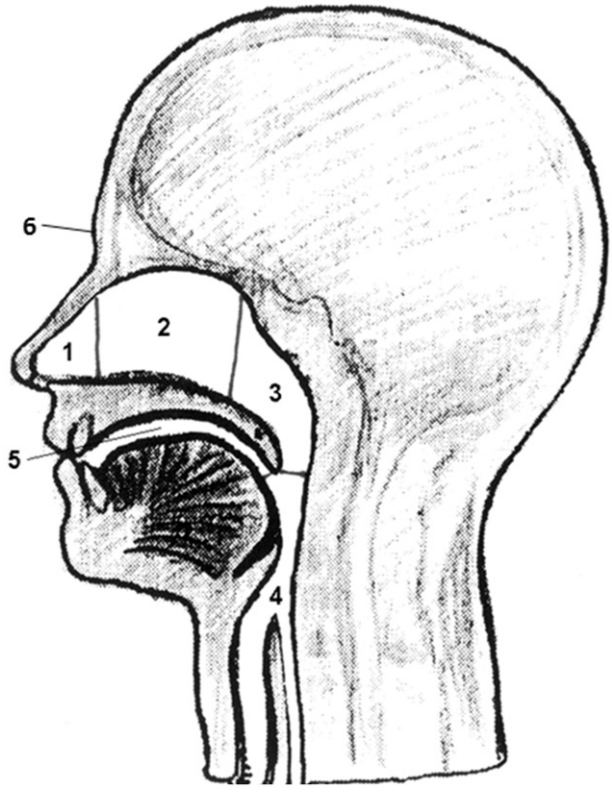

Figure 3. Sagittal diagram used for locating irritation and dryness sensations.

Data analysis

All participants completed all sessions. Subjective symptoms, irritation and dryness ratings were collected from all participants in all exposure conditions. As well, all participants provided data measuring ocular irritation (time to first

blink) and pulmonary function in all conditions. However, ocular photography was only performed on a subset of participants ($n = 6$) in all conditions, with an emphasis on performing this measure before and after the highest exposure concentration. Data were analyzed using Statistica 13 (Tibco Software, Palo Alto, CA). As a first step, all endpoints were analyzed with gender as the main variable; because no differences were observed on any outcome, the two groups were collapsed and all reported analyses were performed on all participants.

Ocular hyperemia analysis

Individuals with no prior training in ocular assessment were recruited, trained and evaluated for their ability to judge ocular redness. During an instructional session, slides were displayed on a computer monitor and individuals evaluated representative slide pairs from the participants. From an analysis of these data, four judges were selected with the highest inter-rater agreement consistent with accepted levels reported in the literature for the assessment of ocular redness (Opiekun et al., 2003). The chosen judges then participated in a single session during which they evaluated the full set of ocular slides from the participants in the study. Judges evaluated the comparative redness of two slide pair combinations per participant in the 100 mg/m^3 and 200 mg/m^3 conditions. Each pair of slides were presented twice: once with the pre-exposure slide on top and once with the post-exposure slide on top. Judges were asked to look at the two slides and determine whether the top slide was definitely more or less red than the bottom slide. The pairwise evaluations were averaged across all four judges for each study participant and exposure condition resulting in two scores per participant. A Chi-square analysis was conducted on the scores to determine whether participants detected an increase in redness following exposure to PG.

Results

Propylene glycol concentrations

The results for both the droplet and total PG concentrations are shown in Table 2. We calculated the average droplet concentration over each session for each participant from the data logs collected during each exposure. Total PG concentrations (droplet + gas phase) were derived from the analysis of the sorbent tube assays. Total PG concentrations were approximately 3–5 times higher than the droplet concentrations.

Table 2. Droplet and gas phase average PG concentrations.

	Concentration (mg/m^3)		
Endpoint	Low	Medium	High
Target (Droplet)	20	100	200
Actual Averaged	30.39	106.12	199.7
(Droplet)	(SE 0.89)	(SE 0.90)	(SE 6.47)
(Droplet + Gas-Phase)	95.57	442.39	870.98

Pulmonary function

Spirometry was performed at baseline, at the midpoint break and post-exposure for the 4-hour exposure, but only pre- and post-exposure for the 30-minute exposure. A multivariate analysis of variance (MANOVA) was performed on the spirometry endpoints. As shown in Table 3, there were no significant changes from pre-exposure to mid-exposure or post-exposure for any of the conditions on forced-expiratory volume in 1 second (FEV_1) for the low $F(10,180) = 0.65$, $p = .93$, medium $F(10,180) = 0.78$, $p = .65$ or high conditions $F(10,180) = 0.79$, $p = .21$. Similarly, there were no significant differences in FEV_1/FVC from pre-exposure to mid- or post-exposure for any of the conditions. The average post-exposure ratio of FEV_1/FVC was above 90% for all exposure conditions, signifying normal pulmonary function (Pellegrino et al., 2005).

