

# Reproductive Toxicity of the Industrial Solvent 2-Ethoxyethanol in Rats and Interactive Effects of Ethanol\*

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The solvent, 2-ethoxyethanol, induced complete embryomortality in pregnant rats exposed to three times the current Federal permissible exposure limit (PEL). Following exposure to ethoxyethanol at a concentration only one-half the current PEL, the offspring evidenced behavioral and neurochemical deviations from controls. Subsequent studies found that ingestion of ethanol with concomitant inhalation of ethoxyethanol vapors early in pregnancy appeared to reduce the number of both behavioral and neurochemical deviations found for ethoxyethanol. In contrast, the concomitant exposure to ethanol and ethoxyethanol later in gestation potentiated the behavioral and neurochemical effects of ethoxyethanol. This research indicates that the industrial solvent 2-ethoxyethanol presents an occupational reproductive hazard and raises the issue of the importance of an interaction of social habits with occupational exposure to such hazards. The results would suggest that occupational physicians should advise pregnant workers in the chemical industry of the adverse effects of ethanol during pregnancy and of the possible interactions with other chemicals and should encourage them to be especially cautious with ethanol consumption since they may be at greater risk.

Concern over workplace contaminants which may pose reproductive hazards has stimulated intensified research into factors such as genetic defects, reduced fertility, spontaneous abortions, infant deaths, and malformations in the offspring of exposed subjects, both in laboratory and epidemiological studies (1), as recently reviewed (2). Of greatest concern are environmental conditions which may preferentially jeopardize reproduction in the absence of apparent toxicity to the parent organism.

Nelson et al. (3) recently reported an example of an industrial solvent, 2-ethoxyethanol (Cellosolve, ethylene glycol monoethyl ether), which altered fetal development at much lower levels than those which affect the mature organism. This chemical is produced and distributed by at least six major American chemical companies and is widely used in industry (e.g., in lacquers, dopes, inks, varnish removers, cleansing solutions and resins). NIOSH estimates that approximately 365,000 American workers are exposed to ethoxyethanol, as are consumers using some commercial products (e.g., certain cosmetics). Ethoxyethanol is relatively nontoxic,

having an oral LD<sub>50</sub> of 3 to 4 g/kg in most common laboratory animal species (4). The lowest concentration affecting laboratory animals via inhalation is on the order of 2000 ppm for an 8-hr exposure (4), with blood constituent changes followed by hepatic and renal alterations. Its current Federal occupational permissible exposure limit is 200 ppm. Thus ethoxyethanol is only moderately toxic to adult animals.

However, recent reports establish the fact that prenatal exposure of rats and rabbits to levels of 600 ppm ethoxyethanol (7 hr/day) results in death of all developing fetuses (3,5,6). Following prenatal exposure to approximately 200 ppm ethoxyethanol, the current U.S. occupational standard, offspring from both rats and rabbits had an increased incidence of congenital malformations (5,6), and rats had increased neonatal deaths (3). Concentrations one-half that level, 100ppm, did not produce neonatal mortality, but did induce behavioral and neurochemical deviations in the offspring of rats (3). More recently, dermal exposure of rats to ethoxyethanol was found to cause fetal wastage and congenital malformations (7). Thus, it appears that developing animals are susceptible to ethoxyethanol toxicity at much lower concentrations than those producing toxicity in the adult rat. In addition, close structural analogs have also been found to produce embryotoxicity (8,9). Further, ethoxyethanol or close structural analogs have recently been shown to be muta-

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genic or to alter testicular function in experimental animals (10-12). These latter findings extend the apparent impact of the ethoxyethanol results to other glycol ethers and to both male and female reproductive processes. Since ethoxyethanol would theoretically be metabolized in a manner very similar to ethanol (i.e., by alcohol and aldehyde dehydrogenase), we were interested in determining if ethanol would alter the prenatal effects of ethoxyethanol. As ethanol is probably the most commonly used (and abused) drug in our culture, such interactive effects would have obvious implications for occupationally related reproductive hazards. Two possibilities were envisioned: ethanol would either reduce the prenatal effects by induction of hepatic detoxification enzymes, i.e., an antagonistic type of interaction; or it might enhance the prenatal effects (since ethanol is itself a teratogen), i.e., a synergistic type of interaction. After first establishing the reproductive toxicology of ethoxyethanol as discussed above, a second study was undertaken to investigate these interaction possibilities. A concentration of ethanol predicted to produce slight effects and the concentration of ethoxyethanol previously shown to produce clear cut neuromotor and neurochemical effects in offspring were chosen for the experiment.

