Journal of Toxicology and Environmental Health, Part A Current Issues

ISSN: 0098-4108 (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/uteh19

Developmental toxicity of nine selected compounds following prenatal exposure in the mouse: Naphthalene, *p*-nitrophenol, sodium selenite, dimethyl phthalate, ethylenethiourea, and four glycol ether derivatives

Michael R. Plasterer , William S. Bradshaw , Gary M. Booth , Melvin W. Carter , Ronald L. Schuler & Bryan D. Hardin

To cite this article: Michael R. Plasterer , William S. Bradshaw , Gary M. Booth , Melvin W. Carter , Ronald L. Schuler & Bryan D. Hardin (1985) Developmental toxicity of nine selected compounds following prenatal exposure in the mouse: Naphthalene, *p*-nitrophenol, sodium selenite, dimethyl phthalate, ethylenethiourea, and four glycol ether derivatives, Journal of Toxicology and Environmental Health, Part A Current Issues, 15:1, 25-38, DOI: 10.1080/15287398509530633

To link to this article: https://doi.org/10.1080/15287398509530633

DEVELOPMENTAL TOXICITY OF NINE SELECTED COMPOUNDS FOLLOWING PRENATAL EXPOSURE IN THE MOUSE: NAPHTHALENE, *p*-NITROPHENOL, SODIUM SELENITE, DIMETHYL PHTHALATE, ETHYLENETHIOUREA, AND FOUR GLYCOL ETHER DERIVATIVES

Michael R. Plasterer, William S. Bradshaw, Gary M. Booth, Melvin W. Carter

Departments of Zoology and Statistics, Brigham Young University, Provo, Utah

Ronald L. Schuler, Bryan D. Hardin

National Institute for Occupational Safety and Health, Robert A. Taft Laboratories, Cincinnati, Ohio

Ethylene glycol dimethyl ether (EGdiME), diethylene glycol dimethyl ether (diEGdiME), triethylene glycol dimethyl ether (triEGdiME), diethylene glycol diethyl ether (diEGdiEE), ethylenethiourea (ETU), sodium selenite (SS), dimethyl phthalate (DMP), naphthalene (NAP), or p-nitrophenol (PNP) were administered by gavage for eight consecutive days to female CD-1 mice. Weight loss was insensitive as an index of sublethal adult toxicity and was inadequate for determining a maximum tolerated dose. LD50 values indicate that SS, NAP, and PNP were more toxic (8.4, 353.6, and 625.7 mg/kg, respectively) than the polyglycol ethers, ETU, and DMP (LD50 values ranged from 2525.8 to 6281.9 mg/kg). Each of the compounds was administered on d 7 through 14 to pregnant animals at a single dose estimated to be at or just below the threshold of adult lethality. In such a reproductive study, each of the compounds could be categorized on the basis of the pattern of maternal lethality and fetotoxicity which it produced. The number of dams with complete resorptions was significantly increased after administration of ETU, and no mice in the EGdiME-, diEGdiME-, or triEGdiME-treated groups delivered any viable offspring. Maternal lethality was significant in the EGdiME, triEGdiME, PNP, and NAP groups. There was a slight reduction in the average number of live pups per litter in the diEGdiEE- and PNP-treated groups and a significant reduction in the NAP group. The number dead per litter was increased with diEGdiEE. SS and DMP had no effect on maternal or fetal survival at the doses administered. Individual pup weight at d 1 postpartum was only significantly reduced by diEGdiEE, and no gross congenital abnormalities were detected in neonates from any treatment group. These results provide guidelines for the subsequent toxicity testing of these chemicals.

This work was conducted as part of a project of the National Toxicology Program using protocols developed by National Institute for Occupational Safety and Health (NIOSH) personnel (contract 210-81-6012 with Minority Enterprise Service Associates and funding from the National Institute of Environmental Health Sciences NTP/NIOSH interagency agreement IA-81-70).

Requests for reprints should be sent to Gary M. Booth, Department of Zoology, 601 WIDB, Brigham Young University, Provo, Utah 84602.

Journal of Toxicology and Environmental Health, 15:25–38, 1985

INTRODUCTION

It is increasingly difficult to adequately assess the toxicity of the extraordinary number of different chemicals used in science and industry. Clearly, the rapid identification of the potentially hazardous chemicals would facilitate this work. Unfortunately, the identification of these compounds is complicated by the fact that many chemicals, which are relatively nontoxic in adults, can be extremely teratogenic (Ruddick and Khera, 1975; Chernoff, 1977; Spiers and Aberd, 1962). This latent teratogenic potential makes it imperative that the toxicity screening of suspect chemicals include reproductive as well as individual adult toxicity parameters.

