Pharmacological Reports
2012, 64, 166–178
ISSN 1734-1140

Hematological effects of exposure to mixtures of selected ethylene glycol alkyl ethers in rats

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Abstract:
Exposure to various ethylene glycol monoalkyl ethers (EGAEs) is known to result in hemolytic effect caused by their metabolites, appropriate alkoxyacetic acids, generated *via* both alcohol dehydrogenase and aldehyde dehydrogenase. It has been shown in many studies that administration of single doses of EGAEs to rats lead to dose- and time-dependent hemolytic anemia. The repeated exposure to isopropoxyethanol (IPE), and butoxyethanol (BE), contrary to methoxyethanol (ME) and ethoxyethanol (EE), resulted in significantly less pronounced hematological changes. While the majority of hematological effects were dramatic at the beginning of the exposure, later these changes clearly regressed despite continued weekly exposure to these ethers. The gradual recovery from the hemolytic anemia may be associated with tolerance development to the hemolytic effect of IPE and BE. ME demonstrated high hematotoxicity, which increased progressively and reached a maximum at the end of 4 week exposure, whereas EE revealed moderate hematological effects. It might be suspected that ME and EE may modified of IPE hemolytic activity in rats simultaneously treated with these compounds. In the rats co-exposed to IPE and ME subcutaneously at a relatively low doses of 0.75 mM + 0.75 mM for 4 weeks, a significantly less pronounced hematological changes at the beginning of the exposure in comparison with animals treated with IPE (0.75 mM) alone were observed. At the later period, i.e., at the end of 4 weeks exposure, the hematological alterations in the same animals were markedly pronounced and progressively elevated with exposure time, except for mean corpuscular volume (MCV) values, which were significantly lower in comparison with IPE group. ME at the higher dose of 1.25 mM/kg and EE at both doses of 0.75 and 1.25 mM/kg did not modify the hematotoxicity of IPE (at doses of 0.75 mM and 1.25 mM) at the beginning of the exposure, whereas increased its harmful effects at the end of the treatment. The amelioration in the majority of the hematological parameters at the beginning of the exposure may be caused by inhibitory effect of ME on IPE metabolism. On the contrary, an accumulation of the methoxyacetic acid and ethoxyacetic acid, toxic metabolites of ME and EE, respectively, and no tolerance development to the hemolytic effect of these two chemicals may be responsible for elevated hematological alterations at the end of the exposure.

Key words:
ethylene glycol ethers, mixtures, hematological changes, toxicodynamic interactions

Introduction

Ethylene glycol alkyl ethers (EGAEs) are used in a variety of industrial and household products, such as a number of paints, varnishes, engine fluids, hydraulic fluids, floor polishes and glass, leather, and upholstery cleaners [6].

EGAEs, i.e., 2-methoxyethanol (ME), 2-ethoxyethanol (EE), 2-isopropoxyethanol (IPE) and 2-butoxyethanol (BE), after uptake into organism may lead to adverse testicular, embriotoxic, teratogenic and hematological effects in animals and humans. The primary systemic toxicity of these chemicals in animals include reproductive, developmental, immunological and hematological disturbances [7, 9, 38, 42]. Recent data indicate that EGAEs can exert the effects on cell viability and on the hydrogen peroxide-induced damage in the human neuroblastoma cells *in vitro* [23]. These chemicals undergo metabolic activation to alkoxyacetic acids (AAAs) *via* alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) in the liver, testicles, and skin [2, 19]. AAAs are responsible for gonadotoxic, embryotoxic, teratogenic, immunotoxic and hemolytic effects [7, 8, 40].

Administration of single doses of EGAEs to rats caused dose- and time-dependent hemolytic anemia [8, 31], which was characterized by an early swelling of erythrocytes as evidenced by an increase in mean corpuscular volume (MCV), resulting in hemolysis and decline in the number of red blood cells (RBC), packed cell volume (PCV), hemoglobin concentration (HGB) and also in the increase of the plasma hemoglobin level and reticulocyte count (Ret) [11, 37]. In addition, BE caused secondary hemoglobinuria positively associated with the dose of this compound [8, 31] and elevated spleen weight/body weight ratio [12]. Spleen enlargement was attributed to sequestration of swelled and deformed RBC, what was directly related to the effect of BE on circulating erythrocytes. Stomatocytes, cup-shaped cells, and spherocytes are the main morphological features of erythrocytes from rats exposed to BE or *in vitro* treated with butoxyacetic acid (BAA), the metabolite of BE [39].

The majority of hematological effects in rats after IPE- or BE-administration at single doses in the range of 0.625–1.25 mM/kg were dramatic and reversible, after a long period of time (25 days), although the increase in MCV was persistent. Acute hematological effects of both ethers were comparable with respect to the profile of changes, their intensity and duration [33, 37]. Under the same conditions, both ME and EE at higher doses (1.25–5.0 mM/kg) did not induce hemolytic anemia [37]. In another study, ME and EE caused weak hemolysis in rats at a higher dose (10 mM/kg). Moreover, ME was significantly less hematotoxic than EE [33].

Also, in the study on the rats, subcutaneous repeated administration of ME, EE, IPE or BE led to distinct hematological alterations evidenced by a decrease in RBC, PCV, mean cell hemoglobin concentration (MCHC) and HGB, and by an increase in MCV value and Ret in peripheral blood. While in rats treated with middle dose of ME (2.5 and 5.0 mM/kg) these changes were strongly pronounced and progressively increased with time, those in animals exposed to EE at the same doses were rather persistent at low constant level for all exposure periods. In contrast, the rats treated with IPE and BE alone demonstrated the dramatic hematological changes at the beginning of exposure, mainly on day 4. Despite of exposure duration for 28 days, these changes were regressed, although the decrease in RBC and MCHC and also the increase in MCV and MCH in rats treated with IPE and BE at dose levels of 0.5–1.25 mM/kg were more persistent, probably due to selective hemolysis of the aged erythrocytes. Hemoglobinuria was observed only in the first day of exposure to BE at a dose of 1.25 mM/kg. The erythrocytes, which were produced during remissions from anemia, tended to be macrocytic and contain more hemoglobin (increased MCH), factors which may also contribute to the persistence of these changes [38].

The literature data indicate that the hemolytic and other adverse effects of EGAEs may be modulated in the metabolic way. The inhibition of ADH by pyrazole or 4-methylpyrazole is known to decrease the hemolytic effect of BE and the urinary excretion of BAA [8, 36]. In the acute experiment simultaneous administration of BE with ethanol, n-propanol or n-butanol to rats almost totally inhibited the hemolytic effect of this compound, and decreased the excretion of BAA in urine. In contrast, the co-administration of ethanol with ME did not modify the urinary excretion of methoxyacetic acid (MAA), but led to accumulation of this metabolite in rats [22, 24].

