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COMPARISON OF THE HEMOLYTIC ACTIVITY OF ISOPROPOXYETHANOL AND PHENOXYETHANOL

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Abstract.
Objectives: Administration of ethylene glycol monoalkyl ethers to rodents causes acute hemolytic anemia. Metabolic activation of these chemicals to alkoxyacetic acids is required to develop hemolytic effect. Current study was undertaken to compare the hemolytic activity of isopropoxyethanol (IPE) and phenoxyethanol (PhE) in male rats. The main goal of this study was to evaluate the role of alkyl and aryl group in hemolytic activity of ethylene glycol ethers. **Materials and Methods:** Rats were treated subcutaneously with single doses of 0, 0.625, 1.25 and 2.5 mmol IPE/kg body weight or 0, 2.5, 5.0 and 10.0 mmol PhE/kg. At 0, 6, 24, 48, 144, 216, and 600 h after dosing, blood samples were collected from end tail of rats and various blood indices were measured. **Results:** Administration of both chemicals resulted in a time- and dose-dependent swelling of erythrocytes as evidenced by an early increase in packed cell volumes and mean cell volume. Subsequently, red blood cells, total hemoglobin concentration, and packed cell volumes decreased when hemolysis progressed. Furthermore, an increase in plasma hemoglobin concentration and reticulocyte counts was observed. The onset of hemolysis induced by IPE was faster than that after PhE administration. The hemolytic activity of IPE was about tenfold higher in comparison with PhE. **Conclusions:** It is likely that the lower hemolytic activity of PhE is associated with inhibitory effect of aryl group on hemolytic action of this compound. Phenyl group, in contrast with alkyl moiety, represents electron acceptor system which exerts resonance and inductive effects and leads to changes in acid strength, also in case of phenoxyacetic acid, a metabolite of PhE.

Key words:
Ethylene glycol ethers, Isopropoxyethanol, Phenoxyethanol, Hemolytic anemia, Dose-effect relationship

INTRODUCTION

Ethylene glycol monoalkyl ethers (EGEs), especially 2-metoxyethanol (ME), 2-ethoxyethanol (EE), isopropoxyethanol (IPE) and 2-butoxyethanol (BE) are extensively produced chemicals [1,2] with a wide range of industrial and domestic applications [3]. Also, monoaryl ether – phenoxyethanol (PhE) – has similar application in practice [4]. The presence of EGEs in paints, lacquers, dyes, and cleaning agents has generated considerable interest in their toxicity.

It is well documented that these chemicals cause acute hemolytic anemia in laboratory animals [5–7] and in humans [8]. Male rats treated with ME, EE or BE demonstrated a time- and dose-dependent increase in packed cell volume (PCV) and mean cell volume (MCV), suggesting an early swelling of erythrocytes. Subsequent hemolysis of erythrocytes leads to a time- and dose-dependent decrease in the

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count of circulating red blood cells (RBCs) and total hemoglobin concentration (HGB_T). These effects of EGEs were accompanied by a significant increase in the number of circulating reticulocytes and plasma hemoglobin concentration (HGB_P) [7,9].

The results obtained in the previous study indicate that the total number of carbons in ether groups, being an indicator of lipophility of these chemicals, is crucial for their hemolytic activity [7].

From the theoretical point of view, the effect of alkyl and aryl groups on hematotoxic activity of glycol ethers is interesting. The current investigation was undertaken to compare the acute hemolytic activity of both IPE and PhE in male rats after a single dose administration and to characterize the differences in toxicity towards the circulatory red blood cells and the reversibility of such effects in the period following the treatment.

MATERIALS AND METHODS

Chemicals

Both IPE and PhE were purchased from Aldrich Chemical Company. All other chemicals of analytical grade were obtained from POCh (Poland).

