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Hematological variations in healthy participants exposed 2 h to propylene glycol ethers under controlled conditions

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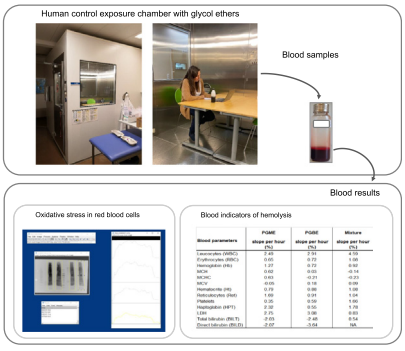
HIGHLIGHTS

- Possible hematotoxicity in humans chronically exposed to PGME and PGBE should be assessed.
- Low propylene glycol derivative air concentration exposures modulated blood parameters.
- RBCs, Hb, Ht, and platelets may increase in response to glycol ether exposure.
- No oxidative stress was observed in RBCs during exposure to propylene glycol derivatives.

GRAPHICAL ABSTRACT



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ABSTRACT

Glycol ethers are solvents used in a plethora of occupational and household products exposing the users to potential toxic effects. Several glycol ethers derived from ethylene glycol induce hematological toxicity, such as anemia in workers. The exposure effects on blood cells of glycol ethers derived from propylene glycol are unknown in humans. The aim of our study was to evaluate blood parameters indicative of red blood cell (RBC) hemolysis and oxidative stress in participants exposed to propylene glycol (propylene glycol monobutyl ether (PGBE) and propylene glycol monomethyl ether (PGME)), two extensively used propylene glycol derivatives worldwide.

Seventeen participants were exposed 2 h in a control inhalation exposure chamber to low PGME (35 ppm) and PGBE (15 ppm) air concentrations. Blood was regularly collected before, during (15, 30, 60, and 120 min), and 60 min after exposure for RBC and oxidative stress analyses. Urine was also collected for clinical effects related to hemolysis.

Under the study conditions, our results showed that the blood parameters such as RBCs, hemoglobin concentration, and white blood cells tended to increase in response to PGME and PGBE exposures. These results raise questions about the possible effects in people regularly exposed to higher concentrations, such as workers.

1. Introduction

Solvents are ubiquitous in professional and domestic products that can threaten human health inducing hematotoxic, neurotoxic, and reprotoxic effects (Sainio, 2015; Arif et al., 2008; Welch and Cullen, 1988; Shih et al., 2000). Glycol ethers are a broad family of chemicals used as solvent to obtain a homogeneous mixture with molecules having different solubility characteristics (OECD SIDS, 2003). Among commonly used glycol ethers

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include these derived from ethylene oxide (ethylene glycol derivatives) and propylene oxide (propylene glycol derivatives) combined with an alcohol (OECD SIDS, 2003). Since the 1970s, several ethylene glycol derivatives have shown to be hematotoxic on either the hematopoietic system in bone marrow (i.e., blood cell production) or the mature peripheral red blood cells (RBCs) (European Centre for Ecotoxicology and toxicology of chemicals, 2005). RBC lysis (i.e., hemolysis) was observed in different animal-model species when exposed to ethylene glycol derivatives (INSERM Collective Expertise Centre, 2000; Starek et al., 2008; Starek-Świechowicz et al., 2012). Hemolysis is defined as the destruction of RBCs and a clinical manifestation of this is anemia. Several investigations have shown that workers exposed to ethylene glycol derivatives can suffer from anemia (Welch and Cullen, 1988; Maksoud et al., 2018; Shih et al., 2003). A decrease in RBC and platelet counts, and in Hb concentration were observed after occupational exposure confirming the hematotoxicity of these glycol ethers derivatives in humans (Welch and Cullen, 1988; Shih et al., 2000; Nagano et al., 1984). The discovery of the hematotoxic but also reprotoxic effects of numerous ethylene glycol derivatives resulted in their ban from domestic products and restrictions in industrial settings (Institut national de la santé et de la recherche médicale (INSERM), 2005). As a result, the use of propylene glycol derivatives increased (Multigner et al., 2005). Based on a few toxicological studies compared to those on ethylene glycol derivatives, the metabolic pathway of the propylene glycol derivatives was considered less toxic and identical for all of them. Some were eye or upper airway irritants and labeled as so (SUVA, 2020). Nowadays, the absence of a comprehensive toxicity database for propylene glycol derivatives continues to support the general assumption that these chemicals are not associated with other effects than local irritation at high concentrations.

