Arch Toxicol (1999) 73: 55–59

SHORT COMMUNICATION

Ulrich Mußhoff · Michael Madeja · Norbert Binding
Ute Witting · Erwin-Josef Speckmann

Effects of 2-phenoxyethanol on N-methyl-D-aspartate (NMDA) receptor-mediated ion currents

Received: 13 October 1998 / Accepted: 24 November 1998

Abstract The actions were examined of 17 frequently used glycol ether compounds on the glutamate receptor-mediated ion currents. The receptors were expressed in *Xenopus* oocytes by injection of rat brain mRNA. Most of the 17 glycol ethers exerted no effects on the glutamate subreceptors activated by kainate and N-methyl-D-aspartate (NMDA), whereas 2-phenoxyethanol (ethylene glycol monophenyl ether) caused a considerable reduction of NMDA-induced membrane currents in a reversible and concentration-dependent manner. The threshold concentration of the ethylene glycol monophenyl ether effect was <10 μmol/l. The concentration for a 50% inhibition (IC_{50}) was ~360 μmol/l. The results indicate a neurotoxic potential for 2-phenoxyethanol.

Key words N-methyl-D-aspartate · Glutamate receptor · Glycol ether · 2-Phenoxyethanol · Ethylene glycol monophenyl ether

Introduction

Glycol ethers (GE) are extensively used as solvents, detergents and emulsifiers alone or as components in industrial and consumer applications. For example, the domestic production of ethylene glycol monoethyl ether in 1986 was estimated to be 122 million pounds (NIOSH 1991). As a result, a high potential for occupational, consumer and environmental exposure to GE exists during manufacturing and use. Inhalation and dermal uptake are the most likely routes of human exposure to GE which have been shown to be toxic by either route (Hardin 1983; Hobson et al. 1986; Piacitelli et al. 1990). Several animal studies indicate that GE may cause embryotoxic, teratogenic and haematotoxic effects as well as atrophy of reproductive and lymphoid organs (Hardin 1983; Grafton and Hansen 1987; Moss et al. 1985; Schuler et al. 1984). Effects on the central nervous system additionally have to be expected, since GE are also capable of rapidly crossing the blood-brain barrier (Ahmed et al. 1983). In fact, disturbances of brain functions, for example headache and persistent cognitive impairment, have been found in humans exposed to GE (Morton 1990; Ohi and Wegman 1978; Parsons and Parsons 1938; Rowe and Wolf 1982).

Little is known about the neuronal mechanisms involved in acute and chronic GE neurotoxicity. In general, it may be possible that voltage-operated ion channels, which underlies the generation of action potentials, or ligand-operated ion channels, which are responsible for the synaptic transmission, or both are involved. The aforementioned clinical data, especially as far as the memory impairment is concerned, can be explained by disturbance of synaptic transmission. In order to gain more insight in this respect, we examined possible effects of GE on the function of the glutamate receptor. Thus, the interaction between 17 frequently used glycol ether compounds and ligand-operated ion channels activated by the glutamate subreceptor agonists kainate (KA) and N-methyl-D-aspartate (NMDA) were investigated. Since studies on the detailed effects of substances on these receptors are often hampered in intact brain tissue by the complexity of the CNS, we chose to use the *Xenopus* oocyte expression system. *Xenopus* oocytes are capable of expressing foreign mRNA (Dascal 1987) and the expressed glutamate and GABA receptors exhibit all of the features of the naturally occurring receptors (Sigel 1990). Thus, the oocyte expression system permits a direct assessment of drug effects on these receptors (Binding et al. 1996; Mußhoff et al. 1995). For these reasons, ligand-operated channels were expressed by injection of rat brain RNA and the currents through the receptor channels were investigated in the presence of the different glycol ether compounds.

U. Mußhoff (✉) · M. Madeja · E.-J. Speckmann
Institut für Physiologie der Universität Münster,
Robert-Koch-Strasse 27a, D-48149 Münster, Germany
e-mail: mushoff@uni-muenster.de; Tel: 251 8355120;
Fax: 254 8355551

N. Binding · U. Witting
Institut für Arbeitsmedizin der Universität Münster,
Robert-Koch-Strasse 51, D-48149 Münster, Germany