Animals and treatment

Male Wistar Krf: (WI) WU rats (10–12 weeks old) obtained from the Jagiellonian University Faculty of Pharmacy Breeding Laboratory (Kraków, Poland) were maintained on standard diet (Murigran, Poland) and water *ad libitum*, and allowed a minimum of 10 days acclimatization to appropriate facilities (12 h dark/light period, 21–24°C ambient temperature, and 40–60% relative humidity) prior to inclusion in the present experiment. In this experiment the Polish law on the protection of animals of was followed [10].

IPE and PhE solutions were prepared immediately before dosing by mixing these chemicals with 0.9% saline or sunflower oil (solubility of PhE in water is 19.3 mmol/dl), respectively, to obtain a dose volume of 2 ml/kg body weight (b.w.), and were administered to rats by subcutaneous injection.

Rats were randomly assigned to groups of 5 animals each treated with IPE or PhE at a single dose of 0.625, 1.25, and 2.50 mmol/kg b.w. or 2.5, 5.0, and 10.0 mmol/kg b.w., respectively. Control animals received 2.0 ml of 0.9% saline or sunflower oil and served as vehicle controls.

Hematologic analysis

At the end of the required period after IPE or PhE administration, i.e. 0, 6, 24, 48, 144, 216, and 600 h, blood samples from end tail of rats were collected.

Blood samples were analyzed manually by means of competence methods. The following parameters were measured: RBCs and reticulocyte counts, HGB_T HGB_P, PCV, MCV, mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC). The HGB_P limit of detection was given in the previous paper [11].

Statistical analysis

Data were evaluated by multiple analysis of variance for dependent variables (repeated measures MANOVA) with simple effects test. These evaluations were made by SPS S for Windows. The regression equations and correlation coefficients were calculated with the STATISTICA – version 5.0 computer program.

For each hematologic parameter the relationship between its value and dose, both dose and time, and time were analyzed separately for two time-period observations. In case of rats treated with IPE, the first time-period for all parameters, except for reticulocyte counts and HGB_P, included 0–24 h, whereas the second time-period 24–600 h. For reticulocyte counts and HGB_P these time-periods were 0–144 h and 0-6 h as well as 144–600 h and 6–600 h, respectively.

In case of rats treated with PhE, the first time-period for RBC, HGB_P, PCV, MCH, and MCHC comprised 0–48 h, when the second time-period was 48–600 h. For reticulocyte counts, HGB_P, and MCV these time-periods were 0–144 h, 0–24 h, and 0–6 h (the first time-period), and 144–600 h, 24–600 h, and 6-600 h (the second time-period), respectively.

Results obtained in the group of rats treated with PhE at a dose level of 10.0 mmol/kg were not statistically analyzed because of too small number of the living animals.

RESULTS

At all time points after IPE administration, the rats were in good condition. In contrast, PhE at the highest dose (10 mmol/kg b.w.) caused drowsiness of rats immediately after dosing and death of 2 animals between 6 and 24 h later. Hematologic changes in the circulating blood were produced by both IPE and PhE, but there were marked quantitative differences in the responses. IPE administration at doses of 1.25 and 2.50 mmol/kg b.w. resulted in early swelling of erythrocytes as evidenced by an increase in PCV and MCV at 6 h of the experiment. Animals treated with the