The most widely used propylene glycol derivatives in the European Union (EU) is propylene glycol monomethyl ether (PGME, CAS # 107-98-2), with a marketed volume ranging from 100,000 to 1,000,000 tons per year (INERIS | Institut national de l'environnement industriel et des risques, 2022; European Chemical Agency (ECHA), n.d.). Propylene glycol monobutyl ether (PGBE, CAS # 5131-66-8) is another commonly used propylene glycol derivative with a marketed volume ≥ 10,000 tons per year in the EU. PGME and PGBE are extensively used worldwide in the industrial manufacture of coating (e.g., paints and varnishes) and in the formulation of a wide range of products such as cleaning products, and anti-freeze, inks, glues, cosmetics, personal care products, and pesticides (Institut national de la santé et de la recherche médicale (INSERM), 2005; Agence française de sécurité sanitaire de l'environnement et du travail (afsset), 2008; Institut national de l'environnement industriel et des risques (INERIS), 2007; Agency for Toxic Substances and Disease Registry, n.d.). The general population, including workers is not immune to inhalation exposures to aerosols when sprayed and to vapor while handling these (European Centre for Ecotoxicology and toxicology of chemicals, 2005). Occupational exposure limit (OEL) values are set to prevent occupational diseases or other adverse effects in workers exposed 8 h, 5 working day over 40 years to some glycol ethers. In Switzerland, the OEL for PGME is 100 ppm in air (SUVA, 2020). There is no Swiss OEL for PGBE, meaning that workers handling products containing PGBE can be exposed to unlimited concentrations with no adverse effect on health. In our last toxicokinetic study with healthy participants, we reported an immediate bioavailability of PGME and PGBE in blood during inhalation exposure (Borgatta et al., 2021). When in blood, these solvents may induce hematological changes such as damages in RBCs' membrane integrity leading to eventual RBC hemolysis (Maurya et al., 2015) or a higher production of reactive oxygen species (ROS) than antioxidants, known as oxidative stress (Sies et al., 2017).

Based on the lack of human toxicological data on glycol ether derivatives and the hematological effects reported in the literature for ethylene glycol derivatives, we aimed to focus on RBCs to determine the presence of hemolysis or oxidative stress in human after the inhalation of the commercial PGME and PGBE. Healthy participants were exposed 2 h via the inhalation route to these glycol ethers alone and in mixture. Blood samples were regularly collected before, during and after exposures.

2. Materials and methods

2.1. Chemicals

Commercial PGME (CAS # 107-98-2, ≥99 %, CAS # 1589-47-5, <0.5 %) and PGBE (CAS # 5131-66-8, ≥99 %) were purchased from Sigma Aldrich (Buchs SG, Switzerland).

2.2. Graphical chart of the method

The study design, including participant recruitment, sample collection times, and type of exposure to glycol ethers, is concisely presented in Fig. 1.

2.3. Exposure design

The study was performed in a 12 m^3 steel exposure chamber that is well described in Devanthéry et al. (2002) and Tomicic et al. (2011). The exposure design is summarized in Fig. 1. Each participant participated to three exposure sessions of 2 h: PGME alone, PGBE alone, and PGME and PGBE in mixture (i.e., Mixture). At least one week elapsed between each session. The PGME air concentration was 35 ppm (i.e., 131 mg/m^3 (Institut national de l'environnement industriel et des risques (INERIS), 2015)) and PGBE 15 ppm (i.e., 83 mg/m^3 (Institut national de l'environnement industriel et des risques (INERIS), 2015)) during the two exposure hours. These air concentrations were representative for occupational exposures (Devanthéry et al., 2000) and because the participants are not used to the odor for these solvents, we set the total of the two glycol ethers correspond to half the Swiss OEL of PGME. The concentrations were similar to those used in our previous study. PGME and PGBE air concentration in the exposure chamber was monitored in real-time and air samples confirmed the target exposure concentration, as described in Borgatta et al. (2021).

2.4. Study population

This non-clinical study was approved in Switzerland by the official ethics committee of canton de Vaud for research studies with humans (Commission cantonale d'éthique de la recherche sur l'être humain, CER-VD, amendment to PB_2017-00043 (343/14) protocol, June 2017) and followed the rules set out by the Declaration of Helsinki.

Both women and men were invited to participate to the study using flyers at the University of Lausanne, the Lausanne University Hospital (CHUV), and an email list from previous studies. Participants who were interested in the study were invited to the Center for Primary Care and Public Health (Unisanté), University of Lausanne, Switzerland, for a health screening. Inclusion and exclusion criteria are presented in Table 1. When the inclusion criteria were met, the participants were invited to participate to the study. Blood was also collected to verify the renal and hepatic function of the participants exposed to PGME and PGBE (i.e., metabolism of glycol ethers): Aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyltransferase (GGT), lactate dehydrogenase (LDH), total bilirubin (BILT) direct bilirubin (BILD), haptoglobin (HPT), creatinine, sodium and potassium levels. Each participant signed a written informed consent form. At the end of the study, the participants were reimbursed for their time and inconvenience.