Behavioral testing is thought to provide a more sensitive technique for assessing prenatal toxicity than standard teratological procedures (13). Accordingly, this research utilized a number of behavioral measures

designed to assess a variety of behaviors at several stages in development, from neonates through sexually mature subjects. The majority of these tests are frequently used in similar studies (14). In addition, levels of four common neurotransmitters were measured in newborn animals and 21-day-old offspring, a time when the development of several neurotransmitter systems plateaus in developing rats. Tables 1 and 2 show the different behavioral tests and neurochemical assays, along with the test ages, included in this research.

Because of the extensive amount of data generated from this study, details have been published elsewhere (3,15,16). This report summarizes the results of the research and presents the conclusions which can be drawn from a synthesis of the data.

The experimental procedures were essentially identical in two experiments, the first on ethoxyethanol alone (3), followed by a study including ethanol and ethoxyethanol (15,16). Virgin Sprague-Dawley female (200-300 g) rats were mated with breeders of the same strain. Between 15 and 20 pregnant rats were assigned to each of the following experimental groups representing the combination of conditions (ethanol, administered in the drinking water, and ethoxyethanol administered via inhalation, at different stages of gestation) included in the two studies: (1) control (sham-exposed) during gestation days 7-13 (C 7-13); (2) control (sham-exposed) during gestation days 14-20 (C14-20); (3) 10% ethanol (and sham-exposed) during gestation days 7-13 (E 7-13); (4) 10% ethanol (and sham-exposed) during gestation days 14-20 (E 14-20); (5) 100 ppm ethoxyethanol during gestation days 7-13 (100 EE 7-13); (6) 100 ppm ethoxyethanol during gestation days 14-20 (100 EE 14-20); (7) 10% ethanol and 100 ppm ethoxyethanol during gestation days 7-13 (E + 100 EE 7-13); (8) 10% ethanol and 100 ppm ethoxyethanol during gestation days 14-20 (E + 100 EE 14-20).

Pregnant rats were individually housed in polycarbonate cages with sawdust bedding, and feed and water were available *ad libitum*, except for the groups given ethanol. Animals were exposed to ethoxyethanol vapor in 0.5 m<sup>3</sup> exposure chambers with the exposure concentration continuously monitored by an infrared analyzer and verified at least weekly by a gas chromatograph. Exposures were conducted 7 hr/day on gestation days 7-13 or 14-20. Alcohol (10% w/w) was given in the drinking water during the time the animals were not in the inhalation chambers. Following birth, litters were culled to eight pups (four females and four males) which were randomly assigned to test groups on postnatal day 10. One female and one male pup per litter were used for each behavioral test (Table 1).

At least ten pups per treatment groups were sacrificed by focused microwave irradiation for neurochemical analyses of whole-brain samples on the day of birth. An equivalent number were sacrificed by focused microwave irradiation on day 21, and the brains were separated into four brain regions (cerebrum, cerebellum, brainstem, and midbrain). Assays on all samples used

**Table 1. Behavioral tests and days of testing in behavioral teratology study (see text for explanation).**

Behavioral test	Function tested	Age at which test administered, days
Ascent on wire mesh	Neuromuscular integrity	10, 12, 14
Rotorod	Neuromuscular integrity	21, 25, 29
Open field	Exploratory activity	16, 17, 18; 30 31, 32, 44, 45, 46; 58, 59, 60
Activity wheel	Circadian activity	32-33
Avoidance conditioning	Aversive learning	Begun days 34, 60
Operant conditioning	Appetitive learning	Begun day 40

**Table 2. Neurochemical tests and days of testing (see text for explanation).**

Neurochemical assay	Site tested	Age at which sample taken, days
Protein	Whole brain	Newborn
Acetylcholine		
Dopamine		
Norepinephrine		
5-Hydroxytryptamine		
Same chemicals	Cerebrum Cerebellum Midbrain Brainstem	21

spectrofluorometric techniques (Table 2). Generally, data were analyzed by multivariate analysis of variance, but, in some selected cases, univariate or nonparametric techniques were more appropriate (3,15,16).