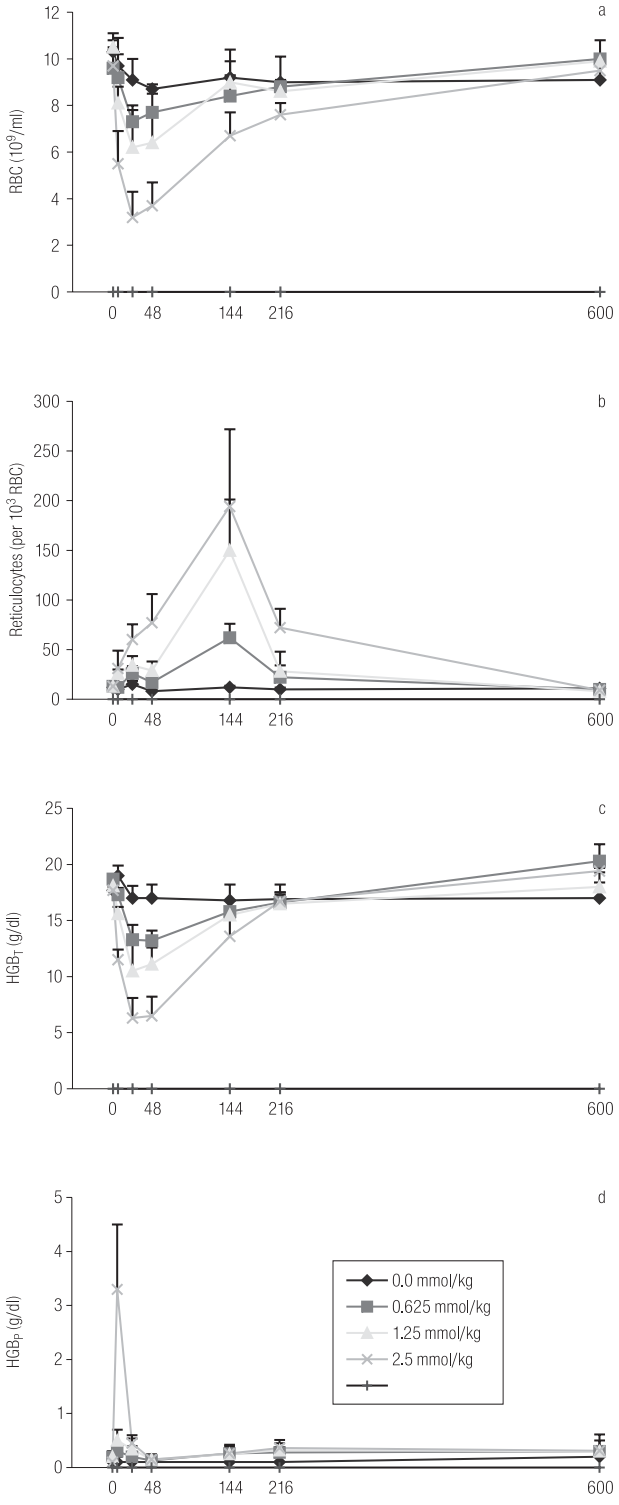

Fig. 1. Time-course (for 600 h) of the effects of IPE at the dose levels of 0–2.5 mmol/kg b.w. on RBC (a), reticulocyte counts (b), HGB_T (c), and HGB_P (d) in rats. Values are expressed as the mean ± SD.