Objective ocular irritation

Time to first blink was not significantly decreased following any of the exposures, with concentration × time interactions for the low, $F(2,36) = 0.378$, $p = .6$; medium $F(2,36) = 1.33$, $p = .27$; and high exposures $F(2,36) = 0.48$, $p = .62$. With respect to the ocular hyperemia assessment, the mean inter-rater reliability was $k = 0.58$ using Cohen's Kappa, deemed to be "moderate" agreement (Landis & Koch, 1977). A chi-square analysis of the pre–post judgments of the ocular photographs revealed that that the panel judgments of the redness of the post-exposure photographs was not significantly different from chance X^2 (1, $n = 158$) = 0.633, $p = .42$. Taken together, we found no evidence of ocular irritation from exposure at these concentrations of PG.

Symptom reports

Symptom scores were generated for each cluster based on the average of ratings made for the corresponding category. Because the focus of the present study was the evaluation of ocular and respiratory irritation, only the eye, nose and throat symptom scores were analyzed.

To be able to compare across all three concentrations of PG, we first analyzed the symptom reports from baseline to 30 (T30) minutes. As shown in Table 4, there was a small, but statistically significant exposure-related effect on symptom reports with higher symptom ratings during the first 30 minutes when participants were exposed to the high concentration of PG, than when exposed to the low or medium PG concentration, $F(2,38) = 13.42$, $p < .001$. When we

Table 3. Percent predicted spirometry values before, during and after exposure to PG aerosol/vapor.

		% Predicted FEV_1			% Predicted FEV_1/FVC		
Exposure		Pre	Mid	Post	Pre	Mid	Post
20 mg/m^3	Mean	97.69	98.75	94.88	96.84	98.51	96.40
	SE	2.60	2.67	3.13	1.65	1.44	1.63
100 mg/m^3	Mean	101.67	101.83	103.10	97.10	97.32	97.65
	SE	3.71	4.15	4.06	1.68	1.63	1.88
200 mg/m^3	Ave	96.48		97.08	95.53		96.47
	SE	2.66		3.02	1.93		1.80

analyzed symptom reports across the entire 4-hour exposure to the low and medium concentration, higher symptom ratings occurred during the medium than the low-exposure condition, $F(1,19) = 8.87$, $p < .01$.

Not all symptoms were equally elevated, however. There was a 3-way interaction of concentration × time × symptom for the first 30 minutes, $F(4,76) = 3.02$, $p < .05$ that showed a slight, but significant increase in perceived nose and throat symptoms from baseline during the medium and high concentration exposures only. In the medium concentration condition, nose symptoms increased from the ratings taken at 30 minutes to the ratings taken a 120 (T12) and 240 (T240) minutes, $F(6,114) = 3.13$, $p < .01$.

Table 4. Symptom reports over time (30, 120 and 240 min) by exposure condition. 1 = None, 2 = Slight, 3 = Moderate, 4 = Very, 5 = Extremely.

			Exposure Concentration		
Symptom	Time point		Low	Medium	High
Eye	Pre	Mean	1.06	1.03	1.08
		SE	0.03	0.02	0.04
	T30	Mean	1.13	1.14	1.35
		SE	0.03	0.05	0.07
	T120	Mean	1.24	1.32	–
		SE	0.06	0.08	–
	T240	Mean	1.24	1.31	–
		SE	0.07	0.09	–
Nose	Pre	Mean	1.13	1.13	1.11
		SE	0.04	0.05	0.07
	T30	Mean	1.10	1.38	1.60
		SE	0.03	0.08	0.08
	T120	Mean	1.21	1.66	–
		SE	0.06	0.12	–
	T240	Mean	1.21	1.63	–
		SE	0.06	0.13	–
Throat	Pre	Mean	1.08	1.10	1.03
		SE	0.03	0.04	0.03
	T30	Mean	1.20	1.38	1.67
		SE	0.06	0.11	0.16
	T120	Mean	1.37	1.55	
		SE	0.11	0.13	
	T240	Mean	1.40	1.53	
		SE	0.10	0.14	

Irritation ratings

There was also a small, but statistically significant exposure-related effect on irritation ratings as shown in Table 5. The High concentration elicited a slight, but significant increase at 30 minutes (T30) relative to the other two exposure conditions $F(2,38) = 17.05$, $p < .001$. During the 4-hour exposure, irritation ratings were higher in the medium versus the low-concentration condition $F(1,19) = 15.05$, $p < .01$.

As was true for the symptom reports, a concentration × time interaction revealed that significantly higher irritation ratings were made during exposure to the high concentration of PG relative to the other concentrations for the first 30 minutes, and during the medium than the low concentration at 2 (T120) and 4 (T240) hours.

