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Behavioral Teratology of Ethylene Glycol Monomethyl and Monoethyl Ethers

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A recent addition to the field of teratology has been the inclusion of functional assessment techniques of offspring after prenatal exposure to exogenous agents. The present paper reviews the behavioral teratogenic effects of ethylene glycol monomethyl ether (EGME, 2-methoxyethanol) and ethylene glycol monoethyl ether (EGEE, 2-ethoxyethanol). Groups of 15 pregnant Sprague-Dawley rats were exposed via inhalation to 25 ppm EGME or to 100 ppm EGEE on gestation days 7 to 13 or 14 to 20. An equal number of sham-exposed controls were included for both periods of gestation. The only effect noted in the maternal animals was a slightly prolonged gestation in the group exposed to 100 ppm EGEE on days 14 to 20. Litters were culled to four female and four male pups on the day of birth. Pups of each sex from all litters were tested on a variety of behavioral tasks (including tests of neuromuscular ability, activity, and learning ability) extending from postnatal days 10 to 90. In addition, brains from newborn and from 21-day-old offspring were removed and analyzed for concentrations of the neurotransmitters acetylcholine, dopamine, norepinephrine, and 5-hydroxytryptamine (serotonin). Both the behavioral testing and the neurochemical evaluations revealed functional alterations in the litter groups experiencing prenatal exposure to EGME and EGEE at concentrations which produced no observable effects in the maternal animals.

Introduction

As teratological testing has evolved, there has been an increasing number of observations made on the offspring following maternal exposure to a variety of test agents. At first, offspring were probably just observed to see if they had the usual number of eyes and ears, and that they were located in the usual places. More sophistication was added when the pregnant animals were sacrificed and fetuses were examined closely for internal defects, whether by serially slicing them and looking for soft-tissue anomalies, or by staining their skeletons and looking for defects. More recently, investigators have recognized that functional assessment may detect deficits not observed by other techniques; proponents of this testing have hypothesized that functional assessment may be a more sensitive indicator of prenatal insult than are conventional techniques which rely on morphological changes. Hence, a variety of functional assessment techniques have been proposed for a number of organ systems: cardiovascular, immune, renal, respiratory and central nervous system (CNS). The last of these has received the greatest emphasis to date; its investigation is commonly referred to as behavioral teratology or developmental neurotoxicology.

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Briefly, behavioral teratology is that branch of science which investigates the effects of perinatal administration of exogenous agents on CNS function; most commonly, this function has been assessed using neurobehavioral, neurochemical, and/or neuropathological techniques. Generally a test agent is administered to experimental animals at some point during gestation. Offspring of these animals are reared either by their own mother or, in cases where the experimental treatment is thought likely to affect seriously the maternal animal, the offspring are fostered to untreated control mothers shortly after birth. Litters are usually culled to a standard number of pups to avoid confounding variables related to nutritional differences or to altered maternal interactions from differing numbers of pups. A dose of test chemical is selected through prior testing, which results in a minimal effect either on mortality or weight of the pups. Following administration of that dose to the maternal animal and her delivery of the pups, the pups are individually identified and are longitudinally tested on any number of tests to determine if there are deviations from similarly handled control pups.

Range-Finding Studies

The present study follows this general pattern of behavioral teratogenic evaluation of the chemicals ethylene glycol monomethyl ether (EGME, 2-methoxyethanol) and ethylene glycol monoethyl ether (EGEE,

2-ethoxyethanol). With both solvents, we first investigated some dose-effect relationships on more conventional teratologic outcomes. For our initial concentration of EGEE, we planned to expose pregnant Sprague-Dawley rats to a concentration 10 times the current Permissible Exposure Limit (PEL) of 200 ppm, or 2000 ppm. Since the highest point on the initial calibration curve was 1200 ppm, we decided to see what effect 1200 ppm exerted before going any higher. To our surprise, after exposure of rats on gestation days 7 to 13, no fetuses survived; they were all resorbed (*1*). Consequently, we reduced that concentration by one-half, and exposed a small number of rats to 600 ppm. However, even after exposure to 600 ppm on gestation days 7 to 13, three-fourths of the fetuses were resorbed, and no pups were born alive. At 600 ppm we also exposed a small number of rats on gestation days 14 to 20, as exposure during this period often results in behavioral disturbances and poor survivability of the offspring in the absence of gross defects. Only one of six mothers so exposed kept pups alive, and these survived only 10 days and appeared very runtlike (growth and developmentally retarded). Even after exposure of maternal animals to 200 ppm EGEE on gestation days 14 to 20, the current PEL, about one-third of the offspring died within the first week of life. We also observed that in those mothers which were allowed to deliver their pups, gestation was prolonged by exposure to EGEE. The animals delivered their offspring about 48 hr later than expected; after exposure at 200 or 300 ppm, delivery was delayed by 12 to 24 hr.