The objective of this investigation was to study the developmental effects of prenatal exposure to nine selected chemicals in a reproductive screen similar to that described by Chernoff and Kavlock (1982). Four laboratories participated in the overall project, which was sponsored by the National Institute for Occupational Safety and Health (NIOSH). The compounds selected by NIOSH for testing in our laboratory included 4 glycol ethers [ethylene glycol dimethyl ether (EGdiME), diethylene glycol dimethyl ether (diEGdiME), triethylene glycol dimethyl ether (triEGdiME), and diethylene glycol diethyl ether (diEGdiEE)], part of a group of 15 whose structure-activity relationships were under study; 2 compounds selected to provide comparisons across all 4 laboratories and with earlier work [ethylenethiourea (ETU), sodium selenite (SS)]; and 3 compounds selected from the list developed by the Chemical Selection Committee of the National Toxicology Program (NTP) and recommended for testing due either to information gaps or suspicion of possible reproductive toxicity [dimethyl phthalate (DMP), naphthalene (NAP), and *p*-nitrophenol (PNP)].

Toxicity information about the polyethylene glycol ethers used in this study is very limited. Indeed, toxicity data for triEGdiME has not previously been reported. However, LD50 values in the rat for diEGdiEE (Lewis and Tatken, 1980) and other polyethylene derivatives indicate that these compounds are moderately toxic and require detailed reproductive hazard analysis. EGdiME has been shown to increase embryonic death and cause skeletal and external anomalies in mice, and diEGdiME has an adverse effect on the male reproductive system (Hardin, 1983).

ETU is a common degradation product of ethylene bisthiocarbamates (Bontoyan and Looker, 1973), which are used extensively in agriculture. ETU has displayed some mutagenic activity in microbial assays (Seiler, 1974; Schupbach and Hummler, 1977), and the results of other studies have demonstrated that ETU is teratogenic in rats (Chernoff et al., 1979; Khera, 1973) at doses producing no maternal toxicity. In mice, ETU caused maternal death but did not produce neonatal toxicity (Chernoff and Kavlock, 1982).

Despite its relatively high degree of cytotoxicity, SS has recently been investigated as an antidote for methylmercury poisoning (Matsumoto et

al., 1979) and as an antitumor agent. The compound reduces tumor growth in mice (Watrach et al., 1982), suppresses the reverse transciptase activity of bovine leukemia virus (Balansky and Argirova, 1981), and increases the longevity of mice exposed to leukemic cells (Milner and Hsu, 1981).

DMP is an ubiquitous environmental contaminant used industrially as a plasticizer in the production of polyvinyl chloride plastics and as an ingredient in insect repellents and perfumes. The extensive world-wide distribution of DMP and other phthalate esters has raised some concern about their potential harmful effects. DMP has been shown to be relatively nontoxic in comparison with other phthalate esters (Sugawara, 1974; Lagente et al., 1979), and it has not displayed carcinogenic properties (Peakall, 1975). However, Singh et al. (1972) found reduction in individual pup weight and litter size, and teratogenic effects such as elongated and fused ribs, incomplete skull bone formation, and the absence of eyes in rats after dams were injected intraperitoneally with DMP.

NAP, a product of petroleum refining and coal-tar distillation, is used in the production of pesticides, dyes, detergents, and moth balls. Although NAP is nonmutagenic in the *Salmonella*/microsome test (McCann et al., 1975), and is believed to be noncarcinogenic (Freeman et al., 1973), it can cross the placental barrier (Anziulewicz et al., 1959) and has been associated with hemolytic anemia and kernicterus (Zinkham and Childs, 1957; Gosselin et al., 1976).

The last compound, PNP, was nonmutagenic both with and without rat-liver microsomal activation in the *Salmonella* assay (McCann et al., 1975) but has been shown to induce DNA damage and inhibit DNA repair mechanisms (Adler et al., 1976; Poirier et al., 1975).

The present study consisted of two phases. First, a maximum tolerated dose (MTD) was determined for each chemical based on lethality and debilitating effects in nonpregnant mice. This corresponded to the dose producing an approximate LD10. Second in the reproductive phase, timed-pregnant animals were treated orally at that dose for each compound over the period of major organogenesis. These relatively high doses were used in order to insure that a false-negative result did not occur that would allow a potentially dangerous chemical to slip through the preliminary screen. The parameters estimated were maternal weight gain and lethality, litter size and weight, gross teratogenicity, and individual pup weight gain up to d 3 postpartum.

MATERIALS AND METHODS

Mice

Adult virgin female CD-1 mice (61-71 d old), used in the MTD determination, and timed-pregnant mice of the same age and strain, used in the

reproductive study, were purchased from the Charles River Breeding Laboratories (Wilmington, Mass.). Day 1 of pregnancy was considered to be the day a sperm plug was found.

Facilities

The mice were maintained at the Brigham Young University animal laboratory facilities for the duration of the study. They were housed in solid-bottomed cages with corn-cob bedding and were fed Wayne Lab Blox and tap water *ad libitum*. Room temperature was kept at 22–24°C, and a 12-h light period began at 6 a.m. each day.