Materials and methods

Total RNA was isolated from rat brain using a guanidine/LiCl method (Cathala et al. 1983). Poly(A)$^+$ RNA was prepared by column chromatography on oligo-(dT) cellulose according to the manufacturer's instructions (Pharmacia Uppsala, Sweden). The RNA was dissolved in distilled water at a concentration of 1 ng/nl and stored at −20 °C until injected into the oocytes. Small sections of the ovary were taken from adult *Xenopus laevis* under anaesthesia (5 mmol/l ethyl *m*-aminobenzoate). Oocytes were manually dissected and each injected with 50 ng poly(A)$^+$ RNA. Injected oocytes were maintained under tissue culture conditions in a solution containing (in mmol/l): NaCl 88, $CaCl_2$ 1.5, KCl 1, $NaHCO_3$ 2.4, $MgSO_4$ 0.8, HEPES 5; pH 7.4; temperature 20 °C. The medium was supplemented with penicillin (100 Units/ml) and streptomycin (100 µg/ml).

Voltage-clamp measurements were performed 3–7 days after injection of poly(A)$^+$ RNA. Membrane currents were measured using a standard two-electrode voltage-clamp technique. The current and voltage electrodes were filled with 2 mmol/l KCl and had resistances of 1 to 2 MOhm. Membrane currents were measured at a holding potential of −70 mV. During the experiments, the oocytes were kept in a frog Ringer's solution composed of (in mmol/l): NaCl 115, KCl 2, $CaCl_2$ 1.8, HEPES 10; pH 7.2. Current responses were measured by simultaneous application of a receptor agonist and the GE compound and normalized to the current induced by the receptor agonist in the absence of the GE compound. Current amplitudes were measured 1 s before termination of substance application. The results were obtained from 44 oocytes. All values represent mean ± SEM. The values obtained under control and test conditions were compared using analysis of variance (ANOVA) followed by Dunnett's test. The value for 50% inhibition (IC_{50}) was determined by fitting mean currents to a simplified Langmuir equation $y = (K_m/c)/(1 + K_m/c)$, where y is the fraction of inhibition of control current, K_m is the dissociation constant and c is the concentration of the respective glycol ether compound. For the experiments a recording and application setup was used, which allows fast and repeated drug applications and precise control of drug concentrations (Madeja et al. 1991, 1995). The glutamate subreceptor ligands KA (50 µmol/l) and NMDA (100 µmol/l, 10 µmol/l glycine added) were added to the control solution. Seventeen glycol ether compounds were tested for their interaction with glutamate receptors (Fig. 1A). All reagents were purchased from regular commercial sources.

Results

After injection of rat brain mRNA, *Xenopus* oocytes acquired responsiveness to KA, and NMDA (Figs. 1, 2).