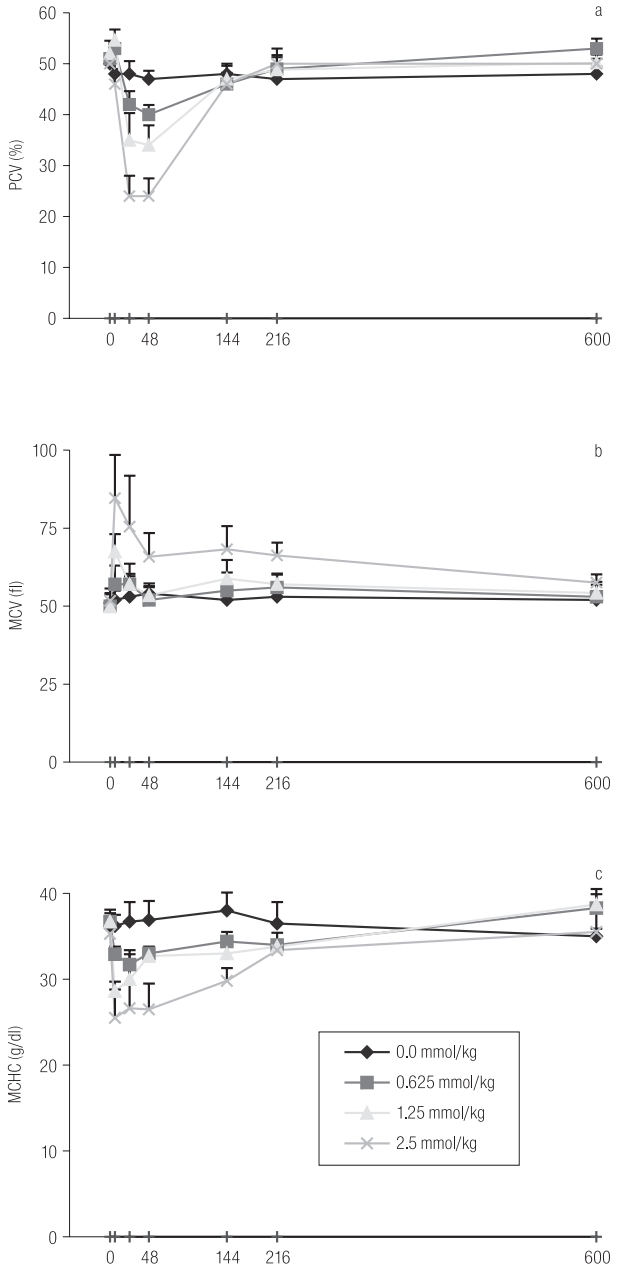

Fig. 2. Time-course (for 600 h) of the effects of IPE at the dose levels of 0–2.5 mmol/kg b.w. on PCV(a), MCV (b), and MCHC (c) in rats. Values are expressed as the mean ± SD.

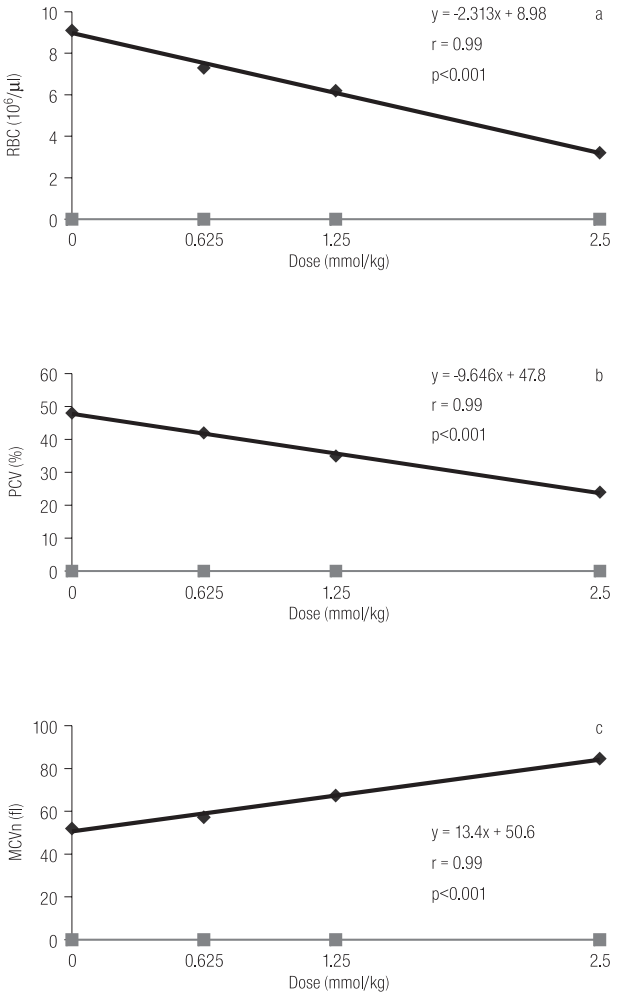

Fig. 3. Dose-effect relationship between IPE administered subcutaneously to rats and RBC at 24 h (a), PCV at 24 h (b), and MCV at 6 h (c) of the experiment.