Chemicals

The chemicals were provided by NIOSH and identified only by coded names, except NAP, which was purchased commercially (Baker Chemical Co.). In order to reduce experimental bias, only toxicity, storage, and handling information about each chemical was provided until the study was completed. All compounds were dissolved in distilled water, except PNP, DMP, and NAP, which were dissolved in corn oil. ETU was insoluble at the concentrations tested and was administered as a water sluffy.

MTD Study

The MTD study was divided into three blocks of three chemicals each. SS, ETU, and diEGdiEE were tested in block I; EGdiME, diEGdiME, and triEGdiME in block II; and PNP, DMP, and NAP in block III. There were 5 dose levels per chemical with 10 mice in each dosage group for a total of 150 treated animals plus 10 shared vehicle controls in each block. Additional dose levels were necessary to determine the toxic threshold for diEGdiEE and NAP in blocks II and III, respectively (nine mice used per dosage level). The mice were housed five to a cage with two cage replications per treatment. The compounds were administered by gavage at the same time each day for 8 consecutive days in 0.25-ml quantities. Treatment concentrations were based on the average animal weight on the day prior to starting the dosing regime. Individual body weights were recorded on d 1 and d 8 of treatment, and on d 4 and d 8 after the final dose (during the recovery phase). Deaths were recorded cumulatively over the experimental period, and necropsies were performed to determine if death was due to puncture of the esophagus during treatment.

Reproductive Study

The reproductive investigation was divided into the same block arrangement as the MTD study. There were 50 pregnant mice randomly assigned

to each treatment group, which received a dose of a particular chemical based on the results of the prior MTD study (150 treated mice plus 50 shared vehicle controls for a total of 200 mice per block). The mice were individually housed in separate cages and assigned shelf locations according to a randomized block design. They were dosed by gavage for 8 consecutive days starting on d 7 of gestation. Treatment concentrations were based on the average d 6 dam weight. Maternal body weights were recorded on d 7 and 18 of gestation, and on d 3 postpartum. Individual pups were formally examined for gross structural abnormalities on d 1. Pups were weighed as entire litters on d 1 (12–24 h after birth) and d 3 (48 h after initial weight) postpartum. Mice failing to deliver by d 23 were killed by cervical dislocation, and their uteri were immersed in a 1% solution of sodium sulfide to determine if implantation had occurred.

Statistical Methods

The weights of the treated groups recorded in the MTD portion of the investigation were compared with the respective control weights for each block using the general linear model with coded linear regressions. Toxicity values (LD50) and confidence intervals were determined using SAS probit analysis program (Barr et al., 1976). The reproductive index, maternal survival, and the total number of live pups per litter were analyzed using chi-square analysis. Maternal weight gains over the gestational period were compared with their respective block controls using one-way analysis of variance.

RESULTS

MTD Study

The original criterion for the MTD determination was to be a dose-dependent weight loss in the treatment groups due to toxicity of the individual compounds. Weight loss, however, proved to be an ineffective indicator of toxicity.

The data summarized in Table 1 show that the nine chemicals tested generally did not induce significant weight loss compared with controls. The one exception was the 250-mg/kg NAP group, in which reduced weight was evident only on d 8 of treatment and was no longer significant at the end of the observation period. In fact, average weights for mice treated at all concentrations of SS, ETU, diEGdiEE, diEGdiME, and triEGdiME were slightly greater (but not significantly so) than those of their control group. There was no evidence of a dose-related change in weight (either loss or gain) with any compound.

Since the criterion of weight loss was not diagnostic for adult toxicity, MTD values were based on gross mortality. Using the cumulative mortality data reported in Table 1, a dose was chosen for the MTD level that