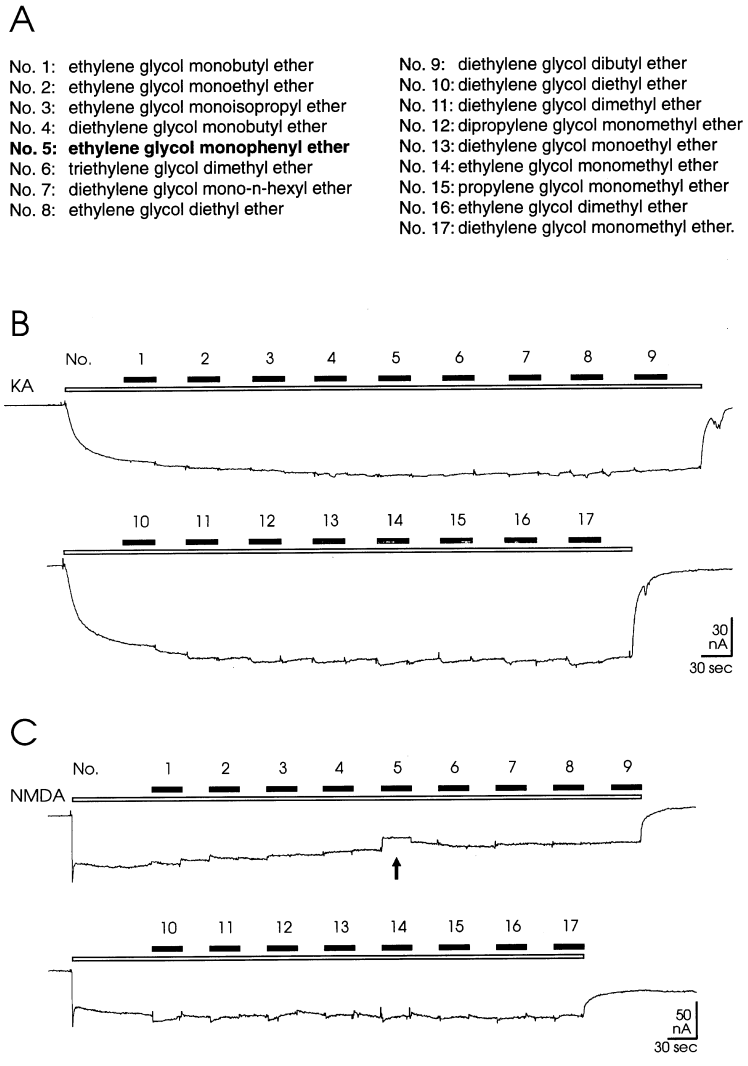


Fig. 1 Effects of 17 glycol ethers (**A**) on kainate (KA)-induced membrane currents (**B**) and on N-methyl-D-aspartate (NMDA)-induced membrane currents (**C**). Original recordings from a *Xenopus* oocyte 3 days after injection of poly(A)$^+$ RNA from rat brain. Holding potential: −70 mV. Administration of 50 µmol/l KA and 100 µmol/l NMDA (*white bars*) and 100 µmol/l of each glycol ether (*black bars*)

The receptor agonists evoked membrane currents in the nanoampere range, which were completely blocked by co-application with their specific antagonists (KA by 100 μmol/l 6-cyano-7-nitroquinoxaline, NMDA by 100 μmol/l aminophosphonovalerate; $n = 3$; data not shown). The agonist responses remained stable and reproducible in amplitude and time course upon successive or prolonged administrations in a given oocyte. The administration of the various GE derivatives alone in concentrations up to 100 μmol/l evoked no detectable membrane currents in the oocytes ($n = 5$).

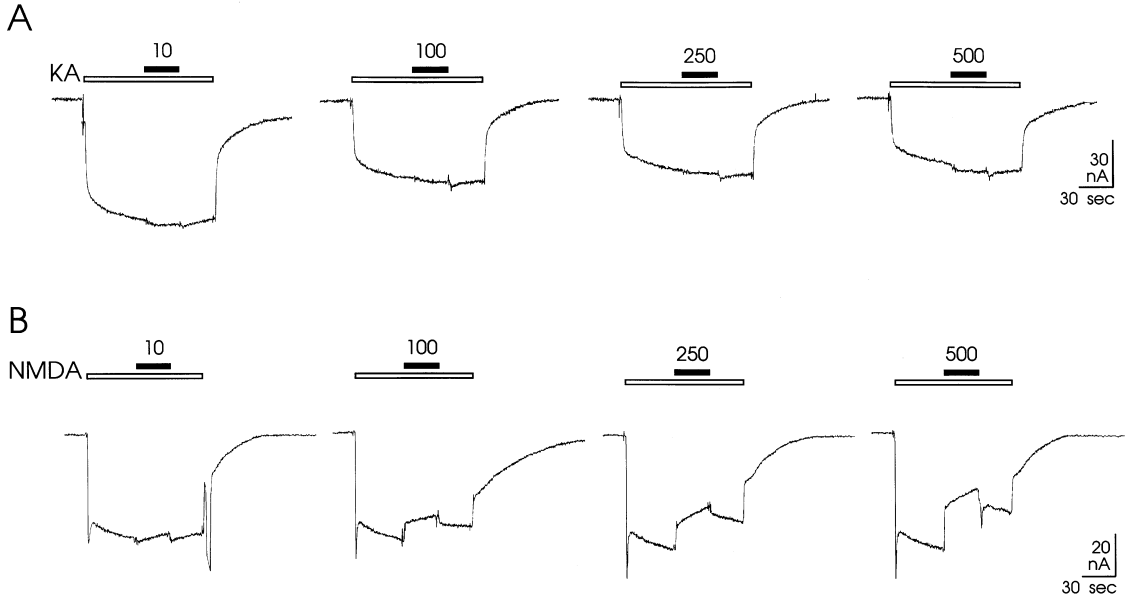
In a first series of experiments, the interaction of the 17 GE derivatives (Fig. 1A) with KA- and NMDA-activated glutamate receptors was tested by the simultaneous application of the agonist with GE. At the beginning of the experiment the receptors were activated by their specific agonists until the resulting membrane current remained stable. During the prolonged application of the receptor agonists the different GE compounds (100 μmol/l each) were successively applied for 30 s. As shown in Fig. 1B, none of the GE tested exerted any significant effect on the KA-induced membrane currents ($n = 6$). When NMDA was used as a receptor agonist solution number 5 (ethylene glycol monophenyl ether = 2-phenoxyethanol) caused a considerable reduction of the agonist response (Fig. 1C; $n = 6$). None of the other GEs exerted any significant effects.