In case of short-term repeated exposure in rats, ethanol intake along with ME, EE and BE administration, only partially protected the animals against hemolytic effects and the alterations in leukocyte sys-

tem induced by these ethers [34]. The co-administration of EE with toluene and xylene (mixture of isomers) to male rats for 4 weeks reduced the extent of testes atrophy and also decreased the highest concentration and total amount of plasma ethoxyacetic acid (EAA), a metabolite of EE [5].

EGAEs are practically used as a mixtures in industrial or domestic solvent composition [41]. In an available literature no data were found on hematological effects of repeated exposure to mixtures of EGAEs in experimental animals and humans. The objective of present study was to evaluate the hematological effects and the changes in leukocyte system in peripheral blood of rats simultaneously treated with IPE and ME, and also IPE and EE for 4 weeks.

Materials and Methods

Chemicals

ME, EE, and IPE were furnished by Sigma-Aldrich Ltd., Poland. Other chemicals were obtained from POCh (Poland). ME, EE, and IPE solutions and their mixtures were made up in saline, immediately before dosing.

Animals

Male Wistar rats (Krf: (WI)WUBR), with an initial body weight of 310 g, purchased from Jagiellonian University Faculty of Pharmacy Breeding Laboratory (Kraków, Poland) were kept under standard animal house conditions (a room temperature of 22°C, a 12/12 h light/dark cycle, the light on at 8:00), with food (Murigram, Motycz, Poland) and water available *ad libitum*. The rats were randomly divided into 11 groups of five animals each. All experiments were carried out accordingly to the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Local Ethics Committee, Kraków, Poland.

Animal treatment

The rats were treated with IPE, ME or EE at doses of 0.75, and 1.25 mM/kg by subcutaneous injections in a fixed volume of 2 ml/kg body weight, regardless of a dose. Another rats were given the mixtures of IPE and ME or IPE and EE in a molar ratio 1:1 in a single doses once per day, 5 days per week, for 4 weeks. Control rats obtained the equivalent volume of saline. The subcutaneous administrations of examined chemicals were because of predominant role of skin absorption of these compounds in industrial conditions and contribution to their metabolic activation. Directly before experiment, during exposure and after its termination, i.e., at 0, 4, 11, 18, and 29 day, blood samples from the tail vein of rats were collected for hematological analyses.

Hematological analyses

Heparin-added whole blood samples, immediately after collection, were used for hematological analyses. RBC, PCV, MCV, HGB, MCHC, mean cell hemoglobin (MCH), and white blood cells (WBC) were analyzed by means of a COBAS MICROS (Roche, Palo Alto, CA, USA) analyzer. Ret was evaluated after staining blood samples (without anticoagulant) with brilliant-cresol blue. The differential white cell count was evaluated after Pappenheim-stained blood films. Hematological analyses were systematically checked as reported previously [38].

Statistical analyses

Results are expressed as the mean ± SD. Data were analyzed by two-way analysis of variance with repeated measurements on one factor (time or dose) and evaluation of simple effects. The analysis was performed with the SPSS 14.0 statistical packet (SPSS Inc. Chicago, IL, USA). Results were considered statistically significant when $p \leq 0.05$.

Results

Effects of IPE, ME, and EE alone and their combinations on hematological parameters

Hematological effects in peripheral blood of rats were quantitatively different depending on the type of EGAEs given. IPE alone, administered at doses of 0.75 and 1.25 mM/kg resulted in a decrease of RBC, PCV, and HGB and also in an increase in MCV and Ret. While the changes in RBC, MCV, and Ret oc-