TABLE 1. Effects of Treatment on Mortality and Weight in Nonpregnant Mice

Compound	Block	Dose (mg/kg)	Cumulative mortality[a]	Average weight[b] (g)
Distilled water	I		0/1	23.29 ± 0.52
Sodium Selenite	I	2.5	1/0	25.31 ± 0.49
		5.0	1/0	24.96 ± 0.52
		10.0	5/0	25.22 ± 0.59
		20.0	10/0	—
		40.0	10/0	—
Ethylenethiourea	I	75.0	0/0	25.10 ± 0.65
		150.0	0/1	25.78 ± 0.72
		300.0	0/0	24.55 ± 0.72
		600.0	1/1	25.65 ± 0.56
		1200.0	1/0	25.61 ± 0.63
diEGdiEE[c]	I	125.0	0/0	25.19 ± 0.58
		250.0	0/0	25.27 ± 0.32
		500.0	0/0	24.75 ± 0.54
		1000.0	0/1	24.15 ± 0.56
		2000.0	0/1	24.35 ± 0.77
	II	3000.0	1/0	25.26 ± 0.82
	II	6000.0	9/0	—
	II	9000.0	9/0	—
Distilled water	II		0/3	23.56 ± 1.06
EGdiME[c]	II	225.0	0/1	23.49 ± 0.85
		450.0	0/1	23.81 ± 0.81
		900.0	0/0	23.38 ± 0.81
		1800.0	1/0	25.00 ± 0.65
		3600.0	9/0	29.10 —
diEGdiME[c]	II	335.0	0/0	25.51 ± 0.64
		670.0	0/0	25.31 ± 0.50
		1340.0	0/0	25.68 ± 0.67
		2680.0	1/0	23.66 ± 0.53
		5360.0	10/0	—
triEGdiME[c]	II	445.0	0/0	27.29 ± 0.60
		890.0	0/0	24.88 ± 0.69
		1780.0	0/0	26.60 ± 0.66
		3560.0	2/0	24.66 ± 0.95
		7120.0	10/0	—
Corn oil	III		0/0	28.87 ± 0.50
p-Nitrophenol	III	62.5	0/0	27.69 ± 0.47
		125.0	0/0	27.66 ± 0.59
		250.0	0/0	27.95 ± 0.44
		500.0	4/0	28.30 ± 0.24
		1000.0	8/0	30.50 ± 0.70
Dimethyl phthalate	III	875.0	0/0	28.13 ± 0.79
		1750.0	0/0	28.45 ± 0.36
		3500.0	1/0	28.90 ± 0.53
		7000.0	5/0	28.48 ± 0.52
		11,890.0	10/0	—

(*Footnotes on page 31.*)

TABLE 1. Effects of Treatment on Mortality and Weight in Nonpregnant Mice (*Continued*)

Compound	Block	Dose (mg/kg)	Cumulative mortality[a]	Average weight[b] (g)
Naphthalene	III	125.0	0/0	28.13 ± 0.29
		250.0	0/0	27.12 ± 0.68[d]
		500.0	10/0	–
		1200.0	10/0	–
		2000.0	10/0	–

[a]Represented as number dead due to treatment/number dead by esophageal puncture.
[b]Average weight of animals on d 8 of treatment (± SE).
[c]Diethylene glycol diethyl ether (diEGdiEE), ethylene glycol dimethyl ether (EGdiME), diethylene glycol dimethyl ether (diEGdiME), and triethylene glycol dimethyl ether (triEGdiME).
[d]Statistically significant at $p < 0.05$.

empirically exhibited a low level (~LD10) of lethality. Table 2 summarizes the MTD and LD50 values for each compound. It should be noted that the LD50 values for diEGdiEE, NAP, diEGdiME, and triEGdiME had to be approximated using 99% and 1% rather than the actual 100% and 0% mortality in order to get at least two nonzero values for the SAS probit analysis.

SS, NAP, and PNP were all highly toxic, with LD50 values of 8.4 mg/kg, 353.6 mg/kg, and 625.7 mg/kg, respectively. Moderate toxicity was noted

TABLE 2. LD50 and Maximum Tolerable Dose (MTD) Values for Nonpregnant Mice

Compound	LD50 (mg/kg)	95% Confidence limits: Lower limit	95% Confidence limits: Upper limit	MTD (mg/kg)
Sodium Selenite	8.4	6.0	12.0	7.0
Naphthalene	353.6[b]	–	–	300.0
p-Nitrophenol	625.7	455.8	894.2	400.0
Glycol ethers[a]				
EGdiME	2525.8	1986.9	3266.7	2000.0
diEGdiME	2978.7[b]	1313.3	6756.2	2000.0
diEGdiEE	3674.3[b]	3079.6	–	3000.0
triEGdiME	4136.7[b]	3509.5	–	3500.0
Ethylenethiourea	5085.0	1354.0	–	600.0[c]
Dimethyl phthalate	6281.9	4176.3	8170.3	3500.0

[a]Ethylene glycol dimethyl ether (EGdiME), diethylene glycol dimethyl ether (diEGdiME), diethylene glycol diethyl ether (diEGdiEE), and triethylene glycol dimethyl ether (triEGdiME).
[b]Values obtained using 99% and 1% rather than the actual 100% and 0% mortality data, in order to get nonzero data points for SAS probit analysis.
[c]This relatively low MTD value for ethylenethiourea was established, in part, by its insolubility in water.

for EGdiME, diEGdiME, diEGdiEE, triEGdiME, ETU, and DMP. The greatest number of deaths for the NAP-, diEGdiEE-, and triEGdiME-treated groups occurred 24–48 h after the initial dose. PNP, DMP, and SS groups, however, produced deaths throughout the treatment schedule. Only two groups, PNP at 500 mg/kg and DMP at 3500 mg/kg, had any posttreatment deaths (one animal died in each group on d 2 of the recovery period).