## Results and Discussion

Tables 3 and 4 present a summary of the behavioral and neurochemical results. Ethoxyethanol, whether alone or in combination with ethanol, generally extended pregnancy duration, but ethanol alone did not. Ethanol on gestation days 14–20 reduced maternal weight gain, feed consumption, and liquid consumption. Pup weights were not affected through the first 5 weeks of life, the period over which they were measured.

Behavioral testing of pups showed that ethanol on gestation days 7–13 was associated with advanced neuromuscular ability on the ascent test, but with poorer performance on the rotorod. Other tests on these pups showed no significant differences from controls. Exposure to 100 ppm ethoxyethanol on gestation days 7–13 caused significant decreases in rotorod performance on each test day and an increase in the latency of leaving the central area of an open field. However, the combination of ethoxyethanol and ethanol during the

same gestational period caused only two changes from controls—a decrease in the number of shuttles in the adaptation period of avoidance conditioning in young rats but an increase in that parameter in older rats. Thus, ethanol early in gestation appeared to reduce the behavioral effects of ethoxyethanol, ameliorating the neuromuscular effects of both exposures.

No behavioral differences were detected in offspring of rats given ethanol alone on gestation days 14–20. Maternal exposure to 100 ppm ethoxyethanol on gestation days 14–20 resulted in pups whose activity wheel measures were depressed and whose performance was retarded in avoidance conditioning. Following combined exposure to ethoxyethanol and ethanol, different measures became affected. That is, open field activity was depressed at each age tested, latency of leaving the central area of the open field was increased, and the number of shuttles in avoidance conditioning was decreased in pups of both ages—during the adaptation period as well as during the conditioning trials themselves. Thus, ethanol later in gestation appeared to potentiate the behavioral effects of ethoxyethanol, depressing both activity and learning.

In neurochemical assays, ethanol on gestation days 7–13 produced a single deviation from controls, viz., an

Table 3. Summary of the behavioral and maternal effects of ethanol, 2-ethoxyethanol and combined exposures on gestation days 7–13 or 14–20, relative to the respective controls.

	E 7–13	EE 7–13	E + EE 7–13	E 14–20	EE 14–20	E + EE 14–20
Maternal weight gain and feed and water consumption				↓		
Pregnancy duration			↑		↑	↑
Behavioral tests						
Ascent, day 10	↑					
12						
14	↑					
Rotorod, day 21		↓				
25	↓	↓				
29	↓	↓				
Open field, L <sup>a</sup>		↑				↑
Day 17						↓
31						↓
45						↓
59				↓		
Activity wheel						
Day					↓	
Night					↓	
Avoidance conditioning						
Young						
CR-5 <sup>b</sup>			↓			↓
CR-20 <sup>c</sup>						↓
Shocks						
Older						
CR-5 <sup>b</sup>			↑			↓
CR-20 <sup>c</sup>						↓
Shocks					↑	
Total deviations	4	4	3	1	4	10

<sup>a</sup>Latency.

<sup>b</sup>Crosses in 5-min warmup.

<sup>c</sup>Crosses in 20 trials.

**Table 4. Summary of the neurochemical effects of ethanol, 2-ethoxyethanol and combined exposures on gestation days 7-13 or 14-20.**

Site and compound <sup>a</sup>	E 7-13	100 EE 7-13	E + 100 EE 7-13	E 14-20	100 EE 14-20	E + 100 14-20
Cerebrum						
ACh		↑			↑	↑
5HT					↑	
DA		↑			↑	
NE		↑				
Protein						↓
Midbrain						
ACh	↑	↑				
5HT						↓
DA						↓
NE		↑				
Protein		↑				
Cerebellum						
ACh		↑	↑			
Ne						
Brainstem						
NE		↑				
Newborn						
ACh				↓		↓
5HT				↓		
DA				↓		↓
NE		↓			↓	
Protein						↑
Total deviations	1	9	3	3	4	7

<sup>a</sup>See Table 2.

increase in midbrain acetylcholine (ACh) in 21-day-old pups. Exposure to 100 ppm ethoxyethanol alone on days 7-13 caused increases in cerebral ACh, dopamine (DA), and norepinephrine (NE), midbrain ACh, NE, and protein, cerebellar ACh, and brainstem NE in 21-day-old pups, and decreases in NE in newborn pups. In contrast, the combination of ethoxyethanol and ethanol on days 7-13 resulted in fewer than one-half that number of deviations: these were increases in cerebral and cerebellar ACh in 21-day-old pups and decreases in DA and 5HT in newborn pups. Thus, ethanol early in gestation appeared to moderate the neurochemical effects of ethoxyethanol.