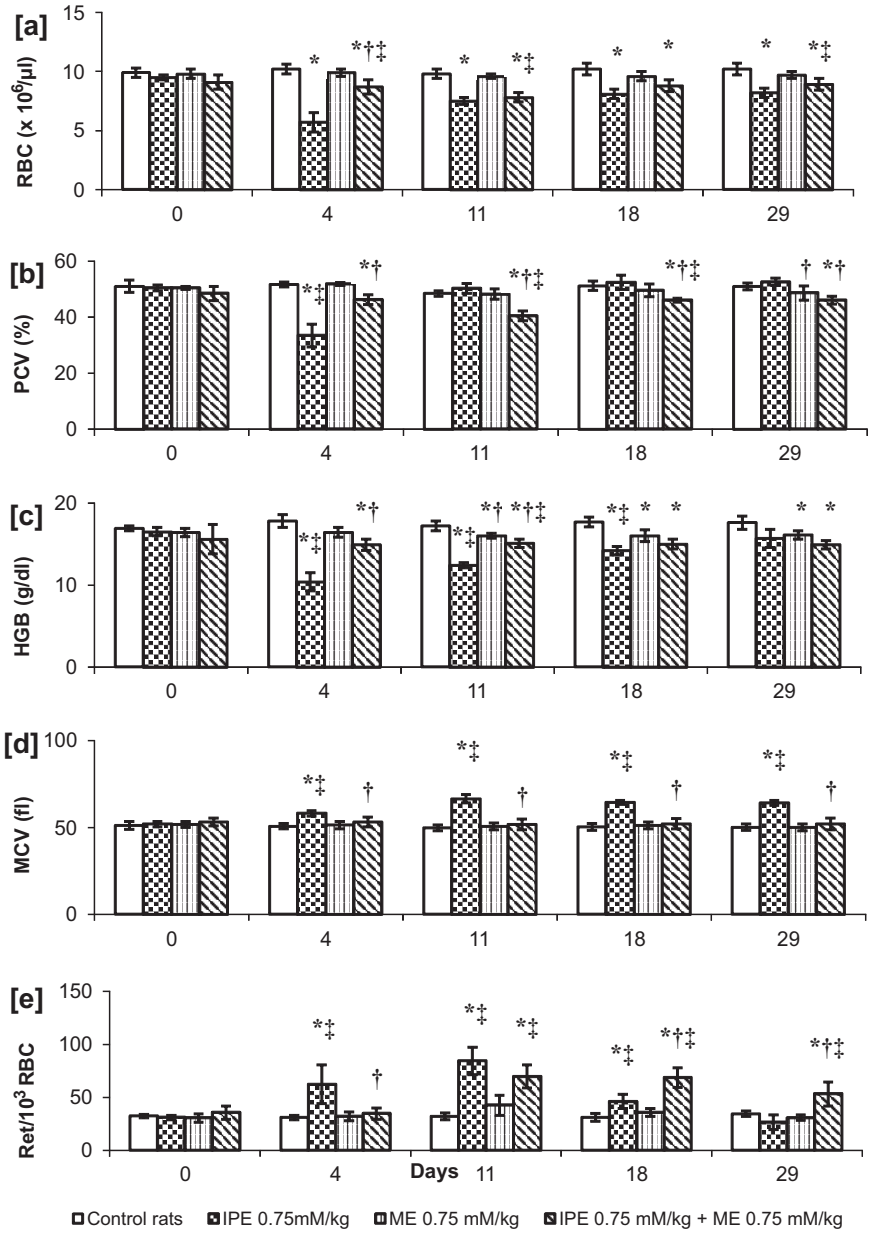

Fig. 1. Effects of combined exposure to isopropoxyethanol (IPE) and methoxyethanol (ME) at doses 0.75 mM/kg b.w. on RBC (**a**), PCV (**b**), HGB (**c**), MCV (**d**) and Ret (**e**) values of the peripheral blood at the designated time points of male rats. Values are the means ± SD of five rats, * – $p \leq 0.05$ significantly different from control rats; † – $p \leq 0.05$ significantly different from rats treated with IPE alone; ‡ – $p \leq 0.05$ significantly different from rats treated with ME alone

curred mainly from the 4th day of exposure to its termination, the alterations in other hematological parameters, i.e., PCV and HGB were most pronounced at the beginning of exposure (at 4th day). ME alone at the low dose of 0.75 mM/kg caused only a decrease in HGB on days 11, 18 and 29 of exposure, whereas at the higher dose of 1.25 mM/kg led to decline in RBC at day 11, PCV on days 11, 18, and 29, and HGB at days 18 and 29 of experiment (Figs. 1 and 2).

In the rats co-exposed to IPE and ME at the lower doses (0.75 mM + 0.75 mM), significantly less pronounced alterations in all hematological parameters were observed on day 4 of exposure in comparison with animals exposed to IPE alone. At the later period, especially on days 18 and 29 of exposure, the RBC, PCV and HGB were significantly lower, whereas Ret was higher in comparison with the control group. The changes in PCV and Ret at this period were more pronounced than in the rats treated with IPE alone. The MCV values at days 11, 18 and 29 did not differ with control group, but were significantly lower in comparison with animals exposed to IPE alone (Fig. 1).

The rats simultaneously treated with the higher doses of IPE and ME (1.25 mM + 1.25 mM) demonstrated the changes in RBC, PCV and HGB similar to

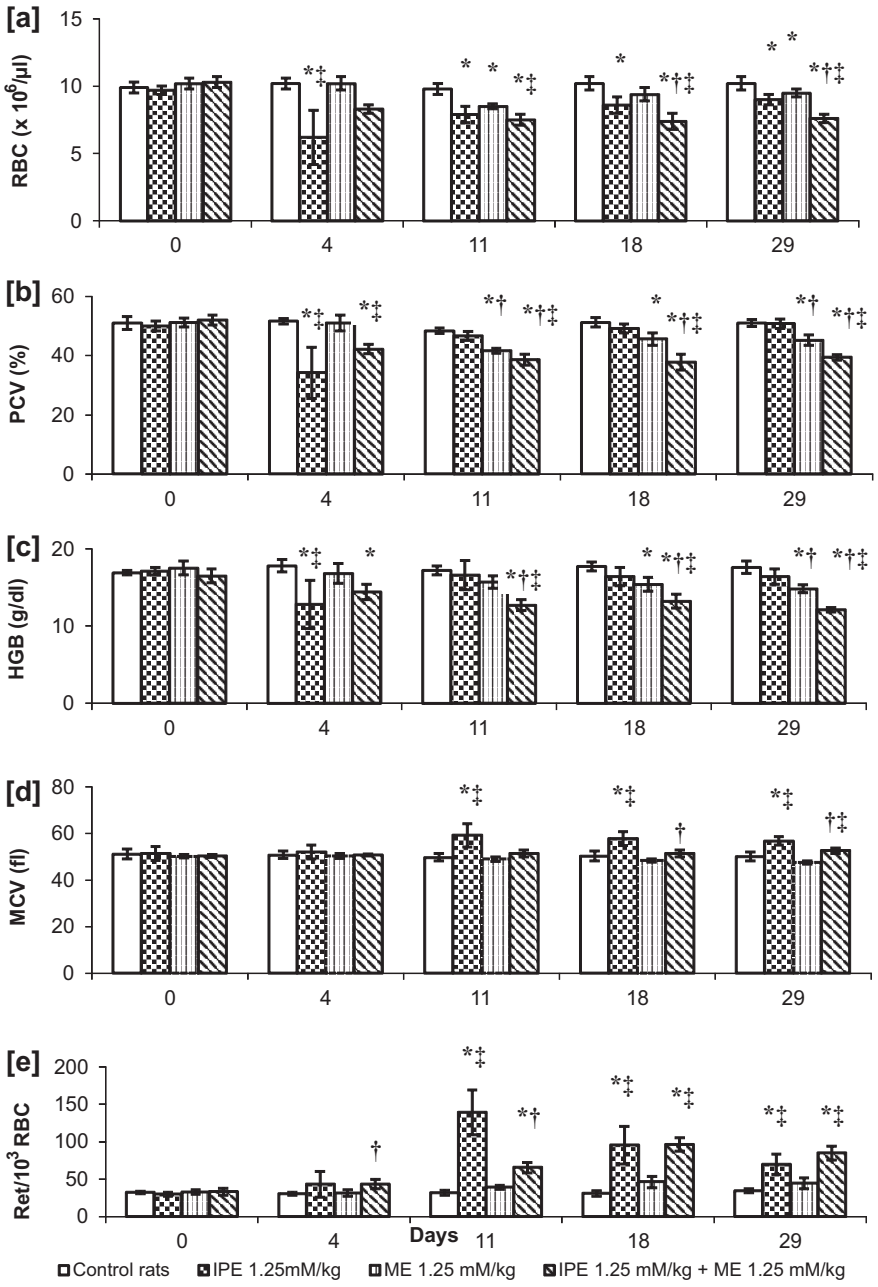

Fig. 2. Effects of combined exposure to isopropoxyethanol (IPE) and methoxyethanol (ME) at doses 1.25 mM/kg b.w. on RBC (**a**), PCV (**b**), HGB (**c**), MCV (**d**) and Ret (**e**) values of the peripheral blood at the designated time points of male rats. Values are the means ± SD of five rats, * – $p \leq 0.05$ significantly different from control rats; † – $p \leq 0.05$ significantly different from rats treated with IPE alone; ‡ – $p \leq 0.05$ significantly different from rats treated with ME alone

the animals exposed to IPE alone at 4th day of exposure. At the later period, i.e., on days 11, 18 and 29, the alterations in RBC, PCV and HGB were more pronounced than in the rats treated with IPE alone. Other hematological parameters, MCV and Ret were significantly lower than in the rats exposed to IPE alone on days 18 and 29 or 11, respectively. Ret at days 18 and 29 did not differ in comparison to IPE alone treated group (Fig. 2).