2.5. Blood samples

Nurses placed a small intravenous catheter (Optiva® II) in the participants' forearm to draw blood. Prior to entering the exposure chamber and directly after the intravenous cannulation, blood was collected and considered the baseline (t0). The exposure session started when the participant entered the exposure chamber. Blood was taken at several time points during exposure: 15 min (t1), 30 min (t2), 60 min (t3), and 120 min (t4). Blood

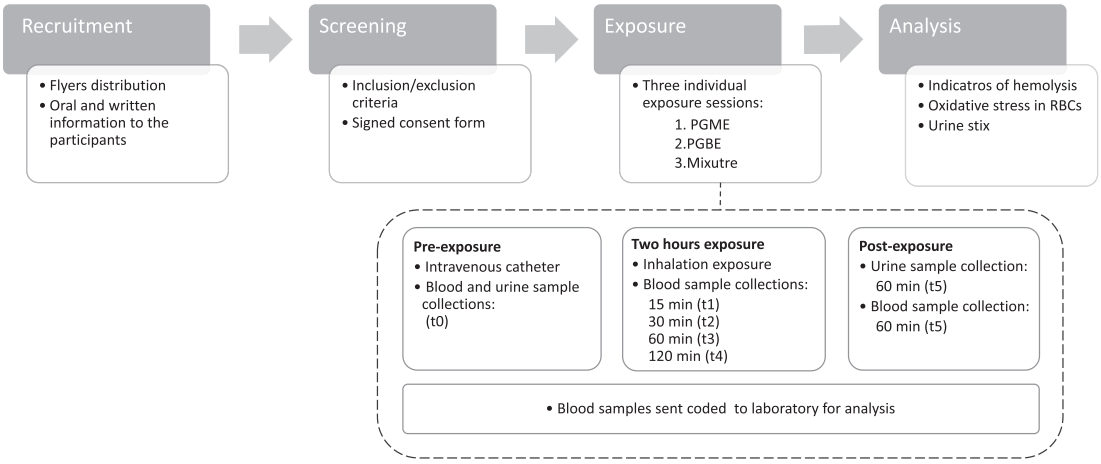

Fig. 1. Exposure and sampling time design of the study with healthy participants.

was also taken 60 min (t5) after the participant left the exposure chamber. The sampling times are summarized in Fig. 1. No tourniquet was used to reduce tourniquet-related risk of hemolysis (Shah et al., 2009).

2.6. Blood analysis

Indicators of hemolysis were assessed with hematological and blood chemistry analysis at t0, t1, t2, t3, t4 and t5. The studied hematological parameters of hemolysis were: complete blood cell count with red blood cell (RBC), reticulocytes (RET), hemoglobin (Hb), hematocrit (Ht), mean cell (or corpuscular) volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC). The laboratory results automatically provided white blood cells (WBC or Leucocytes) counts, although, this was not a primary objective in our study. Blood chemistry parameters included lactate dehydrogenase (LDH), total and direct bilirubin (BILT and BILD), and haptoglobin (HPT). Hematological samples were taken with K3 EDTA S-Monovette® tubes. Blood chemistry samples were collected in Lithium Heparin S-Monovette® tubes. Blood samples were stored in the dark at 4 °C for maximum 3 h before being sent to CHUV routine laboratories (www.chuv.ch/laboratoires). Presence of blood in urine (urinary stix) was checked in fresh urine at t0 and t5.

Table 1
Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Caucasian	Non-Caucasian
Aged between 18 and 65 years old	Aged <18 years
BMI between 18 and 25	BMI below 18 or >25
Absence of concomitant medication	Presence of concomitant medication
Non-smoker	Smoker
No history or presence of liver, kidney, neurological, or lung diseases	History or presence of liver, kidney, neurological or lung diseases
No history of psychiatric disorder	History of psychiatric disorder
No history of asthma	History of asthma
No history of claustrophobia	History of claustrophobia
Not occupationally exposed to glycol ethers	Occupationally exposed to glycol ethers
Man and non-pregnant woman (negative pregnancy test)	Pregnant
Not breastfeeding	Currently breastfeeding
Able to understand the procedure[a]	Not able to understands the procedure[a2]
Has signed the consent form for research project participation	Has not signed the specific consent form for research project participation

[a] Presumed ability to understand/discern the written and oral explanations during the first visit. This capability will be assessed as follows: by asking the participants to repeat what they understood about the study; by asking questions to the participants and assessing their answer (e.g. studied glycol ethers, number of visits, right to accept or reject to participate in the study, etc.).

