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# Evaluation of 60 Chemicals in a Preliminary Developmental Toxicity Test

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The number of chemicals in commerce which have not been evaluated for potential developmental toxicity is large. Because of the time and expense required by conventional developmental toxicity tests, an abbreviated assay is needed that will preliminarily evaluate otherwise untested chemicals to help prioritize them for conventional testing. A proposed short-term in vivo assay has been used in a series of studies in which a total of 60 chemicals were tested. Some were independently tested two or four times each. In this preliminary test, pregnant mice were dosed during mid-pregnancy and were then allowed to deliver litters. Litter size, birth weight, and neonatal growth and survival to postnatal day 3 were recorded as indices of potential developmental toxicity. Results in this assay and conventional mouse teratology tests were generally concordant. Conventional data were available for 14 chemicals (ten teratogens, one fetotoxin, three nonteratogens), of which 11 (nine teratogens, one fetotoxin, one nonteratogen) produced evidence of developmental toxicity. This included conventional data for three chemicals (ethylene glycol, diethylene glycol dimethyl ether, and triethylene glycol dimethyl ether) that were untested before the present study. As high priority candidates for conventional testing on the basis of results here, all were subsequently studied in a standard teratology assay and were confirmed to be teratogenic in mice. Additionally, one of them (ethylene glycol) plus a fourth high priority

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candidate for conventional study (diethylene glycol monomethyl ether) were subsequently tested in rats and were found to be teratogenic in that species.

**Key words: in vivo screen, teratogen, hazard detection, rodent, pregnancy outcome, neonatal viability**

## INTRODUCTION

Conventional developmental toxicity tests are expensive and require specially trained technical and professional staffs to conduct them. With the large number of chemicals in commerce which have never been adequately tested for developmental toxicity, a rapid and inexpensive method is needed to produce preliminary developmental toxicity data which can help to establish relative priorities for the more demanding conventional tests. Preferably, this method could be applied in the general testing environment. However, any such method must also produce few false negative results, since comprehensive testing of low priority chemicals may be delayed. False positive outcomes are also undesirable, but are more tolerable in a preliminary test.

Chernoff and Kavlock [1,2] proposed an approach to this testing need, in which pregnant mice were dosed, then allowed to deliver litters. The number of liveborn pups, their birth weight, and their growth and survival to 3 days of age were used as indices of potential developmental toxicity. At about the time that Chernoff and Kavlock were reporting their initial results, two testing needs developed in the National Toxicology Program (NTP). First, a large number of chemicals had been chosen by the NTP Chemical Selection Committee with a recommendation for "reproductive" testing. The number so recommended was far in excess of the NTP capacity for conventional developmental or reproductive toxicity testing, and some method was needed to determine which of the chemicals warranted highest priority for the limited testing resources. Second, at about that time, two glycol ethers, ethylene glycol monomethyl ether [3] and ethylene glycol monoethyl ether [4], were identified as teratogenic in laboratory animals. These glycol ethers represented a major class of solvents with significant potential human exposure, occupationally and in consumer products. Given these facts, supplemental data were urgently needed on other glycol ethers so that some judgment could be made as to whether additional members of this large chemical family might have similar biological activity, or whether developmental toxicity might be restricted to only these two chemicals. The assay proposed by Chernoff and Kavlock was chosen as a reasonable method to address both testing needs. Six contracts were awarded to five different testing laboratories, and tests were conducted over approximately a 2-year period. Consolidated results are presented here from studies at NIOSH and those contract laboratories.

## MATERIALS AND METHODS

### Experimental Design

Female CD-1 mice were used in all phases of these tests. Several chemicals were evaluated at one time (blocks of chemicals), using a concurrent common control. Initially, nonpregnant mice were orally dosed for 8 consecutive days in a dose-finding study. Based on the outcome of that study, the predicted LD10 was selected for use in

the reproductive phase tests. Those studies employed time-mated mice orally dosed on gestation days (gd) 6–13 (vaginal plug = day 0). Mice were then permitted to deliver litters. As soon as possible after delivery, the number of liveborn pups and total litter weight were recorded. Pups were then returned to their dams until the third postnatal day. At that time the number of live pups was recorded, as were the weights of dams and litters.