We consequently decided on 100 ppm EGEE as the concentration for the behavioral teratology study, a concentration which produced no apparent mortalities or weight reductions in the offspring nor effects in the maternal animal. During this same period of time, Andrew (*2*) was performing the conventional teratology study on EGEE, so we did not look further at the offspring at the higher levels.

With EGME, based primarily on the results with EGEE, we first exposed groups of rats to 200 ppm EGME on gestation days 7 to 15. At that level, all fetuses were resorbed (*3*). At 100 ppm, nearly one-half of the litters were entirely resorbed; surviving fetuses were very small and had an increased incidence of malformations—primarily cardiovascular and skeletal defects. At 50 ppm, there were still increases in resorptions, reductions in fetal weights and an increased incidence of malformations. Consequently, we decided upon the level of 25 ppm, the current PEL, for the behavioral teratology study with EGME.

Procedures and Methods

For both solvents, the behavioral testing paradigm was very similar. Groups of 15 to 20 pregnant Sprague-Dawley rats were exposed either on gestation days 7 to 13 or 14 to 20; the earlier period was included as it encompasses the majority of organogenesis, the period of maximal susceptibility to teratogenic agents. The later period was included as it encloses the primary period during which brain growth and development occurs; as opposed to structural malformations, functional deficits are often induced by exposures during this later period (*4*). Two sets of controls were sham-exposed during these same periods of gestation. Maternal weights, as well as feed and water intake, were measured at weekly intervals. Maternal rats were allowed to deliver their young. The cages of expectant females were checked around 7:30 a.m. and 3:30 p.m. each day for babies. If the maternal rat had completed parturition at either time, we weighed the maternal animal and the entire litter. Four female pups and four male pups were selected without bias and placed back into the cage with the mother rat, and the remaining pups were discarded (except in cases where two were used for neurochemical analysis as will be discussed below). Pups were individually weighed on postnatal days 7, 14, 21, 28 and 35. On day 10, the pups were randomly selected, individually marked, and assigned to test groups shown in Table 1, with one female and one male per litter assigned to each test.

The test procedures have been described in detail for both EGEE (*1*) and EGME (*5*). Consequently, only a brief summary is presented here.

Table 1. Behavioral and neurochemical tests and days of testing in behavioral teratology study.

Tests	Function tested	Age, days
Behavioral tests		
Ascent on wire mesh	Neuromuscular	10, 12, 14
Rotorod	Neuromuscular	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
Neurochemical assays		
Whole brain	Protein, acetylcholine, dopamine, norepinephrine, 5-hydroxtryptamine	Newborn
Cerebrum, cerebellum, brainstem, midbrain	Same chemicals	21

Behavioral Testing

Ascent on a wire mesh screen is a simple test of neuromuscular ability. On days 10, 12 and 14, one female and one male pup from each litter were placed on an inclined screen. On the first day of testing, most rats fall from the screen within 10 to 20 sec; by day 14, however, most rats climb to the top within about 30 sec. The time each animal held onto the screen and the distance climbed within a 60-sec maximum were recorded on each test day.

The rotorod is a more sophisticated test of neuromuscular ability. On days 21, 25 and 29, the same female and male rats used on the ascent test were used for this test. The velocity of a rotating rod was increased as long as the test rats could walk or run upon the rod; the highest velocity attained by the individual rats served as the basis for comparison.

The open field is a commonly used measure of exploratory activity. At 2-week intervals from days 16 to 60, one female and one male rat from each litter were placed in the center of this 1-m field; for 3 min/day on three consecutive days, the rats were scored for the number of sections they entered.

Activity wheels were utilized to measure circadian activity. The same rats used in the open field test were given free access to the activity wheels for approximately 24 hr, and the number of revolutions each hour were recorded.

Avoidance conditioning is a simple, aversively motivated test of learning ability. Briefly, rats from two ages were tested for their ability to learn to avoid electric shock. A buzzer sounded for 5 sec, and, unless the rat moved to the opposite side of the grid floor, it received 0.7 m A electric footshock. Thus, the rats generally learn to avoid the shock after several days of 20 trials per day.

Operant conditioning as we used it was a simple appetitively motivated learning task. Water-deprived rats learned to press a bar for water reinforcement. We then required them to make an increasing number of responses to receive the water reinforcement. The task involves their learning to make the additional number of responses necessary to receive the reinforcement until the animals no longer respond sufficiently to receive reinforcement.

Neurochemical Assays

Within the CNS, messages are passed from one cell to another by the release of a tiny amount of chemicals known as neurotransmitters. Alterations in the concentrations, turnover rates, or receptor binding characteristics of these transmitters are indications of insult to the CNS. We measured the concentrations of four of the neurotransmitters in addition to brain protein: acetylcholine, dopamine, norepinephrine, and 5-hydroxytryptamine or serotonin. In newborn pups, we analyzed whole-brain samples for these chemicals; in 21-day-old offspring, we separated each brain into four general regions: cerebrum, cerebellum, brainstem and midbrain.