For the first 30 minutes of exposure to PG, while irritation ratings for all six symptom areas (nose, sinus, upper throat, lower throat, mouth and eyes) increased significantly from baseline for the high concentration, all but the ratings for the eye area increased for the medium concentration exposure, $F(10,190) = 1.95$, $p < .05$.

Dryness ratings

Due to the known hygroscopic nature of PG, we also asked individuals to report sensations of "dryness" in the six areas

Table 5. Irritation reports over time (30, 120 and 240 min) by exposure condition and location. 1 = None, 2 = Slight, 3 = Moderate, 4 = Very, 5 = Extremely.

			Exposure concentration						Exposure concentration		
Symptom	Time point		Low	Medium	High	Symptom	Time point		Low	Medium	High
Nose	Pre	Mean	1.03	1.00	1.10	Lower	Pre	Mean	1.03	1.13	1.05
		SE	0.02	0.00	0.07	Throat		SE	0.02	0.07	0.05
	T30	Mean	1.20	1.25	2.00		T30	Mean	1.35	1.43	1.75
		SE	0.08	0.08	0.16			SE	0.13	0.15	0.23
	T120	Mean	1.33	1.55			T120	Mean	1.55	1.63	
		SE	0.10	0.13				SE	0.17	0.20	
	T240	Mean	1.43	1.78			T240	Mean	1.65	1.68	
		SE	0.12	0.15				SE	0.21	0.20	
Sinus	Pre	Mean	1.10	1.08	1.10	Mouth	Pre	Mean	1.08	1.10	1.05
		SE	0.05	0.04	0.07			SE	0.05	0.05	0.05
	T30	Mean	1.33	1.43	2.37		T30	Mean	1.28	1.55	1.65
		SE	0.11	0.11	0.22			SE	0.10	0.16	0.22
	T120	Mean	1.33	1.73			T120	Mean	1.58	1.68	
		SE	0.12	0.14				SE	0.21	0.22	
	T240	Ave	1.58	1.85			T240	Mean	1.68	1.65	
		SE	0.17	0.15				SE	0.21	0.21	
Upper	Pre	Mean	1.18	1.10	1.10	Eyes	Pre	Mean	1.10	1.10	1.10
Throat		SE	0.09	0.05	0.07			SE	0.05	0.05	0.07
	T30	Mean	1.40	1.58	2.10		T30	Mean	1.33	1.38	1.90
		SE	0.12	0.16	0.28			SE	0.09	0.13	0.18
	T120	Mean	1.63	1.80			T120	Mean	1.68	1.70	
		SE	0.21	0.21				SE	0.18	0.17	
	T240	Mean	1.80	1.88			T240	Mean	1.73	1.71	
		SE	0.21	0.20				SE	0.22	0.20	

on the diagram. As seen in Table 6, there was a small, but statistically significant exposure-related effect on dryness reports. As with the irritation ratings, we analyzed the dryness ratings during the first 30 minutes of exposure for all conditions and found that ratings of dryness were significantly higher during the highest concentration exposure relative to the other two conditions, $F(2,38) = 7.87$, $p < .01$.

Because we anticipated that the effect of PG on airway and oral mucosa could result in dehydration of the mucosa and elicit corresponding sensations, we were interested to evaluate the concordance between the dryness and irritation ratings for each of the six sites. A series of Spearman rank correlation coefficients were computed to investigate the relationship between reported irritation and reported dryness at each anatomical site (Table 7). Our analysis revealed that ratings of perceived sensory irritation were significantly and positively correlated with ratings of perceived tissue dryness for all areas, ($p = .000$ for all comparisons).

Discussion

Inhalation exposure to PG has been suggested to elicit effects on respiratory function and other irritant symptoms among individuals who are occupationally exposed. However, three out of the previous four studies on human exposure to PG could not support an association between exposure to PG and respiratory function, due to the mixed exposures all cohorts experienced.

Only one previous study in humans (Wieslander et al., 2001) evaluated the association between brief exposures to inhaled PG alone and any adverse effects and concluded that inhaled PG could lead to minor respiratory effects. The current study is the first controlled-exposure study in healthy adults that measured both objective and subjective effects following exposure to three attainable and subject-tolerated concentrations of PG aerosol. In addition, we exposed individuals for occupationally relevant durations at similar exposures to those that were tested for a 1-min duration in the Wieslander et al. (2001) study and found no evidence at the concentrations and durations tested that PG elicited respiratory effects or was an ocular irritant.

In fact, our results are consistent with the results from the Wieslander et al. (2001) study. The latter study revealed a small, exposure-related effect on symptom reports, as did we. It should be noted, however, that in both studies, the ratings for any symptoms never exceeded "slight", even at the highest exposure, which was significantly longer than previously tested. We did not include a clean air condition, as the visual appearance of the PG mist would have informed participants of the exposure condition. As well, the degree of visual occlusion would have provided information as to the exposure concentration and it is acknowledged that symptom reports can be biased by expectations and knowledge of the exposure (Dalton et al., 1997).