## Animals and Housing

Specific-pathogen-free CD-1 mice were purchased from Charles River Breeding Laboratories.* An effort was made to restrict mice to a single colony, but availability problems resulted in shipments representing Kingston, Lakeview, Portage, and Wilmington colonies. With only minor variations, mice were 6–8 weeks of age upon receipt. Lighting was provided on a 12-hr light/dark cycle, and mice had free access to food (BP Nutrition Rat Mouse Breeder Diet No. 3, Purina Certified Rodent Chow No. 5002, Wayne Lab-Blox, or Zeigler Brothers NIH-07 diet) and water.

For dose-finding studies, virgin female mice were group housed (five per cage) throughout treatment, and observation periods (one contractor housed mice singly for dose-finding). Because time-mated mice were to be used in the reproductive phase, an abbreviated quarantine of about 5 days was observed to approximate that in the reproductive studies. At the end of this quarantine, any animals judged unsuitable for the study (eg, underweight) were discarded. Remaining mice were weighed and randomly assigned to experimental groups with stratification by body weight.

In the reproductive phases, time-mated (primigravida) mice were received from the vendor on dg 1–3 (vaginal plug = day 0). On gd 6, mice judged unsuitable for the study were discarded. Remaining mice were weighed and randomly assigned to control and experimental groups with stratification by body weight. Mice were singly housed in solid-bottom boxes. Nesting material (Bed-O-Cobs, Sani-chip, San-i-cel, or sterilized white wood shavings) was changed once weekly, but no bedding change was made later than gd 17 to avoid disturbing mice near parturition.

## Chemicals

Table I lists all chemicals tested, along with the laboratory and experimental block in which the reproductive phase tests were conducted. Some chemicals were supplied to the testing laboratories by NIOSH as coded compounds. In other cases the testing laboratory purchased test chemicals. In all instances, experimental procedures were carried out by personnel without knowledge of the specific dose or treatment groups.

## Procedures

All experiments were conducted in two phases: an initial dose-finding study, followed by a reproductive phase which employed a single dose level for each chemical. In both phases, treatments were administered by gavage using a standard dosing volume of 10 ml/kg body weight (except block 5A, which used 5 ml/kg). The vehicle was distilled water or corn oil, as appropriate. Chemicals with a common

*Disclaimer: Mention of a company name or product does not constitute endorsement by the authors or the National Institute for Occupational Safety and Health.