Reproductive Study

Maternal lethality, as indicated by a reduction in percent maternal survival (Table 3), was significant in the EGdiME, diEGdiME, PNP, and NAP groups. In addition, mice given PNP and NAP showed less maternal weight gain than controls. Reduced maternal weight gain in the absence of maternal death was exhibited by diEGdiEE.

The data in Table 4. show that ETU, EGdiME, diEGdiME, and triEGdiME were the only compounds significantly affecting the reproductive index, a parameter reflecting severe prenatal lethality. In fact, none of the pregnant mice in the EGdiME, diEGdiME, and triEGdiME groups delivered any viable pups. SS- and diEGdiEE-treated groups had moderately reduced reproductive indices, but these values were not significant.

TABLE 3. Effect of Compound Exposure During Pregnancy on Maternal Survival and Weight Gain

Compound	Dose (mg/kg)	Total pregnant	% Maternal survival[a]	Weight gain[b] (g)
Distilled water		45	100	20.16 ± 2.89
Sodium selenite	7.0	45	98	20.22 ± 3.28
Ethylenethiourea	600.0	35	100	21.98 ± 3.20[e]
diEGdiEE[c]	3000.0	42	98[d]	16.68 ± 3.27[e]
Distilled water		43	100	19.89 ± 3.26
EGdiME[c]	2000.0	46	74[f]	–
diEGdiME[c]	3000.0	45	60[f]	–
triEGdiME[c]	3500.0	38	97	–
Corn oil		40	100	22.83 ± 4.02
p-Nitrophenol	400.0	36	81[e]	18.70 ± 6.02[e]
Dimethyl phthalate	3500.0	36	97	21.85 ± 4.66
Naphthalene	300.0	33	85[e]	16.98 ± 4.30[e]

[a]Comparison of the number of pregnant survivors with the total number pregnant (determined by treatment of uteri with 1% sodium sulfide).

[b]Average increase in dams which produced litters (d 7 to 18 ± SD).

[c]Diethylene glycol diethyl ether (diEGdiEE), ethylene glycol dimethyl ether (EGdiME), diethylene glycol dimethyl ether (diEGdiME), and triethylene glycol dimethyl ether (triEGdiME).

[d]Death due to punctured esophagus.

[e]Statistically significant at $p < 0.05$.

[f]Statistically significant at $p < 0.005$.

TABLE 4. Effect of Compound Exposure During Pregnancy on Reproductive Index

Compound	Dose (mg/kg)	Total pregnant[a]	Pregnant survivors (PS)	Survivors delivered (SD)	Reproductive index (SD/PS)
Distilled water		45	45	41	0.911
Sodium selenite	7.0	45	44	39	0.886
Ethylenethiourea	600.0	35	35	26	0.743[b]
diEGdiEE[c]	3000.0	42	41	35	0.854
Distilled water		43	43	42	0.977
EGdiME[c]	2000.0	46	34	0	0.000[b]
diEGdiME[c]	3000.0	45	27	0	0.000[b]
triEGdiME[c]	3500.0	38	37	0	0.000[b]
Corn oil		40	40	40	1.000
p-Nitrophenol	400.0	36	29	28	0.966
Dimethyl phthalate	3500.0	36	35	35	1.000
Naphthalene	300.0	33	28	26	0.929

[a]Determined by treatment of uteri with 1% solution of sodium sulfide.
[b]Significant at $p < 0.05$.
[c]Diethylene glycol diethyl ether (diEGdiEE), ethylene glycol dimethyl ether (EGdiME), diethylene glycol dimethyl ether (diEGdiME), and triethylene glycol dimethyl ether (triEGdiME).

The results of measurements of numbers of live and dead pups and average pup weight, as indices of fetolethality, are shown in Table 5. Naphthalene was the only compound which produced a significant reduction in the average number of live offspring (about 1.5 fewer pups per litter than controls). There was not a concomitant increase in dead pups, however, suggesting the possibility that the smaller total litter size was due to early embryonic resorptions. DiEGdiEE, on the other hand, was overtly fetotoxic, as manifest by significantly more dead pups on d 1. The impact of EGdiME, diEGdiME, and triEGdiME was severe; there were no viable offspring following administration of these compounds.

Pups in the diEGdiEE group suffered a modest 6% average weight deficit at d 1, but showed a percentage increase comparable to controls over the next 2 d. No other compound had an effect on pup weight.