For further clarification, the effects of 2-phenoxyethanol on the glutamate subreceptors were tested with respect to its dependence on the concentration (Fig. 2). The KA-induced membrane currents were not altered by 2-phenoxyethanol applied in concentrations up to 500 μmol/l (Fig. 2A). In contrast, the blocking effect of 2-phenoxyethanol on NMDA responses increased with higher concentrations of the GE derivative (Fig. 2B). The dose-response curve in Fig. 3 shows that the threshold concentration of the 2-phenoxyethanol effect was <10 μmol/l. Application of 1 μmol/l exerted no effect, whereas 10 μmol/l inhibited significantly the NMDA-induced membrane current to 0.92 ± 0.03 ($n = 7$). With administration of 100 and 250 μmol/l of the GE compound, the NMDA response was reduced to 0.75 ± 0.04 and 0.6 ± 0.04, respectively. The concentration for 50% inhibition (IC_{50}), obtained by fitting the mean values to a simplified Langmuir equation, was 362 μmol/l. The blockade of the NMDA currents by ethylene glycol monophenyl ether was completely reversible within 2 min after washing.

Discussion

Several glycol ethers were tested in the oocyte expression assay for their ability to interact with glutamate subreceptors. The findings reported in this study demonstrate that ethylene glycol monophenyl ether (2-phenoxyethanol), caused a considerable reduction of membrane currents elicited by the glutamate subreceptor agonist NMDA. None of the other 16 glycol ether compounds tested exert an effect on the function of this glutamate subreceptor. This result is somewhat surprising considering the structural similarity of the chemicals. It shows that obviously small changes in the chemical structure, in the case of 2-phenoxyethanol probably the aromatic phenylester group, are responsible for selective effects on receptor function. The primary target site could be the channel proteins themselves, the factors that regulate channel activities, or the surrounding lipid bilayer. Since

Fig. 2 Effects of different concentrations of ethylene glycol monophenyl ether (2-phenoxyethanol, solution No 5) on KA-induced membrane currents (**A**) and NMDA-induced membrane currents (**B**). Original recordings from a *Xenopus* oocyte 5 days after injection of poly(A)$^+$ RNA from rat brain. Holding potential: −70 mV. Administration of 50 μmol/l KA and 100 μmol/l NMDA (*white bars*) and 10–500 μmol/l of ethylene glycol monophenyl ether (*black bars*)