**TABLE I. Preliminary Developmental Toxicity Assay Chemicals Tested**

| Chemical name | CAS No. | Lab block[a] |
|---|---|---|
| Allyl chloride | 107-05-1 | 5A |
| Aniline | 62-53-3 | 1C |
| Aniline, p-nitro | 100-01-6 | 1C |
| Aniline, N,N-dimethyl | 121-69-7 | 1C |
| Benzyl alcohol | 100-51-6 | 4B |
| Cinnamaldehyde | 104-55-2 | 6C |
| Decalin | 91-17-8 | 5A |
| 1,3-Dichloro-5,5-dimethylhydantoin | 118-52-5 | 5C |
| Dimethyl methyl phosphonate | 756-79-6 | 6B |
| Disulfiram | 97-77-8 | 5C |
| Ethylenediamine | 107-15-3 | 4B |
| Ethylenethiourea | 96-45-7 | 1A,2A,3A,4A |
| 2-Ethyl-1-hexanol | 104-76-7 | 6D |
| 2-Ethylthioethanol | 110-77-0 | 5C |
| 2-Ethoxyethanethiol | 17362-04-8 | 5C |
| 2-Ethylthioethanethiol | 26750-44-7 | 5C |
| Glycol, ethylene | 107-21-1 | 4B |
| Glycol, ethylene, monomethyl ether | 109-86-4 | 2B |
| Glycol, ethylene, monomethyl ether acetate | 110-49-6 | 5D |
| Glycol, ethylene, monomethyl ether acrylate | 3121-61-7 | 5D |
| Glycol, ethylene, dimethyl ether | 110-71-4 | 3B |
| Glycol, ethylene, monoethyl ether | 110-80-5 | 2B |
| Glycol, ethylene, diethyl ether | 629-14-1 | 1B |
| Glycol, ethylene, monopropyl ether | 2807-30-9 | 5D |
| Glycol, ethylene, monbutyl ether | 111-76-2 | 2B |
| Glycol, diethylene | 111-46-6 | 4B |
| Glycol, diethylene, monomethyl ether | 111-77-3 | 2B |
| Glycol, diethylene, dimethyl ether | 111-96-6 | 3B |
| Glycol, diethylene, monoethyl ether | 111-90-0 | 1B |
| Glycol, diethylene, diethyl ether | 112-36-7 | 3A |
| Glycol, diethylene, monobutyl ether | 112-34-5 | 1A |
| Glycol, diethylene, dibutyl ether | 112-73-2 | 4C,7A |
| Glycol, triethylene | 112-27-6 | 1B |
| Glycol, triethylene, dimethyl ether | 112-49-2 | 3B |
| Glycol, propylene, 3-ethyl ether | 111-35-3 | 5D |
| N-Isopropyl-N′-phenyl-p-phenylenediamine | 101-72-4 | 6B |
| Naphthalene | 91-20-3 | 3C |
| Nitrofurazone | 59-87-0 | 4C |
| p-Nitrophenol | 100-02-7 | 3C |
| D-Phenylalanine | 673-06-3 | 6C |
| Phthalate, dimethyl | 131-11-3 | 3C,7A |
| Phthalate, diethyl | 84-66-2 | 6A |
| Phthalate, di(n-butyl) | 84-74-2 | 6A |
| Phthalate, di(i-butyl) | 84-69-5 | 6A |
| Phthalate, di(n-hexyl) | 84-75-3 | 6E |
| Phthalate, di(n-octyl) | 117-84-0 | 6E |
| Phthalate, di(2-ethylhexyl) | 117-81-7 | 6D |
| Phthalate, mono(2-ethylhexyl) | 4376-20-9 | 6E |
| Phthalate, di(i-decyl) | 26761-40-0 | 6D |

*(continued)*

TABLE I. Preliminary Developmental Toxicity Assay Chemicals Tested (continued)

| Chemical name | CAS No. | Lab Block[a] |
|---|---|---|
| Probenecid | 57-66-9 | 6C |
| Sodium selenite | 10102-18-8 | 1A,2A,3A,4A |
| Tergitol NP-10 | 9016-45-9 | 5B |
| Toluene | 108-88-3 | 2C,7A |
| Toluene, 2,4-diamino | 95-80-7 | 2C |
| Toluene, 2,4-dinitro | 121-14-2 | 2C |
| Trimellitic anhydride | 552-30-7 | 4C,7A |
| Trioctanoin | 538-23-8 | 5A |
| Tris(2-chloroethyl) phosphate | 115-96-8 | 6B |
| Triton X-100 | 9002-93-1 | 5B |
| Tween 60 | 9005-66-7 | 5C |

[a]Corresponds to testing laboratory and block shown in Table II.

vehicle were arranged in experimental blocks consisting of 2–6 chemicals tested concurrently with a vehicle control group. Many experimental blocks contained structurally related compounds.

For dose-finding, chemicals were tested in blocks of three or more chemicals with a single concurrent control group. Each chemical was tested at five dose levels using ten virgin female mice per group. Treatments were administered once daily for 8 consecutive days, with a dosing volume determined on the basis of body weights on the first treatment day. Mice were observed twice daily during treatment, and once daily for 8 days following treatments. Body weights were recorded on the first and last (eighth) day of treatment, and on days 4 and 8 post-treatment. Signs of toxicity were recorded, and dead mice were necropsied to exclude dosing error, eg, instillation into the lungs or perforation of the esophagus, as a cause of death. Dose-finding studies were sometimes inconclusive, making it occasionally necessary to conduct a second study in a later experimental block.