DISCUSSION

The primary purpose of this study was to determine the effectiveness of the NIOSH-modified protocol, originally described by Chernoff and Kavlock (1982), for the initial screening of chemicals for reproductive toxicity potential, and to use the screen in preliminary evaluations of NTP priority chemicals. Those compounds that exhibited toxic properties by this assay could then be prioritized for subsequent, more extensive testing. The essential feature of the procedure is that a chemical is administered to pregnant animals at a single, relatively high

TABLE 5. Effect of Prenatal Exposure on Perinatal Mortality and Pup Weight

Compound	Dose (mg/kg)	Average live per litter (± SD)		Average dead per litter (± SD)		Average pup weight (g ± SD)	
		Day 1	Day 3	Day 1	Day 3	Day 1	Day 3
Distilled water		10.27 ± 1.98	10.07 ± 2.13	0.10 ± 0.30	0.20 ± 0.68	1.61 ± 0.15	2.06 ± 0.22
Sodium selenite	7.0	10.44 ± 1.92	10.26 ± 2.11	0.13 ± 0.41	0.18 ± 0.68	1.61 ± 0.11	2.06 ± 0.25
Ethylenethiourea	600.0	10.96 ± 1.84	10.88 ± 1.75	0.04 ± 0.20	0.08 ± 0.27	1.69 ± 0.14	2.17 ± 0.22
diEGdiEE[a]	3000.0	9.83 ± 2.33	9.54 ± 2.85	0.37 ± 1.00[b]	0.29 ± 1.53	1.52 ± 0.18[b]	1.95 ± 0.28
Distilled water		9.64 ± 2.01	9.43 ± 1.96	0.31 ± 1.26	0.21 ± 0.72	1.60 ± 0.12	2.28 ± 0.26
EGdiME[a]	2000.0	—	—	—	—	—	—
diEGdiME[a]	3000.0	—	—	—	—	—	—
triEGdiME[a]	3500.0	—	—	—	—	—	—
Corn oil		10.75 ± 1.81	10.70 ± 1.80	0 ± 0	0.05 ± 0.22	1.74 ± 0.15	2.50 ± 0.36
p-Nitrophenol	400.0	9.79 ± 3.49	9.71 ± 3.53	0.18 ± 0.67	0.07 ± 0.26	1.71 ± 0.18	2.45 ± 0.33
Dimethyl phthalate	3500.0	10.54 ± 2.56	10.46 ± 2.52	0.29 ± 0.17	0.09 ± 0.37	1.74 ± 0.11	2.49 ± 0.22
Naphthalene	300.0	8.88 ± 2.61[b]	8.69 ± 2.78[b]	0.08 ± 0.39	0.19 ± 0.80	1.71 ± 0.23	2.52 ± 0.55

[a]Diethylene glycol diethyl ether (diEGdiEE), ethylene glycol dimethyl ether (EGdiME), diethylene glycol dimethyl ether (diEGdiME), and triethylene glycol dimethyl ether (triEGdiME).

[b]Significant at $p < 0.05$.

TABLE 6. Categorization of Maternal and Fetal Responses to Toxicant Insult

Category[a]	Response to compound	Priority for future testing	Compound
1	No apparent maternal or fetal effects	(Repeat at higher dose level)	Sodium selenite (SS) Dimethyl phthalate (DMP)
2	High maternal mortality, relatively low feto-toxicity	Middle/Low	*p*-Nitrophenol (PNP) Naphthalene (NAP)
3	High maternal mortality and fetal lethality	High	EGdiME[b] diEGdiME[b]
4	High fetolethality, no maternal mortality	Very high	Ethylenethiourea (ETU) diEGdiEE[b] triEGdiME[b]

[a]Categories listed in order of increasing reproductive toxicity.
[b]Diethylene glycol diethyl ether (diEGdiEE), triethylene glycol dimethyl ether (triEGdiME), ethylene glycol dimethyl ether (EGdiME), and diethylene glycol dimethyl ether (diEGdiME).

challenge dose, determined using a sensitive criterion in a prior study with nonpregnant animals.

The results of the MTD phase of this investigation demonstrate that weight loss in nonpregnant adult female mice was an inadequate measure of toxicity of the particular compounds screened here. The variability within the individual treatment groups was too great to statistically test for differences between the treatment means. Using an alternative criterion, reasonable estimates of the low effect levels for all the chemicals were determined based on gross mortality data (~LD10).

Although the nine compounds tested in this study represent a wide diversity in chemical structure, they can be grouped into four categories based on reproductive toxicity at the modified MTD criterion (Table 6).

SS and DMP belong to a group that, under the conditions used, exhibited neither maternal nor fetal effects. Such an assignment must be tentative, as the dose levels chosen for these compounds may have been below the threshold for reproductive toxicity. An additional trial at a higher concentration may be appropriate, although we note that for SS there was a very narrow range in dose levels to choose from before significant adult lethality occurred. Whereas SS in adults is highly toxic, with a reported rat oral LD50 of 2.5 mg/kg (Lewis and Tatken, 1980) and a mouse oral LD50 of 8.4 mg/kg (this investigation), we observed that a dose near the LD2-3 had relatively no effect on maternal weight gain and only a slight (nonsignificant) reduction in the reproductive index (percentage of pregnant survivors exhibiting 100% fetolethality). Reductions in maternal weight gain and litter size without maternal mortality have been reported in SS-exposed mice at 10 mg/kg (Chernoff and Kavlock,

1982), and a 7% decrease in fetal weight (but no teratogenic effects) was observed in the offspring of dams administered ~1 mg/kg at 30 d prior to mating in addition to d 3-18 of gestation (Nobunga et al., 1979). Singh et al. (1972) found that single intraperiotoneal injections of DMP (402 mg/kg) in pregnant rats increased the resorption rate and significantly reduced the average weight per pup. In our own work, however, the oral administration of large concentrations of DMP (3500 mg/kg) had relatively no effect on maternal weight gain, litter size, or average pup weight in the mouse.