2.7. Oxidative stress in erythrocytes

Oxidative stress in RBC was assessed as described by Renella et al. (2014). In brief, blood was sampled at t0 and t4 with K3 EDTA S-Monovette® tubes. Carbonylation of erythrocyte membrane proteins was quantified as a hallmark of the suspected glycol-ether exposition-induced oxidative stress, using the OxyBlot Protein Oxidation Detection Kit (Sigma-Aldrich S7150). Blood samples were centrifuged for 10 min at 2000*g* at 4 °C. Pelleted erythrocytes were washed with cold 0.9 % NaCl and centrifuged for 15 min at 2000*g* at 4 °C, at least 3-times, or until obtaining a clear supernatant. Erythrocytes were then submitted to hypotonic lysis by incubation of 1 h at 4 °C in a 1/10 PBS solution. Lysates were subsequently centrifuged for 30 min at 20,000*g* at 4 °C to pellet membranes. The supernatant was carefully removed, and the membrane pellet resuspended in lysis buffer and centrifuged under the same conditions to remove free soluble hemoglobin. Washes were repeated at least 3-times, or until obtaining a clear supernatant. Membrane pellets were stored frozen at −80 °C. Membrane proteins were solubilized in 5 volumes of deoxycholate-containing buffer, as described in Delobel et al. (2012), and assayed for protein quantification using the Bradford method.

Samples of 10 to 15 μg were submitted to carbonyl groups-derivatization with 2,4-dinitrophenylhydrazine, as per the kit protocol. Proteins were then separated by SDS-PAGE electrophoresis using precast 4–15 % polyacrylamide gradient gels (BioRad 456-1084). Proteins were then transferred onto PGDF membranes at 100 V for 1 h, and immunodetection of derivatized carbonylated-proteins was performed as per the kit protocol. ECL revelation was realized using the WesternBright Sirius detection kit (Witec K-12043-D10) and automatic image acquisition (Vilber Fusion FX). Images were analyzed using the freeware ImageJ (version 1.52t) by integrating the Area Under the Curve of migration lane signals.

2.8. Statistics

2.8.1. Blood parameters

The statistical analyses were all based on log10 transformed outcomes i.e. total number of RBCs and WBCs and the relative proportions by subtype (eosinophils, neutrophils, lymphocytes, monocytes), Hb, MCH, MCHC, MCV, Ht, Ret, platelets, HPT, LDH, BILT, and BILD. These were modelled assuming a linear trend during exposure (four samples per participants) and a separate indicator for the sample taken 60 min post exposure (t5).

The two key parameters are the slope of outcome by unit of time within the exposure period (parameter A) and the difference between the model-predicted log-transformed biomarker at end of exposure and 1 h

post exposure (parameter B) fitted in the same model. These two parameters were included as random parameters in a linear mixed model and were thus, allowed to vary by participant around central values, which are the main results of this analysis. As the dependent variable is log-transformed, parameter A can be expressed as the % increase (or decrease) per hour during exposure. Similarly, parameter B can be expressed as the % increase (or decrease) in the hour post exposure from the value at the end of exposure. First, we applied this model separately on the three exposure sessions i.e. PGME alone, PGBE alone, and Mixture. In a second analysis, we regrouped all data and rerun the preceding model with inclusion of an interaction parameter between the slope and the experiment. The main output of the latter model is the *p*-value of the Wald test of the absence of difference in the slope between the three parameters. Fig. 2 shows a fictitious example of this model for one participant. In a final analysis, we kept only the outcome value before exposure (t0) and value 1 h after exposure (t5) to test whether the recovery was complete 1 h post exposure (parameter C).

2.8.2. Oxyblots

The results of the oxyblot assays were given as ratios of areas under the curve (AUC) i.e. AUC at end-exposure (t4) divided by the pre- (t0) AUC. Similarly, to the analyses of the blood parameters, these AUC ratios were log-transformed and analyzed using a mixed model with the participant as a random effect and the chemical as fixed effect.

3. Results

3.1. Study population

Six women and eleven men were enrolled in the study (n = 17). Table 2 summarizes the age, BMI, body weight and body height of the participants.

3.2. Hemolysis indicators

Table 3 summarizes the slope (in % per hour) and 95 % confidence interval (CI) of the analyzed blood parameters. For the parameters related to blood hemolysis during exposure (t0 to t5), we observed a statistically significant increasing slope of RBC (1.08 %), Hb (0.92 %), Ht (1.1 %), HPT (1.78 %) and platelets (1.66 %) in the participants exposed to Mixture. Hb and Ht also increased significantly in participants exposed to PGME and PGBE alone. The increase was statistically significant for RETI (1.689 %), MCH (0.621 %), MCHC (0.629 %), and HPT (2.32 %) during PGME exposure. Leucocytes indicated a significant trend toward an increase in slope during exposure, particularly lymphocytes that significantly

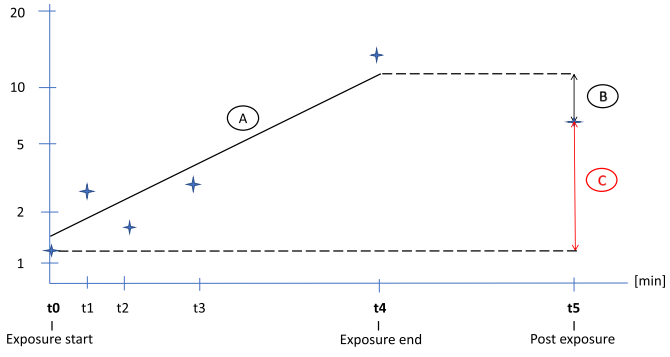

Fig. 2. Parameter A describes the slope of outcome (log-transformed) with exposure time. Parameter B gives the difference between model predicted value of the outcome at end of exposure and 1 h after the exposure ended. Parameter C (in red) is the difference between the outcome before exposure and its value 1 h after exposure. Exposure start (t0), 15 min (t1), 30 min (t2), 60 min (t3) and 120 min (t4) of exposure. Blood was also taken 60 min (t5) after the participant left the exposure chamber.