Ethanol on gestation days 14-20 reduced levels of DA, NE, and 5-hydroxytryptamine (5HT) in whole-brain samples of newborn pups, but no significant differences from control were seen in 21-day-old pups. Exposure to 100 ppm ethoxyethanol alone on gestation days 14-20 produced increased levels of cerebral ACh, DA, and 5HT in 21-day-old pups, and decreased levels of NE in newborn pups. Following combined exposure to ethoxyethanol and ethanol, elevations in cerebral ACh occurred along with decreases in cerebral protein and midbrain ACh and 5HT in 21-day-old pups. There were also decreases in ACh and DA, and increases in NE in newborn pups. Overall, ethanol later in gestation altered the pattern of and appeared to enhance the neurochemical effects of ethoxyethanol.

Table 5 presents a summary of the number of effects observed following exposure to ethoxyethanol alone and when combined with ethanol. Ethanol early in gestation appeared to reduce both the behavioral and neurochemical effects of ethoxyethanol, in that there were only about one-half the number of deviations seen in the

**Table 5. Summary of the number of effects following maternal exposure to ethoxyethanol alone or to ethoxyethanol and ethanol.<sup>a</sup>**

	E	100 EE	E + 100 EE
Days 7-13			
Maternal and behavioral	4	4	3
Neurochemical	1	9	3
Total	5	13	6
Days 14-20			
Maternal and behavioral	1	4	10
Neurochemical	3	4	7
Total	4	8	17

<sup>a</sup>The numbers included below are merely a summary of differences from control (from Tables 3 and 4). Maternal weight gain and feed and liquid consumption are highly correlated; thus the group with decreased values (E 14-20) was given a score of 1 on this variable. All other counts were based on a score of 1 for each test day of difference from control (e.g., if rotorod performance were decreased on all three days of testing, the score would be 3). We used this scoring system because we believe that a consistent pattern of deviations should be given a higher score than if only one day's score is different from controls.

combination condition than were observed after ethoxyethanol alone.\* In contrast, ethanol later in gestation produced over twice as many behavioral and neurochemical alterations as ethoxyethanol alone, thus appearing to potentiate the effects of ethoxyethanol

\*The reduction in the number of defects after concomitant ethanol administration should not be construed to imply that such an effect would be encountered in humans. The point to be emphasized is that an interactive effect was observed during both stages of gestation examined.

(the number of effects were not merely the additive effects of each alone).

The reason a paradoxical prenatal interaction between ethoxyethanol and ethanol was observed is unclear, and would certainly not be predicted. However, the consistency in the patterns of behavioral and neurochemical alterations is striking. It may be that the different events in neural tissue development at different gestation times could account for some of the effects observed. Much of the neural system architecture is formed early in gestation (corresponding to the early exposure period), but neuron development and synaptogenesis take place later in development. Thus it may well be that the chemical interaction affected those processes differently. Further, it is likely that placental transport and biotransformation (17), and emerging fetal biotransformation abilities (18-20) contributed to the effects we observed. However, the mechanism by which ethanol exerted its interaction with the prenatal effects of ethoxyethanol remains to be elucidated.

Overall, the results indicate that 2-ethoxyethanol is embryotoxic in experimental animals and that there may be an interaction between ethanol and ethoxyethanol. The clear implication of these results is that women who work with this solvent would be especially prudent to avoid drinking ethanol while pregnant. Furthermore, our results raise the general possibility that women who work with chemicals while pregnant may place the developing fetus at substantially greater risk through ethanol intake. Occupational physicians should adopt a conservative view and advise women who work with chemicals of the hazards of prenatal ethanol consumption and encourage them to be cautious since they may be at greater risk.

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