For the reproductive phase, the LD10 predicted on the basis of dose-finding results was the single dose used. For several chemicals, the LD10 could not be determined, and 10 ml/kg undiluted compound was established as the maximum practicable dose. Because time-mated mice were to be shipped on gd 0–2, the vendor suggested that pregnancy success might not exceed 50%. In consideration of the expected 10% mortality and potentially low pregnancy rate, initial group size in the reproductive phase was established at 50 time-mated females. This allowed an expectation of at least 20 surviving pregnant mice at term, a group size considered necessary for adequate statistical analysis.

Treatments in the reproductive phase were administered once daily on gd 6–13, with a dosing volume determined on the basis of pretreatment body weights on gd 6. Experimental blocks consisted of 2–6 chemically treated groups with a concurrent vehicle control. Mice were observed twice daily during treatment, and once daily on gd 14–17. Body weights were again recorded on gd 17. At the daily observation, signs of toxicity were recorded. Dead mice were necropsied to exclude dosing error as a cause of death. Beginning on gd 18, mice were observed twice daily for signs of parturition. When delivery was judged to be complete (postnatal day 1), the number of live pups was recorded, and live pups were weighed together as a litter, then returned to the dam. Neither live nor dead pups were systematically examined for malformations. Two days later (postnatal day 3), live pups were again counted and

weighed as a litter, and maternal body weights were recorded. Dams and litters were then discarded. Females that failed to deliver a litter by the presumed gd 22 were killed and uteri were examined. If there was no gross evidence of a failed pregnancy, uteri were placed in 10% ammonium or sodium sulfide to reveal implantation sites as evidence of early termination of pregnancy.

### Data Analysis

Data reported by individual testing laboratories [5–10] were coded into the Parklawn Computer System, and printouts were reviewed by the contractors to verify the accuracy of the data. The Statistical Analysis System (SAS Institute Inc., Cary, NC) was then used to analyze results of each experimental block. Body weights on gd 6 were analyzed by 2-tail ANOVA to verify that there were no group differences in initial body weight. Mortality (excluding death attributed to dosing error) was contrasted between pregnant and nonpregnant mice by 2-tail Fisher's exact test. Nonpregnant mice, as determined by uterine examination, were excluded from all subsequent analyses. The proportion of pregnant survivors that delivered a viable litter (at least one liveborn pup) was compared with the concurrent vehicle control by 1-tail Fisher's exact test. For mice that delivered a viable litter, maternal body weight change from gd 6 to postnatal day (pd) 3, the number of liveborn pups per litter, percent neonatal survival to pd 3, average pup weight at birth, and average pup weight gain by pd 3 were analyzed by pairwise multiple comparisons of control and treated groups, using a 2-tail Mann-Whitney U-test.

## RESULTS

Detailed results of the dose-finding studies are available in reports from each of the testing laboratories [5–10], but are not presented here. Table II lists the results, by experimental block, of all reproductive phase tests (some of these data have been previously reported) [11,12]. Females whose death was attributed to dosing error were omitted, accounting for some denominators in the Dead/treated column being less than 50. The overall variability seen in the 22 control groups, and the associated statistical power afforded by initial groups of 50, is discussed elsewhere [13]. Mortality sometimes varied considerably from 10%, as predicted from the preliminary dose-finding studies. Altered sensitivity of pregnant animals, relative to nonpregnant mice used in the dose-finding studies, might account for some of these discrepancies. Classification of females as pregnant/nonpregnant may not be entirely reliable when death occurred in the first days of treatment. Nevertheless, each dead animal (with death attributed to toxicity) was so classified (data not shown) by three laboratories (2, 3, and 5 in Table II). Based on these data, differential mortality among pregnant and nonpregnant animals could be estimated for 33 tests (31 chemicals). In six of the 33 instances, there was a significant difference ($p < .05$) in mortality of pregnant vs nonpregnant treated mice. Mortality was higher among pregnant mice treated with three chemicals: allyl chloride, 21/28 pregnant mice died versus 4/22 nonpregnant mice (block 5-A); decalin, 7/31 versus 0/17 (block 5-A); and 2,4-diaminotoluene, 16/36 versus 1/14 (block 2-C). Mortality was higher among nonpregnant mice treated with another three chemicals: ethylene glycol monobutyl ether, 4/35 pregnant mice died versus 6/15 nonpregnant mice (block 2-B); propylene glycol 3-ethyl ether, 2/23 versus 11/27 (block 5-D); and 2,4-dinitrotoluene, 6/34 versus 9/16 (block 2-C).