Statistical Analyses

Where possible, data were analyzed by using multivariate analysis of variance; in some cases where assumptions required for parametric analysis were in question, data were analyzed using appropriate nonparametric procedures.

Results

Teratological findings from the higher concentrations of both chemicals were discussed earlier in this paper. At 100 ppm EGEE, we saw no treatment-related effects in the maternal animals except a slight prolongation of gestation in those exposed on gestation days 14 to 20. After exposure at 25 ppm EGME, maternal animals showed no treatment-related effects.

Several of the behavioral tests described above showed significant differences from controls after maternal exposure to 100 ppm EGEE either on gestation days 7 to 13 or 14 to 20 as shown in Table 2. After exposure on days 7 to 13, offspring had impaired performance on the rotorod and in the open field, and had altered performance in avoidance conditioning when tested at the earlier age. After exposure on days 14 to 20, offspring had impaired performance in the activity wheel and in avoidance conditioning when tested at the older age.

Also shown in Table 2 are the behavioral effects seen in the offspring after maternal exposure to 25 ppm EGME. The only significant effects were in the avoidance conditioning test, where there were alterations in the performance of the offspring from the group exposed on days 7 to 13.

Table 3 presents the significant differences in neurochemical results. As may be seen, numerous alterations were detected after exposure to 100 ppm EGEE on gestation days 7 to 13, with fewer effects after exposure on days 14 to 20. However, maternal exposure to 25 ppm EGME during either period of gestation resulted in

Table 2. Results of behavioral testing in behavioral teratology study.[a]

	EGME		EGEE	
Test	7–13 days	14–20 days	7–13 days	14–20 days
Ascent	0	0	0	0
Rotorod	0	0	–	0
Open field	0	0	+	0
Activity wheel	0	0	0	–
Avoidance conditioning	–	0	–	+
Operant conditioning	0	0	0	0

[a]0 = effect not significantly different from controls ($p < 0.05$); – = significant effect lower than control value; + = significant effect higher than control value.

Table 3. Results of neurochemical assays in behavioral teratology study.[a]

	EGME		EGEE	
	7–13 days	14–20 days	7–13 days	14–20 days
Newborn				
Ach	0	0	0	0
DA	0	0	0	0
NE	0	0	−	−
5-HT	0	0	0	0
21-day-old, cerebrum				
Ach	−	−	+	+
DA	+	+	+	+
NE	−	−	+	0
5-HT	0	−	0	+
21-day-old, cerebellum				
Ach	0	0	+	0
DA	−	−	0	0
NE	0	+	0	0
5-HT	+	+	0	0
21-day-old, brainstem				
Ach	+	+	0	0
DA	−	−	0	0
NE	+	+	+	0
5-HT	+	+	0	0
21-day-old, midbrain				
Ach	0	0	+	0
DA	0	0	0	0
NE	0	−	+	0
5-HT	+	+	0	0

[a]0 = concentration of neurotransmitter not significantly different from control ($p < 0.05$); − = concentration of neurotransmitter significantly reduced from controls; + = concentration of neurotransmitter significantly elevated from controls.

numerous neurochemical alterations, particularly in the brainstem and cerebrum.

Discussion

Our results indicate that maternal exposure of rats to 100 ppm EGEE or to 25 ppm EGME during gestation still exerts some effect which leads to alterations in the CNS of the offspring, though the low number of behavioral alterations after exposure to 25 ppm EGME may indicate that this concentration is near the threshold for producing effects in our test system. Though we did not test these concentrations for conventional teratogenic effects and thus cannot be certain, our findings suggest that these behavioral effects occurred at exposure levels lower than those required to produce conventional teratogenic effects. This result is consistent with reports in the literature for a number of other chemicals (*6*). It lends support to the hypothesis that functional assessment may be a more sensitive indicator of prenatal damage than are conventional techniques.

Our results with the neurochemical assays are also interesting. With both EGEE and EGME, alterations were produced quite consistently in the neurochemical assays as opposed to the behavioral tests. Whereas additional studies investigating other neurochemical parameters (e.g., other transmitters, turnover rates, receptor binding) will be required to elucidate the functional significance of the changes we observed, it does appear that neurochemical evaluations may serve as even more sensitive indicators of prenatal damage then behavioral ones.

Finally, our results highlight the susceptibility of the developing organism to the toxic effects of these glycol ethers. Our observations of alterations in the CNS of the offspring after exposure during prenatal life occurred at concentrations which produced no observable adverse effect in the maternal animals.

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