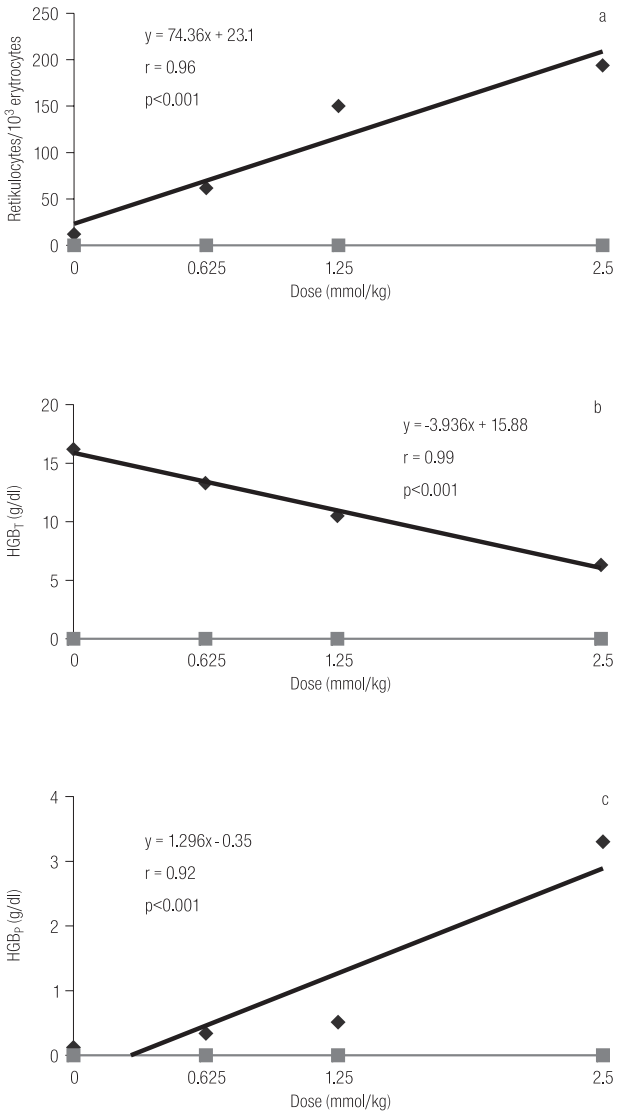

Fig. 4. Dose-effect relationship between IPE administered subcutaneously to rats and reticulocyte counts at 144 h (a), HGB_T at 24 h (b), and HGB_P at 6 h (c) of the experiment.

highest dose of this compound (2.5 mmol/kg b.w.) had significantly elevated PCV also at the end of the experiment (600 h). At 24 and 48 h of experiment, after IPE administration at each dose levels, a decrease in PCV was observed. IPE produced marked reductions in RBC, and MCHC as late as 600 and 216 h, respectively. Other hematologic parameters, i.e. reticulocyte counts and HGB_P, were markedly elevated with maximum values at 144 and 6 h, respectively. The greatest changes in other parameters, i.e. RBC, PCV, MCV, and HGB_T occurred at 24, 24, 6, and 24 h, respectively (Figs. 1 and 2). These maximum values correlated well with IPE-doses (Figs. 3 and 4). All hematologic parameters demonstrated dose, both dose and time, and time dependence. These hematologic changes returned to normal at 600 h, except for RBC, HGB_T, HGB_P, and PCV.

In contrast, PhE caused less pronounced effects on circulating red cells than IPE (Figs. 5 and 6). Rats treated with PhE at a dose of 2.5 mmol/kg b.w. demonstrated the time-dependent increase in PCV and MCV, and also the decrease in HGB_T. Rats given this compound at a dose of 5.0 mmol/kg b.w. had significantly elevated HGB_P at 6 and 24 h of the experiment, reticulocyte counts at 48 and 144 h, PCV and MCV at 6 h and significantly diminished HGB_T at 48 h, and MCHC at 6 h. Dose-dependent significant changes were observed in reticulocyte counts, HGB_P, PCV, and MCV. On the other hand, both dose and time, and time-dependent changes were seen in reticulocyte counts,