Mean maternal body weights before treatments began (gd 6) were similar across groups in all experimental blocks (data not shown). Because gd 17 body weights are markedly influenced by litter size, possible treatment-related effects on maternal body weight were evaluated in terms of body weight gain between gd 6 and pd 3 (weight change in Table II). Body weight gain was affected by 18 chemicals, three of which did not affect any other endpoint: diethylene glycol diethyl ether, block 3-A; tris (2-chloroethyl)phosphate, block 6-B; and probenecid, block 6-C. Perhaps more notable is an absence of effects on body weight gain with eight chemicals, despite maternal mortality of 10% or more. Extreme examples are: diethylene glycol monobutyl ether, 25% mortality, block 7-A; propylene glycol 3-ethyl ether, 26% mortality, block 5-D; 2,4-dinitrotoluene, 30% mortality, block 2-C; allyl chloride, 50% mortality, block 5-A. Decalin (block 5-A) produced 10% maternal mortality and was the only chemical associated with a significant increase in maternal body weight gain.

Six chemicals (Table III) were tested by more than one laboratory, each selecting a dose and testing these chemicals independently. Sodium selenite and ethylenethiourea (ETU) had been reported by Chernoff and Kavlock [1,2] to be positive and negative chemicals, respectively. Sodium selenite had no apparent adverse effect on the maternal or reproductive endpoints at doses of 7 mg/kg or less. At 14 mg/kg, it caused 44% maternal mortality, significantly reduced maternal body weight gain, and adversely affected four of the five reproductive endpoints. The only endpoint not affected by that dose of sodium selenite was the average number of liveborn pups per viable litter.

ETU presented significant problems in dose preparation. Chernoff and Kavlock [1,2] reported administering 300 mg/kg in water, but the very limited solubility of ETU made delivery of doses greater than 100–150 mg/kg very difficult. Constant agitation was required to keep undissolved ETU in suspension, and there was a tendency for ETU to adhere to the sides of containers or to plug gavage needles. High doses were attempted, but the actual delivered dose probably varied considerably, especially at the highest dose levels. That may account for the somewhat inconsistent observations in two of the testing laboratories: litter size was reduced in the 300 mg/kg dose group, while at 600 mg/kg, litter size was normal, but the number of viable litters was reduced. The only other significant effect with ETU was an increase in pup birth weight at 300 mg/kg, but that may be secondary to the signficantly reduced average litter size at that dose.

Also tested more than once (Table III) were four chemicals (diethylene glycol monobutyl ether, dimethyl phthalate, toluene, and trimellitic anhydride) which produced no mortality and no adverse effects on reproductive indices when first tested. Those chemicals were retested at higher doses to investigate whether the initial negative findings resulted from inadequate challenge. Maternal mortality in the repeat tests ranged from 2–28%, but there still were no adverse effects on reproductive endpoints.

One of the reasons for employing this test system was to develop preliminary data for a large number of glycol ethers. A total of 20 glycols and glycol ethers were tested, as were three sulfur-substituted analogues of ethylene glycol monoethyl ether (Table IV). Neither diethylene nor triethylene glycol showed evidence of developmental toxicity, but ethylene glycol adversely affected all measures of reproductive success. The seven methyl-substituted glycol ethers tested were dramatically positive: six produced 100% intrauterine death, and the seventh (diethylene glycol monomethyl