EE given to rats at the lower dose of 0.75 mM/kg was ineffective. EE administered at the higher dose of 1.25 mM/kg led to a decrease in PCV at day 18, and in HGB on days 18 and 29 of exposure. Other values of the hematological parameters were similar as in the control group (Figs. 3 and 4).

In the rats co-exposed to IPE and EE at the lower doses (0.75 mM + 0.75 mM), the values of RBC, PCV and HGB at 4th day of exposure were higher, whereas MCV, and Ret were markedly lower in comparison to the rats treated with IPE alone. At the later period, i.e., at days 11, 18 and 29, the hematological alterations in principle were similar to the changes ob-

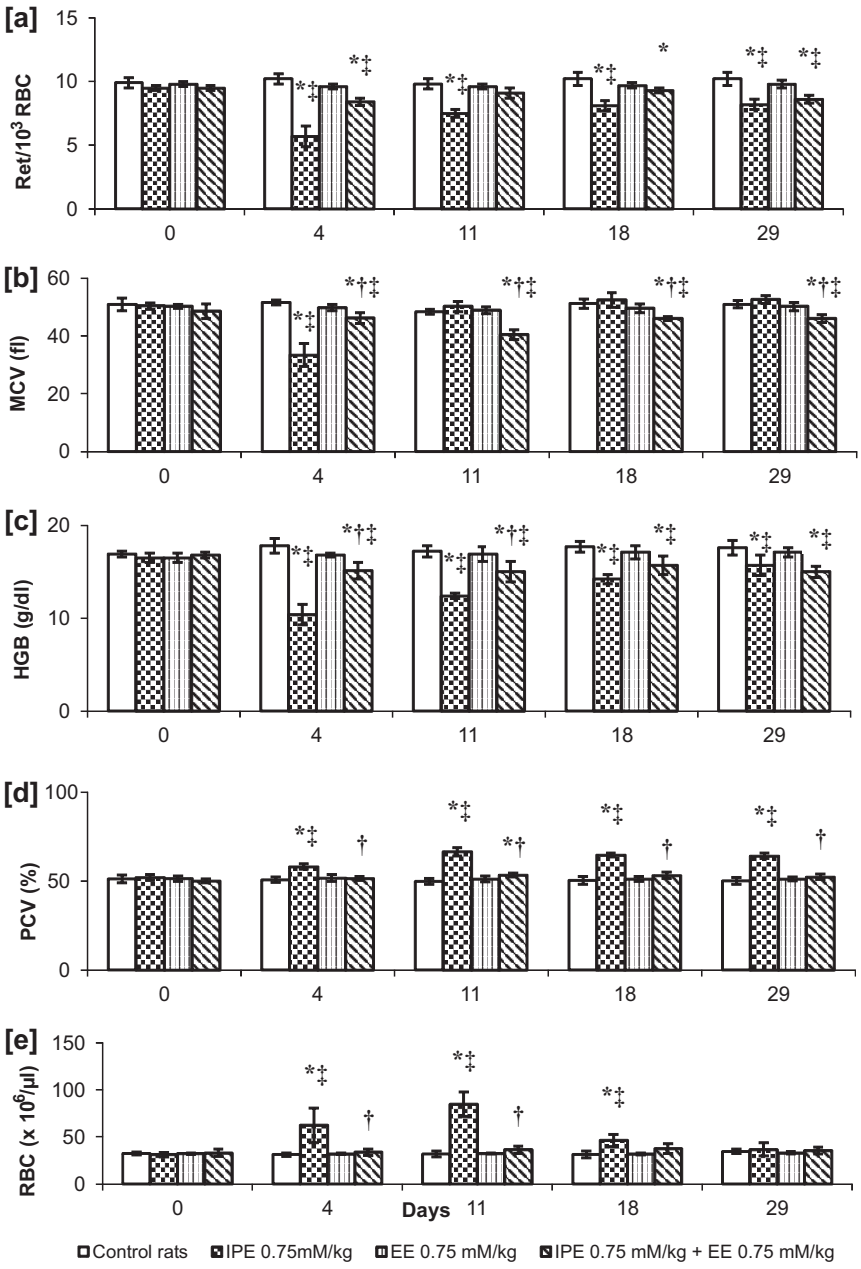

Fig. 3. Effects of combined exposure to isopropoxyethanol (IPE) and ethoxyethanol (EE) at doses 0.75 mM/kg b.w. on RBC (**a**), PCV (**b**), HGB (**c**), MCV (**d**) and Ret (**e**) values of the peripheral blood at the designated time points of male rats. Values are the means ± SD of five rats, * – $p \leq 0.05$ significantly different from control rats; † – $p \leq 0.05$ significantly different from rats treated with IPE alone; ‡ – $p \leq 0.05$ significantly different from rats treated with EE alone

served at day 4 of exposure. The values of RBC and HGB did not differ in comparison to the IPE group. PCV values at days 11, 18 and 29 were significantly lower than in the control, IPE alone and EE alone treated groups. Both MCV and Ret were persistent at levels of the control group and were significantly lower than in the rats treated with IPE alone on days 11, 18, 29 and 11 of exposure, respectively (Fig. 3).

In the rats simultaneously treated with IPE and EE at the higher doses (1.25 mM + 1.25 mM), RBC, PCV and HGB at 4th day of exposure did not differ in comparison to the rats exposed to IPE (1.25 mM/kg) alone, whereas MCV and Ret were significantly higher. PCV values at days 11, 18 and 29 were markedly lower than in other groups. The values of others hematological parameters, i.e., HGB, MCV and Ret did not differ in comparison with IPE alone group, but were significantly lower (HGB) or higher (MCV and Ret) than in control and EE groups (Fig. 4).