We also observed that the results of pulmonary function testing are consistent with the previous study in that they showed no clinically significant exposure-related decrease in either FEV_1 or FEV_1/FVC. In the Wieslander et al. (2001) study, the finding of a 5% decrease in FEV_1, shown by only 4 out of 27 healthy volunteers can be interpreted as within the normal variation expected with repeated spirometric measurements in a research study (Pellegrino et al., 2005) As well, post-exposure spirometric values were 102% of predicted for this healthy cohort Moreover, the small, albeit significant, decrease in the FEV_1/FVC ratio was also not indicative of impairment of lower airways as the ratio was greater than 80% both pre- and post-exposure, indicating the absence of any obstructive defect (Pellegrino et al., 2005).

The subjective symptom reports of upper airway and ocular dryness may well represent a consequence of the hygroscopic nature of PG, rather than effects of sensory irritation on mucosal tissue, via the trigeminal nerve (Doty, 1975). Our results demonstrated that both irritation and dryness ratings were highly correlated. That is, as ratings of irritation increased with PG exposure, ratings of dryness also increased. It is plausible that naïve participants confuse sensations associated with the dehydrating effects of hygroscopic compounds (e.g. difficulty swallowing, globus, increased phlegm, dry eye) to fall under the larger category of "irritation". Therefore, the sensory distinction between irritation and dryness is important to consider when relying on subjective ratings to investigate the irritant effects from chemical exposures. Previous literature regarding histologic changes using animal models following PG exposure (Suber et al., 1989) may, at least in part, be associated with cell and tissue responses to mucus membrane drying. Specifically, squamous metaplasia has been observed in patients with dry eye syndrome (Murube & Rivas, 2003) as well as eye dessication challenges in mice (De Paiva et al., 2007).

For the exposure conditions, we were able to control the concentration of respirable (~2–5 micron) droplets generated. However, the aerosol (droplet + gas phase) concentration was significantly higher than what was generated and maintained in droplet form. Given that the Wieslander et al. (2001) study only measured total PG concentration, it is unknown what fraction of the mist they generated was

Table 6. Dryness ratings over time (30, 120, 240 min) by exposure condition. 1 = None, 2 = Slight, 3 = Moderate, 4 = Very, 5 = Extremely.

		Exposure concentration		
Time point		Low	Medium	High
Pre	Mean	1.09	1.10	1.13
	SE	0.04	0.04	0.06
T30	Mean	1.33	1.40	1.74
	SE	0.09	0.11	0.15
T120	Mean	1.55	1.66	
	SE	0.15	0.17	
T240	Mean	1.73	1.72	
	SE	0.20	0.16	

Table 7. Spearman rho correlations for irritation versus dryness.

Region	Spearman Correlation	*p* value
Oral cavity	0.72	.000
Nasal vestibule	0.44	.000
Nasal cavity	0.80	.000
Nasopharynx	0.77	.000
Oropharynx and laryngopharynx	0.80	.000
Eyes	0.65	.000

composed of aerosol, nor the size of any aerosols that were generated. Nevertheless, the total PG concentrations we measured in the medium and high conditions spanned the range of concentrations tested in the earlier study.

While the current study is limited in that participants were only exposed on one occasion to each of three concentrations, the absence of any objective findings on respiratory tract effects at these concentrations and durations suggests that PG does not elicit acute changes in respiratory function. Given the potential for more widespread exposure among humans to heated PG aerosol and vapor from e-cigarettes than from theatrical or occupational exposures, we wondered how the concentrations used in our study related to exposures to PG from vaping. An analysis of the concentration of inhaled heated PG mist from one variety of e-cigarette (shisha-pen, (Kienhuis et al., 2015)) reported concentrations comparable to our medium and high exposures (430–603 mg/m^3) and suggested on the basis of the Wieslander et al. (2001) study that the concentration of PG in one type of e-cigarette was sufficient to cause irritation of the airways (Kienhuis et al., 2015). Due to differences in delivery route (oronasal inhalation in our study versus oral inhalation from vaping) and exposure duration (acute versus repetitive exposures), our study cannot inform about the long-term respiratory exposure to PG in e-cigarettes. However, the current study does provide evidence that acute exposures to high concentrations of PG aerosol/vapor comparable to those emitted by one type of e-cigarette were well tolerated by participants and did not elicit respiratory effects.

In conclusion, we exposed 20 healthy adults to three different concentrations of PG droplets and vapor for acute, occupationally relevant durations. From this study, we suggest that the available scientific data do not provide support that PG elicits changes in respiratory function or ocular irritation in humans.

Disclosure statement

Marcy Banton was employed by Lyondell Chemical Company. The other authors report no conflicts of interest.

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