Table 2
age, body mass index, body weight and body height of the six women and eleven men enrolled in the study.

Participants		Age [years]	Body weight [kg]	Body height [cm]	Body mass index
Women	Mean	29	58	140	21
	SD	11	25	65	9
Men	Mean	29	76	178	24
	SD	13	12	10	3

increased during PGBE (4.47 %) and Mixture (3.65 %) exposures. A descriptive table (Table A1) with Q1, median, and Q3 at prior- (t0), end- (t4), and post- (t5) exposures to PGME, PGBE, and Mixture are provided as supplementary data. Blood was not found in the participant urine samples in any exposure conditions (data not shown).

Post- (t5) vs end-exposure (t4) comparisons showed that WBCs significantly increased by 4.59 % and 2.91 % per hour when the participants were exposed to the Mixture and PGBE alone. After these two exposures had stopped, eosinophils continued to increase by 14.95 % and 25.21 %, lymphocytes by 10.67 % and 11.82 %, and neutrophils by 8.43 % and 8.28 %. Eosinophils and lymphocytes also increased by 18.38 % and 8.33 % respectively, after PGME exposure stopped.

Post- (t5) vs pre-exposure (t0) comparisons for PGME (Table 3A) showed that all blood parameters returned to pre-exposure level. The pattern was not so clear for PGBE (Table 3B) and Mixture (Table 3C). In both exposure conditions, WBCs did not return to pre-exposure level. An hour post exposure, the number of WBCs was still elevated by 8.26 % and 12.78 % for PGBE and Mixture respectively. For PGBE, the number of eosinophils was increased by 26.13 % and HPT by 2.54 %. For Mixture it was the neutrophils that were still high by 14.86 % an hour post exposure. No statistically significant difference was observed between the three exposure conditions: PGME, PGBE and Mixture (data not shown).

3.3. Oxidative stress in erythrocytes

Fig. 3 summarizes oxidative stress responses in RBC membrane at the end of the glycol ether exposures. The ratio results showed that PGME and PGBE exposures (alone or in mixture) did not significantly alter the oxidative metabolism balance in the RBC membrane under our studied conditions. RBC membrane proteins were neither less or more oxidized at the end of the exposure to PGME, PGBE, and Mixture.

4. Discussion

Our results showed exposure-related effects on hemolysis indicators in humans when exposed 2 h to low PGME and PGBE air concentrations. RBCs, Hb, Ht, HPT, and platelets increased mainly during exposure and these responses dropped to the pre-exposure (t0) values when the participants were no longer exposed. The tendency was different for WBCs, as some lineages increased mainly after exposure, whether or not they were elevated during exposure, indicating a slower response to exposure than RBCs. No oxidative stress in RBCs was observed meaning that PGME and PGBE do not alter the oxidative metabolism in RBCs' membrane when these solvents are inhaled. Our results on RBCs and WBCs are opposite to those recorded in peer-reviewed studies with workers and animals exposed to ethylene glycol and propylene glycol derivatives. In workers chronically exposed to ethylene glycol derivatives, blood analyses showed a decrease in RBCs, WBCs, platelets, and Hb (Welch and Cullen, 1988; Shih et al., 2000; Nagano et al., 1984), and an anemia (Welch and Cullen, 1988; Maksoud et al., 2018; Shih et al., 2003). Hemolysis or hemolytic anemia were also observed in animal-models and in vitro RBC studies but the exposure time to these solvents was not specified (European Centre for Ecotoxicology and toxicology of chemicals, 2005). High oral exposure dose to ethylene glycol derivatives decreased RBCs, WBCs, platelets, and Hb in mice after five weeks of exposure (Nagano et al., 1984). No hematological change was reported in both rabbits exposed 13 weeks and in rats and mice

Table 3
Blood parameter gradual differences during exposure (i.e., from t0 to t4), post- vs end-exposure (i.e., t5 vs t4), and post- vs before exposure (i.e., t5 vs t0). The differences are expressed as the slope per hour in % with 95 % confidence interval (CI). The participants were exposed 2 h to PGME (A.), PGBE (B.) and PGME-PGBE in Mixture (C.). Significant results are shown in bold.