Effects of EGAEs on leukocyte system

In the rats treated with IPE alone at dose of 0.75 mM/kg, the decrease in WBC and reduction in lymphocyte

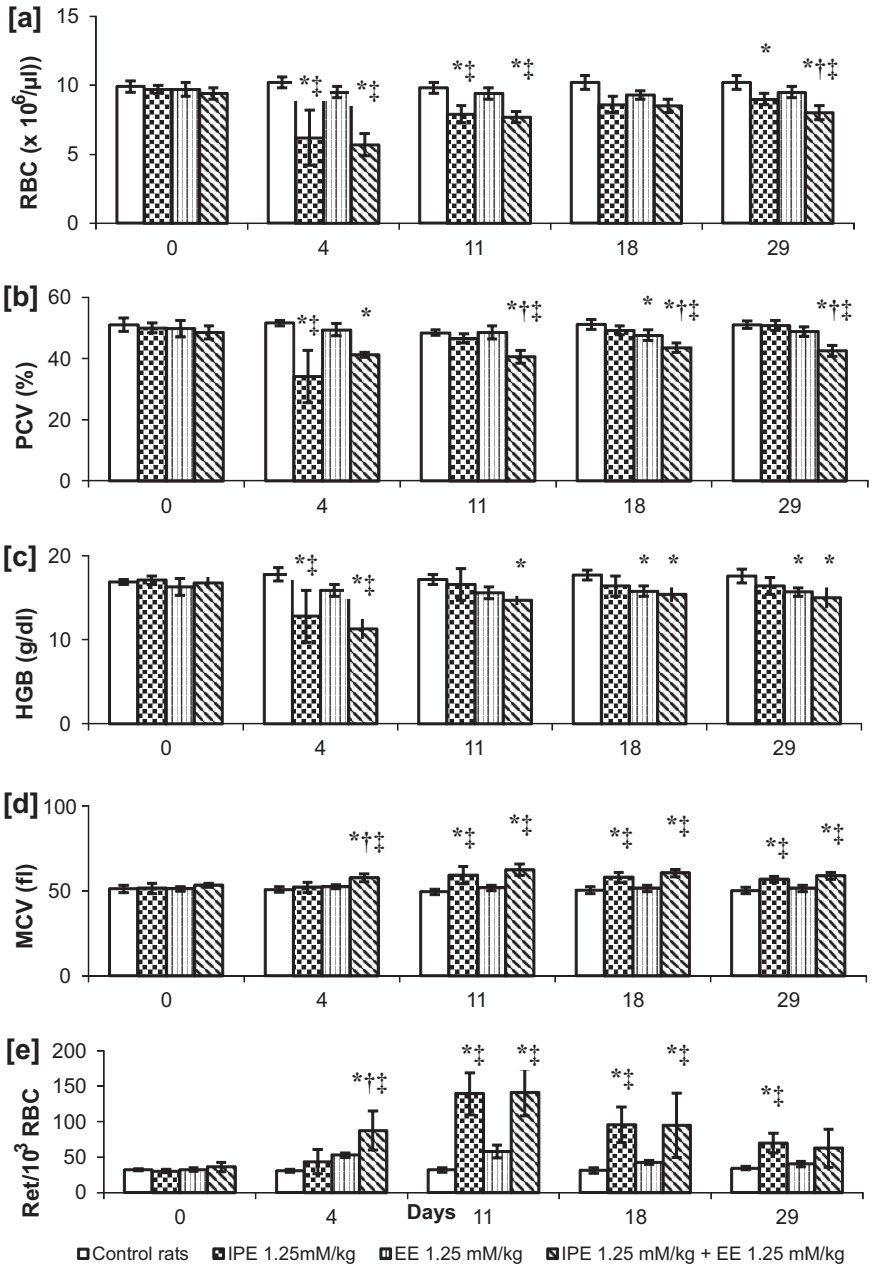

Fig. 4. Effects of combined exposure to isopropoxyethanol (IPE) and ethoxyethanol (EE) at doses 1.25 mM/kg b.w. on RBC (**a**), PCV (**b**), HGB (**c**), MCV (**d**) and Ret (**e**) values of the peripheral blood at the designated time points of male rats. Values are the means ± SD of five rats, * – $p \leq 0.05$ significantly different from control rats; † – $p \leq 0.05$ significantly different from rats treated with IPE alone; ‡ – $p \leq 0.05$ significantly different from rats treated with EE alone

count at day 29 or days 4, 18 and 29, respectively, were observed. No significant alterations in WBC and lymphocyte counts in rats treated with ME alone at dose of 0.75 mM/kg were seen. The rats co-exposed to the lower doses of IPE and ME (0.75 mM + 0.75 mM) demonstrated the decrease in these cells mostly at days 4 and 29 of the experiment. The drop in WBC at day 4 was significantly higher than in the rats treated with IPE alone (Fig. 5).

No significant alterations in WBC count in the rats treated with the higher dose of IPE (1.25 mM/kg) were observed. The number of these cells in the rats exposed to ME alone was lower than in the control group at day 4 of the experiment, whereas in the animals simultaneously treated with IPE and ME at the higher doses (1.25 mM + 1.25 mM) were diminished at days 4, 18 and 29. WBC count at day 18 was significantly lower in comparison with IPE alone group. At different time of exposure the lymphocyte counts were reduced. In the rats treated with IPE alone, the reduction in these cells on days 4 and 29 of exposure was observed. ME administration at dose of 1.25 mM/kg resulted in a decline in lymphocyte count only at day 4 of the experiment. Co-exposure to IPE and ME

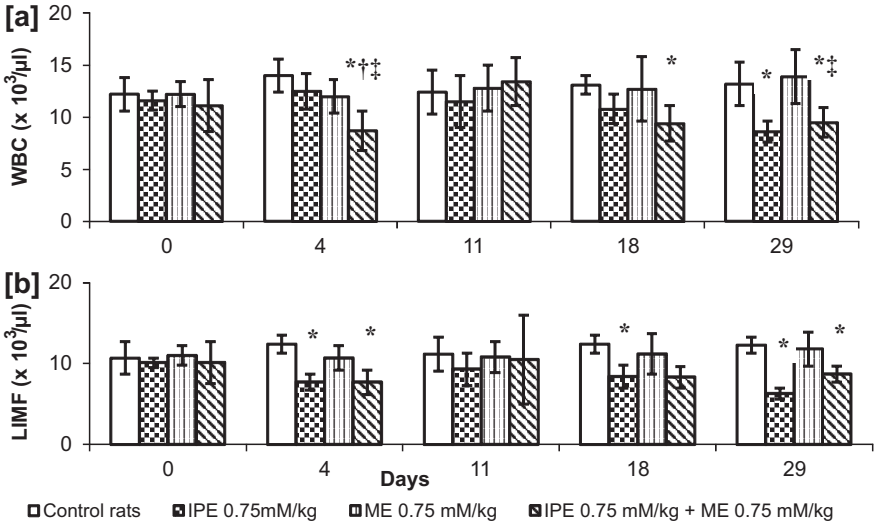

Fig. 5. Effects of combined exposure to isopropoxyethanol (IPE) and methoxyethanol (ME) at doses 0.75 mM/kg b.w. on WBC (**a**) and LYM (**b**) values of the peripheral blood at the designated time points of male rats. Values are the means ± SD of five rats, * – $p \leq 0.05$ significantly different from control rats; † – $p \leq 0.05$ significantly different from rats treated with IPE alone; ‡ – $p \leq 0.05$ significantly different from rats treated with ME alone