Chemicals in the second and third categories of toxic response both produced high maternal mortality but differed in the severity of fetolethality. Both the PNP and NAP groups (category 2) had reductions in maternal survival, average litter size, and maternal weight gain, yet those pups that were delivered appeared healthy and normal compared with controls. Naphthalene administered intraperitoneally to rats did not produce teratogenic effects at maternally nontoxic doses (Hardin et al., 1981).

EGdiME- and diEGdiME-treated groups (category 3), however, had an even greater reduction in maternal survival and 100% fetolethality. As maternal mortality was near 25% for both of these chemicals, it would be appropriate to retest them at doses closer to the LD10 in order to determine if fetotoxicity is independent of a maternal effect. Abnormal sperm morphology in the mouse and male infertility in the rat have been reported following exposure to diEGdiME, and EGdiME has been observed to increase intrauterine death, exencephaly, and skeletal defects at 490 mg/kg in mice (Hardin, 1983).

ETU, diEGdiEE, and triEGdiME fall into a fourth category, chemicals that deserve high priority for further testing. Although the expression of toxicity differed for each of the three compounds, all were fetotoxic, and none produced a significant effect on maternal survival. Fetolethality was manifest with ETU as a significant number of pregnant mice that did not deliver (probably due to early death and resorption of conceptuses), with triEGdiME as zero viable pups, and with diEGdiEE indirectly, as reduced maternal weight gain, and directly, as increased numbers of dead pups and reduced birth weights. Studies with rats utilizing a protocol very similar to that followed in the present work have demonstrated a strong positive correlation between maternal weight loss and developmental toxicity (Rands et al., 1982). Interestingly, none of the three chemicals induced any obvious congenital abnormalities. In a similar study, Chernoff et al. (1979) reported that ETU induced a decrease in body weight and ossification, and increased the incidence of cleft palate formation in rats at concentrations as low as 80 mg/kg, but they found no evidence of teratogenicity in the mouse (Chernoff and Kavlock, 1982). An explanation for this discrepancy was proposed by Ruddick et al. (1977) following their observation that ETU was metabolized much more rapidly in the mouse than in the rat.

Although no gross abnormalities were detected in any of the treatment groups, these compounds certainly cannot be ruled nonteratogenic without further, more detailed investigation.

Four of the nine compounds tested in this investigation, the glycol ethers, were similar enough chemically to compare their structure-activity relationships. The high fetotoxicity of these compounds was clearly linked to whether they were methyl- or ethyl-substituted ethers. All three of the glycol ethers with terminal methyl groups—EGdiME, diEGdiME, and triEGdiME—caused 100% fetolethality, yet diEGdiEE, an ethyl-substituted derivative, had a much less significant effect on fetal survival.

REFERENCES

Adler, B., Braun, R., Schoneich, J., and Bohme, J. 1976. Repair-defective mutants of *Proteus mirabilis* as a prescreening system for the detection of potential carcinogens. *Biol. Zentralbl.* 95:463-469.

Anziulewicz, J. A., Dick, H. J., and Chiarolli, E. E. 1959. Transplacental naphthalene poisoning. *Am. J. Obstet. Gynecol.* 78:519-521.

Balansky, R. M., and Argirova, R. M. 1981. Sodium selenite inhibition of reproduction of some oncogenic RNA viruses. *Experientia* 37:1194-1195.

Barr, A. J., Goodnight, J. H., Sall, J. P., and Helwig, J. T. 1976. *User's Guide to SAS 76.* Raleigh, N.C.: SAS Institute.

Bontoyan, W. R., and Looker, J. B. 1973. Degradation of commercial ethylene bisthiocarbamate formulations to ethylenethiourea under elevated temperature and humidity. *J. Agric. Food Chem.* 21:338-341.

Chernoff, G. F. 1977. The fetal alcohol syndrome in mice: An animal model. *Teratology* 15:223-230.

Chernoff, N., and Kavlock, R. J. 1982. An *in vivo* screen utilizing pregnant mice. *J. Toxicol. Environ. Health* 10:541-550.