**TABLE II. Test Results (Mean ± SD; NA = not available) by Experimental Laboratory and Block**

| Chemical name | Lab block[a] | Dose (mg/kg/d) | Maternal response variables: No. dead/ treated | Maternal response variables: Weight change (g) | Maternal response variables: Viable litters | Neonatal response variables: Liveborn per litter | Neonatal response variables: Percentage survival | Neonatal response variables: g per pup: Birth weight | Neonatal response variables: g per pup: Weight gain |
|---|---|---|---|---|---|---|---|---|---|
| Ethylenethiourea | 1-A | 100 | 0/50 | 8.5 ± 2.9 | 21/21 | 10.0 ± 2.1 | 90.6 ± 24.5 | 1.5 ± 0.2 | 1.2 ± 0.3 |
| Sodium selenite | 1-A | 3.5 | 0/50 | 8.7 ± 2.2 | 23/23 | 10.2 ± 1.9 | 100 | 1.5 ± 0.2 | 1.2 ± 0.3 |
| Diethylene glycol monobutyl ether | 1-A | 500 | 0/50 | 8.6 ± 2.1 | 36/37 | 10.3 ± 2.5 | 98.3 ± 4.5 | 1.6 ± 0.2 | 1.1 ± 0.3 |
| Control | 1-A | Water | 0/49 | 9.1 ± 2.9 | 30/31 | 9.7 ± 2.3 | 96.0 ± 13.3 | 1.6 ± 0.3 | 1.1 ± 0.5 |
| Ethylene glycol diethyl ether | 1-B | 2,955 | 5/50 | 0.2 ± 4.4* | 4/35* | 2.0 ± 2.0* | 45.0 ± 52.6* | 1.3 ± 0.1* | 0.6 ± 0.4 |
| Diethylene glycol monoethyl ether | 1-B | 5,500 | 7/50 | 5.8 ± 2.6* | 32/33 | 9.5 ± 2.1 | 98.0 ± 6.0 | 1.5 ± 0.2 | 0.9 ± 0.2 |
| Triethylene glycol | 1-B | 11,270[b] | 2/50 | 6.6 ± 2.1* | 36/36 | 9.4 ± 2.3 | 99.0 ± 3.3 | 1.5 ± 0.1 | 1.0 ± 0.3 |
| Control | 1-B | Water | 0/50 | 7.9 ± 1.7 | 42/42 | 10.1 ± 2.4 | 99.3 ± 2.4 | 1.6 ± 0.2 | 1.0 ± 0.3 |
| Aniline | 1-C | 560 | 6/50 | 6.7 ± 4.0* | 25/25 | 10.3 ± 2.2 | 94.1 ± 16.5 | 1.5 ± 0.1* | 0.9 ± 0.2* |
| p-Nitroaniline | 1-C | 1,200 | 21/50 | 3.6 ± 2.2* | 11/16* | 5.0 ± 3.5* | 78.0 ± 35.0* | 1.5 ± 0.2 | 0.8 ± 0.3 |
| N,N-dimethylaniline | 1-C | 365 | 3/50 | 8.2 ± 2.5 | 36/37 | 8.6 ± 2.8 | 98.4 ± 4.1 | 1.7 ± 0.2 | 1.1 ± 0.3 |
| Control | 1-C | Corn oil | 0/50 | 9.0 ± 3.9 | 41/41 | 9.5 ± 3.3 | 99.3 ± 2.6 | 1.6 ± 0.2 | 1.1 ± 0.4 |
| Ethylenethiourea | 2-A | 300 | 0/50 | 7.7 ± 3.4 | 29/31 | 8.8 ± 2.6* | 97.3 ± 12.5 | 1.7 ± 0.1* | 0.5 ± 0.3 |
| Sodium selenite | 2-A | 5 | 0/50 | 7.0 ± 2.4 | 30/34 | 9.0 ± 2.9 | 100 | 1.6 ± 0.2 | 0.5 ± 0.2 |
| Control | 2-A | Water | 0/50 | 8.0 ± 2.7 | 31/34 | 10.2 ± 1.8 | 95.5 ± 18.4 | 1.6 ± 0.1 | 0.5 ± 0.3 |