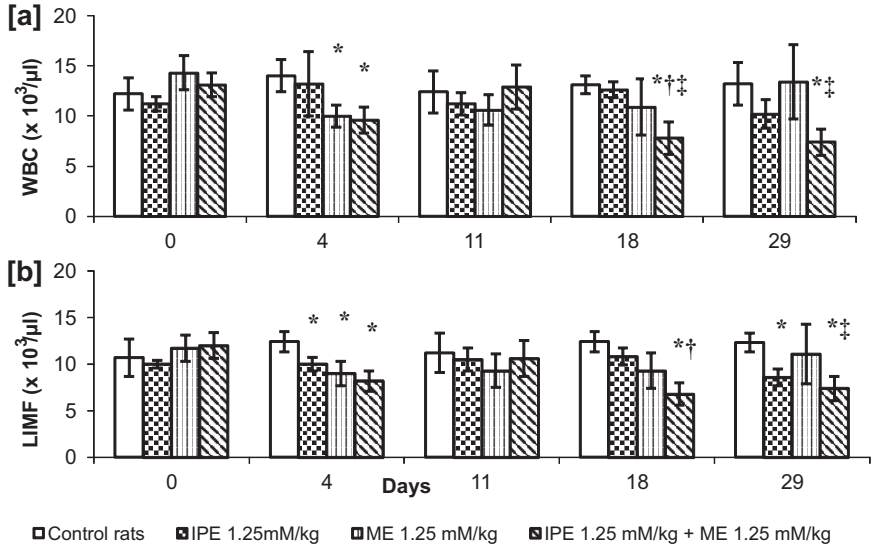

Fig. 6. Effects of combined exposure to isopropoxyethanol (IPE) and methoxyethanol (ME) at doses 1.25 mM/kg b.w. on WBC (**a**) and LYM (**b**) values of the peripheral blood at the designated time points of male rats. Values are the means ± SD of five rats, * – $p \leq 0.05$ significantly different from control rats; † – $p \leq 0.05$ significantly different from rats treated with IPE alone; ‡ – $p \leq 0.05$ significantly different from rats treated with ME alone

at the higher doses (1.25 mM + 1.25 mM) led to the decreases in lymphocytes at days 4, 18 and 29. The number of lymphocytes in those animals at day 18 was significantly lower than in the control group and in the rats exposed to IPE alone (Fig. 6).

EE alone at dose of 0.75 mM/kg caused reduction in both WBC and lymphocytes at day 29 of exposure. In the rats simultaneously treated with IPE and EE at the lower doses (0.75 mM + 0.75 mM) any alterations in WBC and lymphocyte counts were not observed (Fig. 7). EE alone at the higher dose of 1.25 mM/kg caused the drop in number of both WBC and lymphocytes at days 11 and 29 of the experiment. No changes in the number of these cells in the rats co-exposed to IPE and EE at the higher doses (1.25 mM + 1.25 mM) were observed (Fig. 8).

Discussion

In the present study, the assessment of the interactive effects of combined exposure to IPE and ME, and also IPE and EE, on the hematological parameters of peripheral blood in rats were evaluated. The protocol of this experiment aimed at the constitution of a model of conditions that may take place in an industry and in a household.

In this study, it was observed that subcutaneous repeated administration of each of three EGAEs at low dose levels led to distinct hematological changes, but there were marked qualitative and quantitative differences in the responses. These changes were evidenced by a decrease in RBC, PCV and HGB, and also by an increase in MCV and Ret in peripheral blood.

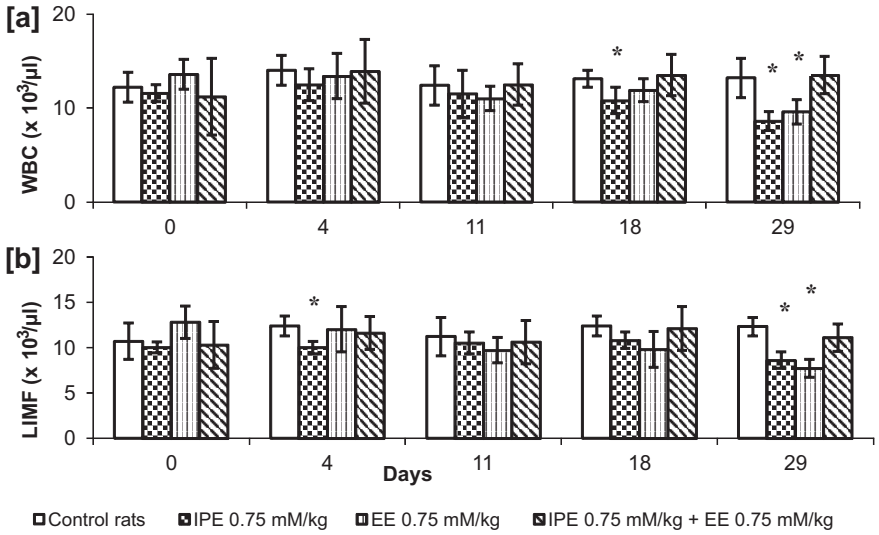

Fig. 7. Effects of combined exposure to isopropoxyethanol (IPE) and ethoxyethanol (EE) at doses 0.75 mM/kg b.w. on WBC (**a**) and LYM (**b**) values of the peripheral blood at the designated time points of male rats. Values are the means ± SD of five rats, * – $p \leq 0.05$ significantly different from control rats