A. PGME

Blood parameters	During exposure			Post- vs end- exposure			Post- vs before exposure	
	Diff. %	95 % CI		Diff. (%)	95 % CI		Diff. (%)	*p*
Leucocytes (WBC)	2.49	−0.46	5.52	**4.58**	0.21	9.13	3.96	0.34
Eosinophils	−3.39	−10.75	4.58	**18.38**	1.94	37.47	−1.16	0.88
Neutrophils	2.66	−0.81	6.26	4.14	−0.26	8.74	6.46	0.18
Lymphocytes	2.38	−1.40	6.31	**8.33**	1.26	15.88	2.71	0.60
Monocytes	1.90	−1.33	5.24	−4.25	−10.86	2.85	−5.33	0.31
Erythrocytes (RBC)	0.65	−0.07	1.37	−0.88	−1.83	0.07	0.38	0.70
Hemoglobin (Hb)	**1.27**	0.73	1.82	**−1.32**	−2.05	−0.58	1.07	0.13
MCH	**0.62**	0.23	1.01	−0.45	−1.28	0.39	0.68	0.16
MCHC	**0.63**	0.32	0.94	−0.60	−1.23	0.04	0.70	0.09
MCV	−0.05	−0.31	0.21	0.25	−0.32	0.81	0.16	0.61
Hematocrit (Ht)	**0.79**	0.15	1.43	−0.75	−1.76	0.26	0.61	0.47
Reticulocytes (Ret)	**1.69**	0.08	3.32	−2.14	−4.88	0.68	−0.16	0.93
Platelets	0.35	−0.90	1.63	−0.38	−2.62	1.91	0.96	0.57
Haptoglobin (HPT)	**2.32**	1.32	3.33	−0.84	−3.40	1.79	2.85	0.07
LDH	2.75	−0.42	6.02	−4.63	−9.44	0.44	−3.77	0.32
Total bilirubin (BILT)	−2.03	−9.08	5.56	6.96	−8.82	25.48	NA	NA
Direct bilirubin (BILD)	−2.07	−6.36	2.41	0.74	−8.42	10.82	NA	NA

B. PGBE

Blood parameters	During exposure			1 h post- vs end- exposure			1 h post- vs before exposure	
	Diff. %	95 % CI		Diff. (%)	95 % CI		Diff. (%)	*p*
Leucocytes (WBC)	**2.91**	0.62	5.25	**8.33**	3.50	13.38	**8.26**	0.04
Eosinophils	1.39	−3.38	6.39	**25.21**	5.83	48.13	**26.13**	0.04
Neutrophils	1.80	−1.47	5.17	**8.28**	2.79	14.06	6.88	0.16
Lymphocytes	**4.47**	0.80	8.26	**11.82**	6.18	17.77	**13.10**	0.02
Monocytes	2.32	−0.34	5.05	−2.25	−11.15	7.55	−4.53	0.33
Erythrocytes (RBC)	0.72	−0.03	1.48	−0.82	−2.36	0.74	−0.38	0.74
Hemoglobin (Hb)	**0.72**	0.10	1.34	−0.13	−1.15	0.91	0.79	0.36
MCH	0.03	−0.54	0.59	0.67	−0.62	1.98	1.22	0.07
MCHC	−0.21	−0.68	0.25	0.51	−0.51	1.55	0.59	0.20
MCV	0.18	−0.11	0.46	0.29	−0.31	0.89	**0.67**	0.04
Hematocrit (Ht)	**0.88**	0.15	1.61	−0.71	−2.18	0.78	0.24	0.81
Reticulocytes (Ret)	0.91	−0.70	2.56	−1.54	−4.86	1.90	0.71	0.70
Platelets	0.59	−0.47	1.67	0.94	−0.96	2.87	0.73	0.61
Haptoglobin (HPT)	0.55	−0.44	1.55	**1.82**	0.05	3.63	**2.54**	0.01
LDH	**3.08**	1.28	4.91	−1.32	−4.96	2.46	−0.77	0.73
Total bilirubin (BILT)	−2.48	−7.38	2.68	−3.23	−13.46	8.20	NA	NA
Direct bilirubin (BILD)	−3.64	−8.03	0.96	−3.87	−13.04	6.27	NA	NA