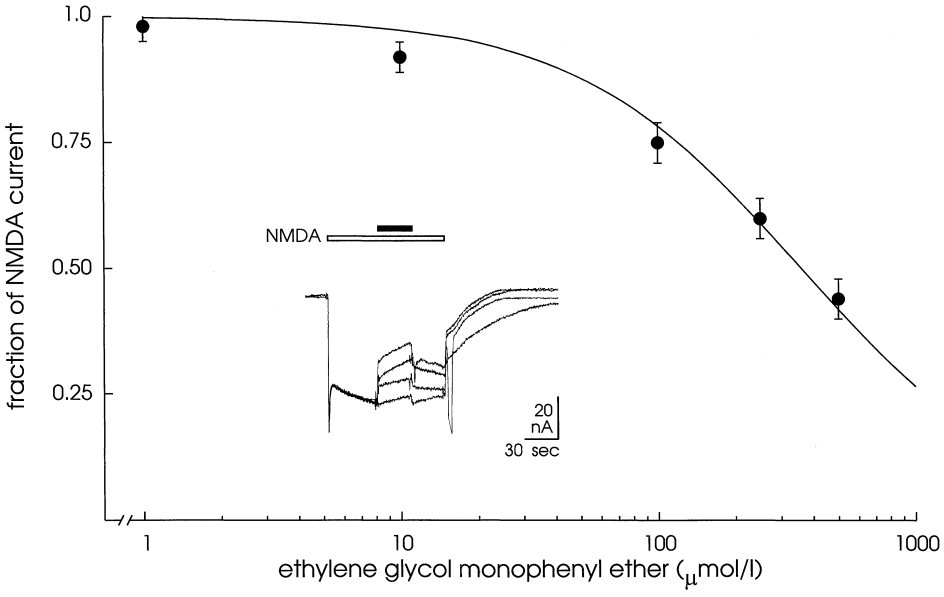


Fig. 3 Inhibition of N-methyl- D-aspartate (NMDA)-induced membrane currents by different concentrations of ethylene glycol monophenyl ether (2-phenoxyethanol, solution No 5). Each point represents mean +SEM of 7 experiments (7 oocytes; 3 frogs). *Inset*: Original recordings from one *Xenopus* oocyte are superimposed. Concentration of NMDA 100 μmol/l; ethylene glycol monophenyl ether was administered in concentrations of 1–500 μmol/l. The currents are shown as a fraction of the current obtained in the absence of GE

the effect of 2-phenoxyethanol occurs immediately and is restricted to the NMDA receptor, whereas KA and GABA receptors are not impaired by this substance, it is likely that this compound interacts directly with the NMDA receptor and not via a nonspecific interaction, for example with the surrounding neuronal membrane. This is further supported by the findings that the other glycol ethers also have no effects on the function of the different receptors nor on the membrane potential of the oocytes.

The effect of 2-phenoxyethanol on NMDA receptors indicates a strong neurotoxic potential of this compound. Generally, impairment of NMDA receptors may lead to several neurological disorders, including a general decrease in neuronal activity and disturbances in the processes of learning and plasticity in man (Stoltenburg-Didinger 1994). This is in line with observations on three patients, showing an immediate and delayed neurotoxic effect of this compound. 2-phenoxyethanol, used as an anaesthetic for handling small fish at a salmon hatchery, caused three women to experience headache and symptoms of intoxication during use, followed by diminished sensation and strength of hands and fingers. After 1 to 2 years of exposure, the patients manifested gradual onset of symptoms of impairments of central cognitive functions that persisted (Morton 1990).

There are no data available concerning the concentration of ethylene glycol monophenyl ether or other GE compounds in brain tissue after exposure. Therefore, the dose-response data determined in the present investigation can hardly be related to the neurotoxic symptoms described by Morton (1990). In general, GE neurotoxicity is dependent on many factors, including species, age, dose, and route of administration. Ahmed and coworkers showed in animal experiments that ethylene glycol monomethyl ether and/or its metabolites could rapidly cross the blood-brain barrier at an early time period after treatment, but a considerable amount was eliminated 24 h after treatment (Ahmed et al. 1983). From the case report above one can conclude that ethylene glycol monophenyl ether also enters the brain tissue and, during longer periods of exposition, may reach concentrations, which are able to impair brain function. This may be to block NMDA-dependent synaptic transmission.

It could not be excluded that, besides 2-phenoxyethanol, metabolites of this substance are responsible for the neurotoxic effects, since all GE undergo extensive in vivo metabolism. Alkoxyacetic acid metabolites appear to be also responsible for the toxic effects of several GE in vivo (Bartnick et al. 1987; Foster et al. 1984; Ghanayem et al. 1987; McGregor et al. 1983; Moss et al. 1985). It is possible that other metabolites of the different GE compounds have special effects on the metabolism of neurons or glial cells, which are in many aspects different to that of oocytes. However, the data presented in this study indicate that not only GE metabolites but also parent molecules may be responsible for neurotoxic effects. In addition, it has been taken into account that metabolites of other GE parent compounds, found not to be effective in the present study, may also exert effects on excitatory or inhibitory receptors. In summary, 2-phenoxyethanol has been shown to counteract the effects of NMDA. This could provide a basis for explanation of neurotoxic signs described in the literature. Nevertheless, the effects of metabolites of 2-phenoxyethanol, especially phenoxyacetic acid, on glutamate receptors need to be further investigated.