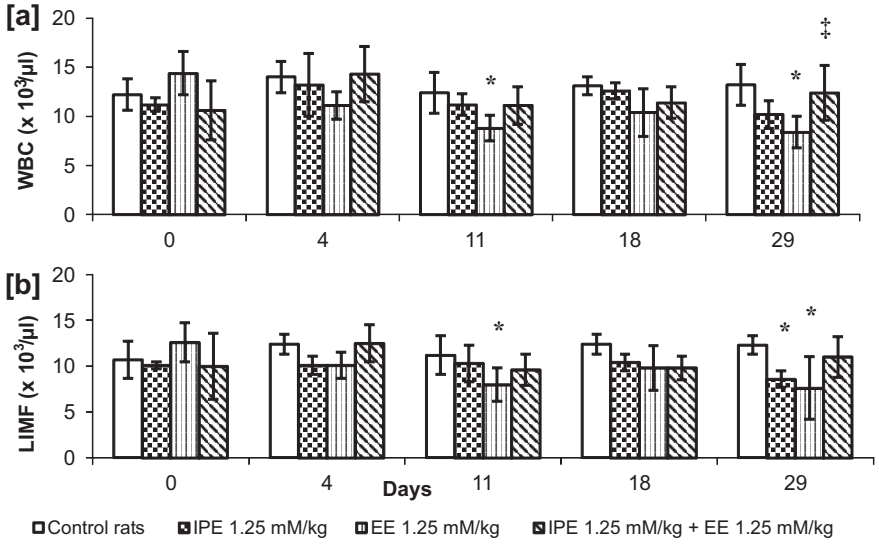

Fig. 8. Effects of combined exposure to isopropoxyethanol (IPE) and ethoxyethanol (EE) at doses 1.25 mM/kg b.w. on WBC (**a**) and LYM (**b**) values of the peripheral blood at the designated time points of male rats. Values are the means ± SD of five rats, * – $p \leq 0,05$ significantly different from control rats; ‡ – $p \leq 0.05$ significantly different from rats treated with EE alone

The rats treated with IPE alone at both doses (0.75 and 1.25 mM/kg) demonstrated the strong pronounced hematological alterations at the beginning of exposure, i.e., on day 4. In the later period, these alterations were regressed despite of exposure duration, although the decrease in RBC and HGB, and the increase in Ret were more persistent, probably due to the selective hemolysis of the aged erythrocytes, leaving a population of young red cells [4]. It is well evidenced that aging of erythrocytes results in several biochemical and biophysical changes, e.g., in glutathione, ATP, and 2,3-diphosphoglycerate diminished levels, in a decrease of antioxidant enzymes activity, and in an increase of lipid peroxides concentration [3, 13, 44]. In addition, erythrocytes became more osmotically fragile and less deformable during the aging process [26]. These features of aged erythrocytes are responsible for their hemolysis expressed in hemoglobinuria and an increase in plasma hemoglobin concentration. In contrast to BE [8, 31, 38], no increase in plasma hemoglobin concentration in the rats exposed to IPE, ME or EE in the present study were observed. Distinct hemolysis in form of elevated plasma hemoglobin level, induced by IPE after subcutaneous administration of this compound at a single dose of 2.5 mM/kg, was seen in previous study [32].

In contrast, the rats treated with ME or EE alone at dose of 1.25 mM/kg demonstrated the lack of hemato-

logical alterations at the beginning of exposure and markedly pronounced and progressively elevated with exposure time hematological changes at the end of exposure, i.e., on days 18 and 29. These changes included only RBC, PCV and HGB in the rats treated with ME, and also HGB and Ret in animals exposed to EE. The lower dose of both ME and EE (0.75 mM/kg) was ineffective.

The progressive recovery from the hemolytic anemia, observed in the rats treated with IPE alone, may be associated with tolerance development to the hemolytic effect of this compound. This tolerance was characterized by a gradual removal of hematological changes manifested in an increase in RBC, PCV and HGB, and also a decrease in Ret. The tolerance development, so-called autoprotection [28], was also observed in other studies [10, 18, 38]. On the other hand, present experiment and previous study [38] demonstrated no tolerance to ME- and EE-induced intravascular hemolysis developed under experimental conditions. It seems that the selective hemolysis of older erythrocytes in association with the development of reticulocytosis during the process of active blood regeneration may lead to tolerance to the hemolytic effects of both IPE and BE. This hypothesis was supported by investigation of the effect of BE and BAA on erythrocytes of anemic/recovered rats *in vitro* and *in vivo*, respectively. Hemolytic anemia was induced either by treatment of rats with BE or by bleeding. The results demonstrated that anemic/recovered rats were significantly less sensitive to BE-induced hemolytic effects than control animals. However, tolerance to BE was more pronounced in BE-pretreated/recovered rats than in bled/recovered animals. This difference in the responses was connected to the fact that bleeding led to the loss of red cells of all ages while BE-pretreatment resulted in the selective removal of the sensitive older cells by hemolysis [10].

In other study, when the rats were pretreated with a non-lethal dose of BE, these animals were able to survive the lethal dose of this compound administered subsequently. Also, bled/recovered rats demonstrated considerable higher survival in comparison with control group after administration of lethal dose of BE. *In vitro*, the erythrocytes from the bled/recovered rats showed remarkable resistance to deformation (deform and swell) caused by BAA in comparison with cells from control group. The mechanism of this autoprotection was related to the inherent resilience of newly formed erythrocytes that replaced the cells lost due to hemolysis [28].

Due to relatively low doses of EGAEs, the changes in the leukocyte system of rats were poorly pronounced. These changes were expressed mainly in the reduction in leukocyte and lymphocyte counts. In previous study [34], it was found that ME and EE at higher doses (2.5 and 5.0 mM/kg) strongly suppressed leukocyte system causing leukopenia, lymphocytopenia, and neutrocytopenia. The literature data [14, 43] indicate that ME is immunotoxic. Dermal exposure of rats to this compound at doses in a range of 2–16 mM/kg/day for 4 consecutive days produced drops in thymus and spleen weight, enhanced the lymphoproliferative responses to mitogens, and a reduction in the antibody plaque-forming cell response. Moreover, it was observed lymphoid tissue atrophy after inhalation of ME in male New Zealand white rabbits [21]. Also, a marked immunosuppressive effects of this chemical and both MAA and methoxyacetaldehyde, an intermediate metabolite of ME, on thymus weight and lymphoproliferative functions in rats were found [29, 30].

There is still too little knowledge on the interactive effects of combined exposure to EGAEs and other compounds, especially in relation to the hematological alterations. The co-administration of aliphatic alcohols, i.e., ethanol, n-propanol, and n-butanol at a dose of 30 mM/kg and BE at doses of 1, and 5 mM/kg almost completely inhibited the hemolysis and reduced the urinary excretion of BAA by 31–43% [22]. In the other study [34], it was found that ethanol consumption along with exposure to EGAEs for 4 weeks only partially protected the rats against hemolytic effects and the alterations in leukocyte system of peripheral blood induced by these ethers. The preventive effects of ethanol were seen at both doses of ME (2.5 and 5.0 mM/kg) and BE (0.75 and 1.25 mM/kg), and also at the higher dose of EE (5.0 mM/kg). In the rats simultaneously consumed ethanol and treated with ME or EE at the lower dose (2.5 mM/kg) the protection from the alterations mainly in leukocyte system was observed. On the contrary, the rats co-exposed to ethanol and ME or EE, at the higher dose (5.0 mM/kg) demonstrated the amelioration of some hematological parameters, such as RBC, PCV and HGB in comparison with the animals treated with EGAEs alone. Also, the ethanol orally intake along with exposure to BE at doses of 0.75 and 1.25 mM/kg markedly ameliorated hematological parameters, es-

pecially RBC, PCV, HGB, MCV and Ret in peripheral blood. It was suggested that competitive ethanol inhibition of ADH may lead to the changes in BE metabolism expressed in the decrease of BAA level [34].