C. Mixture

Blood parameters	During exposure			1 h post- vs end- exposure			1 h post- vs before exposure	
	Diff. %	95 % CI		Diff. (%)	95 % CI		Diff. (%)	*p*
Leucocytes (WBC)	**4.59**	1.72	7.53	**9.11**	4.63	13.78	**12.78**	0.00
Eosinophils	−7.04	−14.14	0.65	**14.95**	3.90	27.18	−6.03	0.52
Neutrophils	**4.94**	1.04	8.99	**8.43**	2.44	14.77	**14.86**	0.02
Lymphocytes	**3.65**	0.23	7.19	**10.67**	4.12	17.63	9.28	0.05
Monocytes	4.55	−0.18	9.50	0.35	−6.64	7.87	3.36	0.58
Erythrocytes (RBC)	**1.08**	0.41	1.75	−0.70	−2.10	0.72	1.26	0.22
Hemoglobin (Hb)	**0.92**	0.39	1.45	−0.81	−1.85	0.25	0.19	0.81
MCH	−0.14	−0.70	0.42	−0.14	−1.38	1.12	**−1.07**	0.04
MCHC	−0.23	−0.70	0.24	−0.09	−1.09	0.92	**−0.84**	0.02
MCV	0.09	−0.16	0.34	0.09	−0.44	0.62	−0.14	0.60
Hematocrit (Ht)	**1.08**	0.44	1.73	−0.69	−1.96	0.60	0.88	0.34
Reticulocytes (Ret)	1.04	−0.66	2.78	−2.82	−6.28	0.76	−2.33	0.22
Platelets	**1.66**	0.42	2.91	−0.60	−2.96	1.82	2.53	0.18
Haptoglobin (HPT)	**1.78**	1.00	2.56	−1.51	−3.13	0.13	1.28	0.18
LDH	0.83	−1.19	2.90	−0.13	−4.14	4.06	−3.18	0.15
Total bilirubin (BILT)	0.54	−6.54	8.15	−11.01	−23.86	4.00	NA	NA
Direct bilirubin (BILD)	NA	NA	NA	NA	`NA	NA	NA	NA

exposed nine days to PGME via inhalation (Miller et al., 1984; Miller et al., 1981). Our results showed an exposure-related effect (i.e., marker of exposure) on RBCs and WBCs after a single inhalation exposure to PGME and PGBE, whereas the results found in workers and animal-model studies were clinically relevant (i.e., markers of effect) showing for example an anemia. Discrepancy between clinical and non-clinical responses exists with

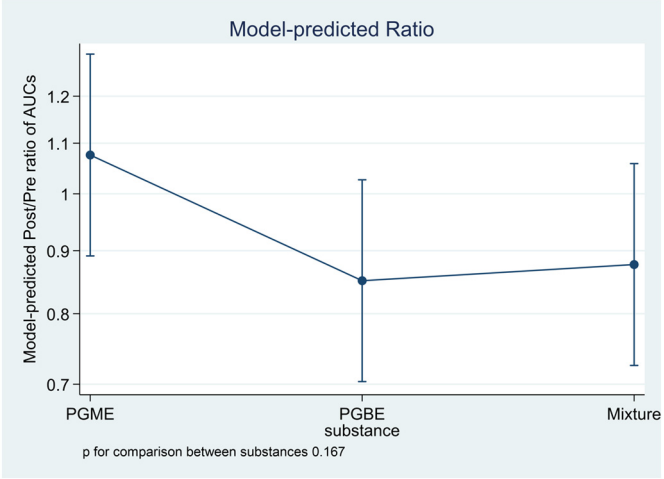

Fig. 3. Oxyblot results in RBCs after PGME, PGBE and Mixture exposures.

other chemicals known as hematotoxic. For instance, benzene is a solvent with well-known toxic effects on peripheral blood cells and bone marrow in humans and animal models. However, some studies demonstrated a lack of clinical signs of hematotoxicity in workers exposed to low air concentrations (Wilburn et al., 2007), although biological changes may have been already initiated. In our study, the short exposure time and low dose used for ethical reasons did not trigger clinical responses but our results suggest that the hematological effects reported in workers suffering from anemia may be induced when the exposure is chronic or at high doses, such as during cleaning, painting, varnishing activities. Meaningful exposure-related changes at low exposure concentrations may be predictive of toxic effects and studies are encouraged to not be restricted to clinically observable effects.