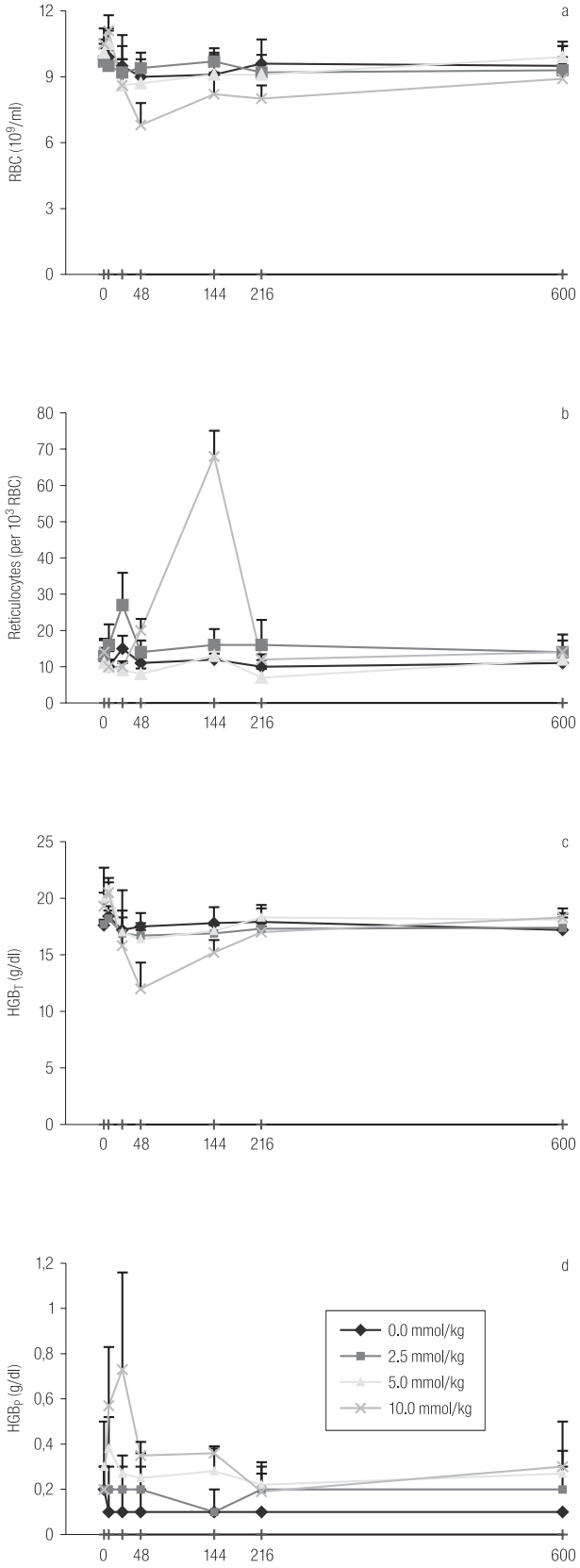

Fig. 5. Time-course (for 600 h) of the effects of PhE at the dose levels of 0–10.0 mmol/kg b.w. on RBC (a), reticulocyte counts (b), HGB_T (c), and HGB_P (d) in rats. Values are expressed as the mean ± SD.