The combined administration of EE (2.2 mM/kg) with toluene (2.7 mM/kg) and xylene isomers (4.7 mM/kg) to male rats for 4 weeks has reduced the extent of testes atrophy by 25% and also diminished both the highest concentration and the total amount of plasma EAA, by 45 and 25%, respectively. These results suggested that testicular toxicity caused by EE may be decreased during exposure to this compound in the form of solvent mixture containing toluene and xylene isomers [5].

The data cited above indicate that when animals are exposed to a compound in mixture or simultaneously with others, the toxicity of the chemical can be increased or decreased by additive, synergistic or antagonistic effects provided by mixture compounds. The results mentioned above represent an antagonistic effects of the examined chemicals.

The results obtained in the present study clearly indicate that the effects of ME and EE on hematological alterations induced by IPE in rats are complex. At the beginning of the co-exposure to IPE and ME at a relatively low doses of 0.75 mM + 0.75 mM, when IPE alone at the same dose led to the most pronounced hematological alterations, ME showed a protective effect against erythrocyte damage. At the later period, i.e., at the end of 4 weeks exposure, the hematological alterations in the same animals were markedly pronounced and progressively elevated with exposure time, except for MCV values, which were significantly lower in comparison with IPE group. ME at the higher doses of 1.25 mM/kg and EE at both doses of 0.75 and 1.25 mM/kg did not modify the hematotoxicity of IPE at doses of 0.75 and 1.25 mM/kg at the beginning of the exposure, whereas increased its harmful effects at the end of the treatment. Also, the changes in leukocyte system in the rats simultaneously treated with IPE and ME at both dose levels were occasionally similar or more pronounced than in animals exposed to IPE alone. Co-exposure to IPE and EE practically did not exert an influence to WBC and lymphocyte counts in peripheral blood. Thus, the combined exposure to EGAEs may lead to less or more pronounced hematological alterations than when these compounds are given separately.

The explanation of the biological basis of the interactions between EGAEs is difficult. The interactions may be related to the metabolism of these chemicals. Distinct differences in the metabolism of the ME, EE and BE as a function of alkyl chain length were observed [20, 25]. After administration of these compounds in drinking water to male rats, 34% of the ME dose was eliminated in the urine as MAA, 25–45% of the EE dose was excreted as EAA, and 50–60% of the BE dose was eliminated as BAA during 72 h [20]. These data suggest that the amount of the glycol ether metabolized *in vivo* to appropriate AAA increased with growing alkyl chain length from ME to BE. However, the fraction of the dose excreted as AAA was elevated with increasing doses of ME and EE, whereas was diminished with growing a dose of BE. These results may indicate that metabolic pathway *via* ADH was saturated and correlated well with the ability of rat liver ADH to oxidize the EGAEs. It was shown in male rat liver ADH that the K_m value for BE was approximately tenfold lower than for ME and EE, while the V_{max} values were almost the same [2]. Consequently, at low and similar glycol ether concentrations, the metabolism of BE would be more efficient than that of ME and EE. The low K_m value for BE compared to ME and EE indicates that at the same time the metabolism of BE *via* ADH will be saturated at lower concentrations than those of ME and EE. This may explain why BE metabolism to BAA *in vivo* is more efficient than ME and EE to MAA and EAA, respectively, despite the fact that the activity of cytosolic ADH was increased by repeated ME treatment [17].

In addition, the elimination half-life of MAA from plasma of male rats was longer (13.2 ± 0.4 h) than of EAA one (9.4 ± 3.7 h). Also, the total clearance of EAA is higher than that of MAA [1]. In humans, urinary MAA has a long half-life (77.1 h). In workers exposed to ME at constant level of 13.5 mg/m^3 for six consecutive days, following a one-week cease in the exposure, urinary MAA was increased significantly from 10.6 mg/g creatinine on Monday (prior to work) to 45.6 mg/g creatinine on Saturday (after work) [27]. These data indicate that MAA is accumulated in the organism. On the contrary, no accumulation of BE and BAA was found in humans and rats exposed to BE. In male rats exposed to BE vapor at concentrations of 100 or 500 mg/m^3 for 1–12 days, the total blood clearance of parent compound averaged 2.6 l/h per kg, corresponding to a hepatic extraction ratio of about 0.75. The renal clearance of BAA (average 0.53 l/h per kg) corresponded to ~15% of the renal blood flow.

The recovery of BAA in urine was 64% of the inhaled amount of BE [15]. Similar results were obtained in human inhalation experiments [16]. Also, Ghanayem et al. [10] revealed that no qualitative or quantitative alterations in BE metabolism and disposition were caused by repeated exposure to BE compared to those found in rats treated with a single dose of this ether. It seems that physicochemical and metabolic features of IPE are markedly similar to BE. IPE and BE are weakly lipophilic compounds with octanol-water partition coefficients (log K_{ow}) 0.05 and 0.83, respectively, whereas ME and EE are hydrophilic with log K_{ow} amounting to –0.77 and –0.32, respectively (United States National Library of Medicine: http://toxnet.nlm.nih.gov). Moreover, it was found a direct quantitative relationship between the physicochemical properties of AAAs and their hemolytic activity *in vitro* [35].

Summary and conclusions

In conclusion, the short-term repeated administration of IPE to rats at relatively low doses level leads to distinct hematological alterations which are typical of hemolytic anemia. On the contrary, the exposure to ME or EE alone in the same manner resulted in significantly less pronounced hematological changes in the rats. The alterations in leukocyte system of rats treated with EGAEs were expressed mainly in the reduction of leukocyte and lymphocyte counts. The obtained results on EGAEs co-exposure suggest that ME and EE may modified the hematological effects of IPE in the rats. The amelioration in the majority of the hematological parameters in the rats observed at the beginning of the exposure may be caused by inhibitory effect of ME at low dose on IPE metabolism. It may be supposed that slowly generated and accumulated both MAA and EAA [1, 27], toxic metabolites of ME and EE, respectively, and intolerance development to the hemolytic effect of these two chemicals, may be responsible for increased hematological alterations at the end of 4 weeks exposure. From practical point of view, there is possible only partial counteraction against adverse hematological effects of an individual ethylene glycol ethers by application of their appropriate mixtures in accordance with occupational safety principles.

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Received: July 6, 2011; **in the revised form:** September 7, 2011; **accepted:** September 23, 2011.