The internal concentrations of PGME and PGBE trigging non-clinical or clinical responses on blood cells are unknown. In our study, we monitored different blood parameters along exposure time and highlighted any changes from the baseline (i.e., before exposure). In a toxicokinetic study with participants exposed to PGME (35 ppm) and PGBE (15 ppm), the maximal internal blood concentrations after two hour exposure were 1.4 mg/L for PGME and 0.26 mg/L for PGBE (Borgatta et al., 2021). Blood concentration at the mg/L range may initiate hematological responses. Organic solvents are reported to have nonspecific physicochemical effects and can modulate cell membrane fluidity and influence the hydrophobic force regulating macromolecular interactions (Heipieper et al., 1994; Quinn, 1981). These properties explain the use of organic solvent to dissolve membrane lipids and release the integral proteins and subcellular components. In blood, PGME and PGBE concentration at the mg/L range may initiate effects on the lipid bilayer membrane of RBC membrane for instance. As a response to the aggression, RBC count increased in our study. Another possibility explaining the increase in RBCs may be an effect of the solvents at the respiratory level. PGME is a recognized irritant for human airways (nothing is known for PGBE) (SUVA, 2020). Irritants can trigger lung inflammation and decrease oxygen diffusion from the alveoli into blood. Both mechanisms can modulate RBC and WBC counts (Doerschuk, 2000; Wong et al., 2016; Bhutta et al., 2022). In the toxicokinetic study (Borgatta et al., 2021), the absorption in blood of PGME and PGBE slowed down (i.e., diffusion effect) during exposure. This toxicokinetic response was assumed to be related to lung inflammation decreasing the absorption rate of gases, including oxygen (Borgatta et al., 2021). The primary function of RBCs is to carry oxygen from alveoli to tissues. When oxygen level in blood is low, a negative feedback loop induces the production of RBCs (de Back et al., 2014; Ho et al., 2000), and thus, a possible increase of the cell count in blood. Inflammation also modulates WBC count (*Leukemoid Reaction - an overview*, 2022). An increase in WBCs was reported in female rats exposed to low PGME concentration (300 ppm) and a decrease in those exposed to high concentration (3000 ppm) (Miller et al., 1984). In our study, PGME low exposure-related effect was also an increase in white blood cell counts, which is consistent with the results found in female rats at 300 ppm. The increase in eosinophils observed post exposure may be related to the irritant effect of PGME and possibly PGBE. Eosinophils play a central role in asthma (Casas and Nemery, 2014; Busse and Sedgwick, 1992) and this pathology has been observed in workers exposed to glycol ethers such as professional cleaners (Folletti et al., 2017; Vincent et al., 2017; Kogevinas et al., 1999; Vizcaya et al., 2015). Neutrophils and lymphocytes have important effector functions in inflammation (Doerschuk, 2000). In rats exposed to ethylene glycol derivatives, the number of these cells showed a negative trend toward neutrophils and a positive in lymphocytes (Miller et al., 1981). In our study, neutrophils and lymphocytes were also increased and we assume this response related to irritant effects with an inflammatory response possibly starting in lungs. Although hematological effects after exposure were significantly different than controls, we cannot exclude that some of these effects were sporadic occurrences unrelated to exposure. Our study was first designed to focusing on RBCs to determine the presence of hemolysis and oxidative stress. An exposure session free of chemical would be relevant to perform to confirm our results on WBCs since nychthemeral rhythm and stress can also modulate these cells (Oishi et al., 2006; Nishitani and Sakakibara, 2014). The participants were sitting 2 h in 12 m^3 exposure chamber that may induce a certain stress, although, they self-reported as not suffering from claustrophobia (exclusion criteria).

Publications on hematological changes from participants exposed to propylene glycol derivatives such as PGME and PGBE in a controlled environment are absent. Neither peer-reviewed toxicological data in animal-models exist for PGBE. Therefore, our results are unique and currently not comparable. Moreover, our results provide human data based on a reasonably large number of participants (n = 17) and are free of interspecies variability. Our results indicated an exposure-related effect on RBCs involved in anemia, hemolysis, and inflammatory responses in humans exposed one time to low PGME and PGBE air concentrations alone and in mixture. Therefore, we recommend a biomonitoring as well as medical monitoring of potential hematological effects in workers that use regularly products containing propylene glycol derivatives. We also recommend additional human studies to understand the dose-response relationship between PGME and PGBE internal doses and hematological changes. The responses observed on WBCs must be taken with caution. One limitation of our study was that we did not include non-exposure sessions meaning performing the exposure sessions without exposure to any solvent to observe if this influences WBC counts. Toxicological studies on PGBE, among other glycols ethers, are encouraged to better understand possible hematological effects. An increase in toxicological knowledge on propylene glycol ethers will help in OEL setting, determining potential personal protective equipment use and/or occupational work ship alternatives to decrease the exposure.

5. Conclusion

Our results showed an exposure-related increase in some hemolysis indicators and inflammatory cells in healthy participants exposed 2 h in a control inhalation exposure chamber to low PGME (35 ppm) and PGBE (15 ppm) air concentrations. These hematological changes were related to low exposure concentrations, indicative of possible health effects in individuals regularly exposed to higher concentrations. In workers chronically using products based on these glycol ethers, clinical effects such as anemia or chronic respiratory inflammation cannot be excluded. The concentration and frequency of exposure, as well as the absorbed dose, influence the development of clinical symptoms of most chemicals. We recommend medical follow-up of workers exposed to propylene glycol derivatives, with special attention to PGBE for which toxicological data are lacking.

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CRediT authorship contribution statement

Borgatta Myriam	Conceptualization, Methodology, Investigation, Writing - Original draft preparation, Visualization, Project administration, Funding acquisition.
Wild Pascal	Formal analysis, Visualization, Review & editing
Julien Delobel	Investigation
Raffaele Renella	Conceptualization, Resources, Reviewing
Nancy Hopf	Methodology, Visualization, Writing - Review & editing, Supervision

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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