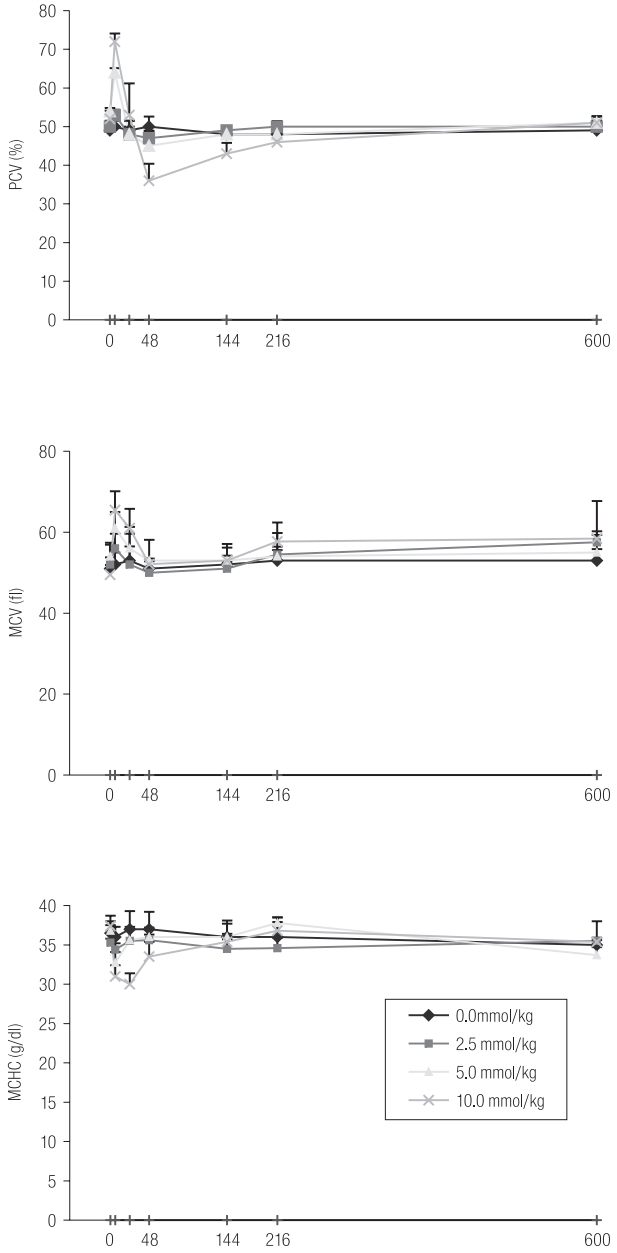

Fig. 6. Time-course (for 600 h) of the effects of PhE at dose levels of 0–10.0 mmol/kg b.w.on PCV (a), MCV (b), and MCHC (c) in rats. Values are expressed as the mean ± SD.

HGB_T, PCV, MCV, and MCHC. All hematologic parameters, except for HGB_P and MCHC demonstrated time trend, especially at the highest dose of PhE (5.0 mmol/kg). The majority of maximum values of these changes correlated well with PhE doses (Table 1). The hematologic changes observed in the rats treated with PhE disappeared over the recovery period and returned to normal at 216 h of the experiment, except for reticulocyte counts, HGB_P and PCV.

Table 1. Dose-effect relationship between PhE administered subcutaneously to rats in doses of 0–10.0 mmol/kg b.w. and some hematologic parameters

Hematologic parameter	Maximum change	Regression equation	Correlation coefficient	P
RBC (10^6/µl)	at 48 h	y = -0.219x + 9.4	0.84	≤0.001
Reticulocytes (per 10^3 RBC)	at 144 h	y = 5.29x + 6.6	0.86	≤0.001
HGB_P (g/dl)	at 6 h	y = 0.046x + 0.118	0.99	≤0.001
PCV (%)	at 6 h	y = 2.48x +48.4	0.97	≤0.001
MCV (fl)	at 6 h	y = 1.35x + 52.7	0.98	≤0.001
PCV (%)	at 48 h	y = -1.154x + +48.8	0.94	≤0.001

DISCUSSION

The two ethylene glycol ethers, IPE and PhE that differ only in their alkyl and aryl groups, produced markedly dissimilar changes in circulating red blood cells of rats after acute subcutaneous treatment. Both chemicals caused distinct hemolytic anemia, more pronounced after IPE-administration than after PhE-treatment. As expected, and in agreement with previous reports [7,11], IPE and PhE given to rats resulted in early swelling of erythrocytes as demonstrated by an increase in PCV and MCV values. This effect was seen only at 6 h of the experiment and preceded apparent hemolysis. Hemolysis was evidenced by reductions of RBC, HGB_T (except for the PhE-treated rats), PCV, and MCHC and by an increase in HGB_P. While the increase in HGB_P after IPE-administration was of short duration, that after PhE-treatment was prolonged. Subsequently, reticulocyte counts considerably raised as a result of regenerative process.