References

Ahmed AE, Farooqui MYH, Upreti RK, Elshadbrawy O (1983) Comparative toxicokinetics of 2,3-^{14}C and 1-^{14}C-acrylonitrile in the rat. J Appl Toxicol 3: 39–47

Bartnick FG, Reddy AK, Klecak G, Zimmermann V, Hostynek JJ, Kunstler K (1987) Percutaneous absorption, metabolism, and hemolytic activity of *n*-butoxyethanol. Fundam Appl Toxicol 8: 59–70

Binding N, Madeja M, Musshoff U, Neidt U, Altrup U, Speckmann E-J, Witting U (1996) Prediction of neurotoxic potency of hazardous substances with a modular in vitro test battery. Toxicol Lett 88: 115–120

Cathala G, Savouret J-F, Mendez B, West BL, Karin M, Martial JA, Baxter JD (1983) A method for isolation of intact translationally active ribonucleic acid. DNA 2: 329–335

Dascal N (1987) The use of *Xenopus* oocytes for the study of ion channels. Crit Rev Biochem 22: 317–387

Foster PMD, Creasy DM, Foster JR, Gray TJB (1984) Testicular toxicity produced by ethylene glycol monomethyl and monoethyl ethers in the rat. Environ Health Perspect 57: 207–218

Ghanayem BI, Burka LT, Matthews HB (1987) Metabolic basis of ethylene glycol monobutyl ether (2-butoxyethanol) toxicity: role of alcohol and aldehyde dehydrogenase. J Pharmacol Exp Ther 242: 222–231

Grafton TF, Hansen DK (1987) In vitro embryotoxic effects of ethylene glycol in rats. Teratog Carcinog Mutagen 7: 483–489

Hardin BD (1983) Reproductive toxicity of the glycol ethers. Toxicology 27: 91–102

Hobson DW, D'Addario AP, Bruner RH, Uddin DE (1986) A subchronic dermal exposure study of diethylene glycol monomethyl ether and ethylene glycol monomethyl ether in the male guinea pig. Fundam Appl Toxicol 6: 339–348

Madeja M, Mußhoff U, Speckmann E-J (1991) A concentration-clamp system allowing two-electrode, voltage-clamp investigations in oocytes of *Xenopus laevis*. J Neurosci Methods 38: 267–269

Madeja M, Mußhoff U, Speckmann E-J (1995) Improvement and testing of a concentration-clamp system for oocytes of *Xenopus laevis*. J Neurosci Methods 63: 211–213

McGregor DB, Willins MJ, McDonald P, Holmstrom , McDonald D, Niemeier RW (1983) Genetic effects of 2-methoxyethanol and bis(2-methoxyethyl) ether. Toxicol Appl Pharmacol 70: 303–316

Morton WE (1990) Occupational phenoxyethanol neurotoxicity: a report of three cases. J Occup Med 32: 42–45

Moss EJ, Thomas LV, Cook MW, Walters DG, Foster PMD, Creasy DM, Gray TJB (1985) The role of metabolism in 2-methoxyethanol-induced testicular toxicity. Toxicol Appl Pharmacol 79: 480–489

Mußhoff U, Madeja M, Binding N, Witting U, Speckmann E-J (1995) Lead-induced blockade of kainate-sensitive receptor channels. Naunyn-Schmiedeberg's Arch Pharmacol 353: 42–45

NIOSH (1991) Criteria for a recommanded standard: occupational exposure to ethylene glycol monomethyl ether, ethylene glycol monoethyl ether, and their acetates. US Department of Health and Human Services, Public Health Service, Center for Disease Control, National Institute of Occupational Safety and Health, Division of Standards Development and Technology Transfer. Publ No 91–119, Cincinnati, Ohio

Ohi G, Wegman DH (1978) Transcutaneous ethylene glycol monomethyl ether poisoning in the work setting. J Occup Med 20: 675–676

Parsons CB, Parsons MM (1938) Toxic encephalopathy and granulopenia due to volatile solvents in industry: report of a case. J Ind Hyg Toxicol 20: 124–130

Piacitelli GM, Votaw DM, Krishnan ER (1990) An exposure assessment of industries using ethylene glycol ethers. Appl Occup Environ Hygiene 5: 107–114

Rowe VK, Wolf MA (1982) Derivatives of glycols. In: Clayton GD, Clayton FE (eds) Patty's industrial hygiene and toxicology, 3rd edn Wiley, New York, pp 3911–3919

Schuler RL, Hardin BD, Niemeier RW, Booth G, Hazedlen K, Piccirillo V, Smith K (1984) Results of testing fifteen glycol ethers in a short-term in vivo reproductive toxicity assay. Environ Health Perspect 57: 141–146

Sigel E (1990) Use of *Xenopus* oocytes for the functional expression of plasma membrane proteins. J Membrane Biol 117: 201–221

Stoltenburg-Didinger G (1994) Neuropathology of the hippocampus and its susceptibility to neurotoxic insult. NeuroToxicology 15: 445–450