Chernoff, N., Kavlock, R. J., Rogers, E., Carver, B., and Murray, S. 1979. Perinatal toxicity of maneb, ethylene thiourea, and ethylenebisisothiocyanate. *J. Toxicol. Environ. Health* 5:821-834.

Freeman, A. E., Weisburger, E. K., Weisburger, J. H., Wolford, R. G., and Maryak, J. M. 1973. Transformation of cell cultures as an indication of the carcinogenic potential of chemicals. *J. Natl. Cancer Inst.* 51:799-807.

Gosselin, R. E., Hodge, H. C., Smith, R. P., and Gleason, M. N. (eds.). 1976. *Clinical Toxicology of Commercial Products—Acute Poisoning,* 4th ed. Baltimore, Md.: Williams and Wilkins.

Hardin, B. D. 1983. Reproductive toxicity of the glycol ethers. *Toxicology* 27:91-102.

Hardin, B. D., Bond, G. P., Sikov, M. R., Andrew, R. P., Beliles, R. P., and Niemeier, R. W. 1981. Testing of selected workplace chemicals for teratogenic potential. *Scand. J. Work. Environ. Health* 7(suppl. 4):66-75.

Khera, K. S. 1973. Ethylenethiourea: Teratogenicity study in rats and rabbits. *Teratology* 7:243-252.

Lagente, M., de la Farge, F., and Valdiguie, P. 1979. The influence of phthalate esters on human plasma lecithin cholesterol acyl transferase. *Lipids* 14:533-534.

Lewis, R. J., and Tatken, R. L. (eds.). 1980. *Registry of Toxic Effects of Chemical Substances.* U.S. Dept. of Health and Human Services, Washington, D.C.

Matsumoto, N., Shimizu, M., and Iljima, S. 1979. Modifying effect of Na_2SeO_3 on the toxicity of CH_3HgCl. *Teratology* 20(1):A153.

McCann, J. C., Edmund, C., Yamasaki, E., and Ames, B. N. 1975. Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Assay of 300 chemicals. *Proc. Natl. Acad. Sci. U.S.A.* 72:5135-5139.

Milner, J. A., and Hsu, C. Y. 1981. Inhibitory effects of selenium on the growth of L1210 leukemic cells. *Cancer Res.* 41:1652-1656.

Nobunaga, T., Satoh, H., and Suzuki, T. 1979. Effects of sodium selenite on methylmercury embryotoxicity and teratogenicity in mice. *Toxicol. Appl. Pharmacol.* 47:79-88.

Peakall, D. B. 1975. Phthalate esters: Occurrence and biological effects. *Residue Rev.* 54:1-41.

Poirier, M. C., De Cicco, B. T., and Lieberman, M. W. 1975. Nonspecific inhibition of DNA repair synthesis by tumor promotors in human diploid fibroblasts damaged with *N*-acetoxy-2-acetylaminofluorene. *Cancer Res.* 35:1392-1397.

Rands, P. L., Newhouse, C. L., Stewart, J. L., and Bradshaw, W. S. 1982. Indicators of developmental toxicity following prenatal administration of hormonally active compounds in the rat. II. Pattern of maternal weight gain. *Teratology* 25:45-51.

Ruddick, J. A., and Khera, K. S. 1975. Pattern of anomalies following single oral doses of ethylenetiourea to pregnant rats. *Teratology* 12:277-282.

Ruddick, J. A., Newsome, W. H., and Iverson, F. 1977. A comparison of the distribution, metabolism, and excretion of ethylenethiourea in the pregnant mouse and rat. *Teratology* 16:159-162.

Schupbach, M., and Hummler, H. 1977. A comparative study on the mutagenicity of ethylenethiourea in bacterial and mammalian test systems. *Mutat. Res.* 56:111-120.

Seiler, J. P. 1974. Ethylenethiourea (ETU), a carcinogenic and mutagenic metabolite of ethylenebisdithiocarbamate. *Mutat. Res.* 26:189-191.

Singh, A. R., Lawrence, W. H., and Autian, J. 1972. Teratogenicity of phthalate esters in rats. *J. Pharm. Sci.* 61:51-55.

Spiers, A. L., and Aberd, M. D. 1962. Thalidomide and congenital abnormalities. *Lancet* 1:303-305.

Sugawara, N. 1974. Effects of phthalate esters on brine shrimp. *Bull. Environ. Contam. Toxicol.* 12:421-424.

Watrach, A. M., Milner, J. A., and Watrach, M. A. 1982. Effects of selenium on growth rate of canine mammary carcinoma cells in athymic nude mice. *Cancer Lett.* 15:137-143.

Zinkham, W. H., and Childs, B. 1957. Effects of vitamin K and naphthalene metabolites on glutathione metabolism of erythrocytes from normal newborns and patients with napthalene hemolytic anemia. *Am. J. Dis. Child.* 94:420-423.

Received August 26, 1984
Accepted November 11, 1984