The results obtained in the present study indicate that PhE is about tenfold less hematotoxic than IPE. On the other hand, this chemical exerted evident depressive effect on the central nervous system and led to animal death at a dose level corresponding with a lower limit of LD_{50} *per os* [12]. However, the acute IPE toxicity in rats, expressed by LD_{50} *per os*, is about 5 times lower than that of PhE [13].

Hematologic activities of IPE and well known BE are comparable with respect to the profile of changes, their intensity, and duration [7]. Various changes in circulating red blood cells observed after BE administration are typical of hemolytic anemia with an associated reticulocytosis and hyperplasia of both bone marrow and spleen [14–16]. The majority of alterations in red blood cells were reversible after a long period of time, although the increase in MCV was persistent, probably due to the selective hemolytic action of IPE and BE on the aged erythrocytes, leaving a population of young red cells [15]. Moreover, erythrocytes produced during remission from severe acute hemolytic anemia tend to be macrocytic and normoblastic with the pronounced anisocytosis and polychromasia as well as with the presence of Howell Jolly bodies [14], factors which may also contribute to the persistence of these alterations. Additionally, erythrocytes from animals treated with BE exhibited a tendency to aggregate as evidenced by the appearance of rouleaux in blood smears [15]. Also, experiments *in vitro* demonstrated that male rat erythrocytes exposed to butoxyacetic acid, a metabolite of BE, showed marked morphological changes, including spherocytosis, spheroechinocytosis and a tendency for erythrocytes to adhere to one another [17].

The onset of BE-induced hemolysis was faster in female than in male rats. These effects were also accompanied by a time-dependent increase in a relative spleen weight, which indicate that the sequestration of deformed or damaged erythrocytes by the spleen has taken place [15, 16].

Data presented in this report clearly demonstrate the difference between the IPE and PhE hemolytic activity.

These chemicals significantly differ in their lipophilicity. IPE is a low lipophilic compound with octanol-water partition coefficient (log K_{ow}) of 0.43, whereas PhE is more lipophilic with log K_{ow} amounting to 1.16 [18]. Therefore, lipophilicity may play a secondary role in hemolytic activity of EGEs. This point of view is inconsistent with our previous observation on hemolytic action of three EGEs (ME, EE, and BE) [7]. In the present study the relationship between total carbons in alkyl moiety and their hemolytic activity was observed. Thus, the hemolytic activity of ethylene glycol alkyl ethers raised together with the increase in hydrophobic properties. Log K_{ow} (25°C) of ME, EE, and BE amounted to -0.77, -0.54, and 0.81, respectively [18].

Metabolic activation of IPE and PhE to isopropoxyacetic acid (IPAA) and phenoxyacetic acid (PhAA), respectively, is required to develop the hemolytic effect. The literature data indicate that male rats orally treated with these ethers metabolize IPE to IPAA less efficiently than PhE to PhAA [19,20]. The percentage of doses of parent compounds eliminated from the body and the duration of their elimination were similar, but the IPAA elimination rate was higher than that of PhAA. These data indicate that differences in hemolytic activity of IPE and PhE are not caused by their toxicokinetics.

The mechanism(s) underlying this differential hematotoxicity between the two glycol ethers are unknown. However, it is likely that the structure parameters such as resonance and inductive effects, apart from physicochemical properties (e.g., solubility, partition coefficients, and ionization), may be responsible for differences in their bioactivity [21]. The phenyl group, in contrast with alkyl moiety, represents an electron acceptor system, which exerts resonance and inductive effects and lead to changes in acid strength, also in case of PhAA. It seems that elevated acidity may inhibit the hemolytic activity of such chemicals.

In conclusion, it is clear that there are considerable differences between IPE and PhE in acute hemolytic activity, most of the hematologic changes resolve within 3 weeks or less following the treatment. The reasons for the difference in toxicity between the two glycol ethers are unknown, however, it is likely that events related to the structure of both chemicals, but not to their lipophilicity, and metabolism to ether derivatives of acetic acid appear to be largely responsible for difference in their hematotoxic effects.

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