| Ethylene glycol monomethyl ether | 2-B | 1,400 | 7/49 | NA | 0/30* | 0 | NA | NA | NA |
| Ethylene glyclol monoethyl ether | 2-B | 3,605 | 5/50 | NA | 0/32* | 0 | NA | NA | NA |
| Ethylene glycol monobutyl ether | 2-B | 1,180 | 10/50 | 1.7 ± 3.5* | 24/31* | 9.8 ± 2.7 | 98.3 ± 6.4 | 1.6 ± 0.2 | 0.4 ± 0.1 |
| Diethylene glycol monoethyl ether | 2-B | 4,000 | 5/50 | −0.8 ± 3.4* | 5/32* | 3.2 ± 2.2* | 23.3 ± 30.8* | 1.5 ± 0.3 | 0.1 ± 0.2* |
| Control | 2-B | Water | 0/50 | 5.0 ± 3.5 | 31/32 | 10.1 ± 2.8 | 99.7 ± 1.6 | 1.6 ± 0.2 | 0.4 ± 0.1 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Toluene | 2-C | 2,350 | 1/50 | 6.2 ± 2.2 | 28/30 | 9.6 ± 3.4 | 99.5 ± 1.9 | 1.6 ± 0.2 | 0.7 ± 0.2 |
| 2,4-Dinitrotoluene | 2-C | 390 | 15/50 | 6.1 ± 2.6 | 23/28 | 10.3 ± 3.2 | 98.0 ± 8.4 | 1.6 ± 0.2 | 0.6 ± 0.1 |
| 2,4-Diaminotoluene | 2-C | 150 | 17/50 | 1.1 ± 3.2* | 5/20* | 5.4 ± 2.7* | 85.7 ± 31.9 | 1.5 ± 0.4 | 0.6 ± 0.3 |
| Control | 2-C | Corn oil | 0/50 | 6.3 ± 2.8 | 30/33 | 10.6 ± 2.4 | 94.5 ± 15.3 | 1.6 ± 0.2 | 0.6 ± 0.2 |
| Ethylenethiourea | 3-A | 600 | 0/50 | 9.0 ± 2.7 | 26/35* | 11.0 ± 1.8 | 96.8 ± 18.0 | 1.7 ± 0.1 | 0.6 ± 0.2 |
| Sodium selenite | 3-A | 7 | 1/50 | 8.2 ± 4.1 | 39/44 | 10.4 ± 1.9 | 98.6 ± 7.6 | 1.6 ± 0.1 | 0.6 ± 0.3 |
| Diethylene glycol diethyl ether | 3-A | 3,000 | 0/50 | 7.8 ± 2.9* | 35/41 | 9.8 ± 2.3 | 99.6 ± 1.8 | 1.5 ± 0.2 | 0.5 ± 0.2 |
| Control | 3-A | Water | 0/50 | 9.5 ± 2.3 | 41/45 | 10.3 ± 2.0 | 99.8 ± 1.3 | 1.6 ± 0.2 | 0.5 ± 0.1 |
| Ethylene glycol dimethyl ether | 3-B | 2,000 | 13/50 | NA | 0/34* | 0 | NA | NA | NA |
| Diethylene glycol dimethyl ether | 3-B | 3,000 | 20/49 | NA | 0/27* | 0 | NA | NA | NA |
| Triethylene glycol dimethyl ether | 3-B | 3,500 | 2/50 | NA | 0/37* | 0 | NA | NA | NA |
| Control | 3-B | Water | 0/50 | 6.2 ± 3.4 | 42/43 | 9.6 ± 2.0 | 98.3 ± 6.4 | 1.6 ± 0.1 | 0.7 ± 0.2 |
| p-Nitrophenol | 3-C | 400 | 9/50 | 8.2 ± 2.8 | 28/29 | 9.8 ± 3.5 | 99.6 ± 1.8 | 1.7 ± 0.2 | 0.7 ± 0.3 |
| Dimethyl phthalate | 3-C | 3,500 | 0/49 | 8.3 ± 2.0 | 35/35 | 10.5 ± 2.6 | 99.7 ± 1.9 | 1.7 ± 0.1 | 0.7 ± 0.2 |
| Naphthalene | 3-C | 300 | 10/50 | 5.1 ± 2.6* | 26/28 | 8.9 ± 2.6* | 98.1 ± 8.5 | 1.7 ± 0.2 | 0.8 ± 0.4 |
| Control | 3-C | Corn oil | 0/50 | 8.3 ± 2.6 | 40/40 | 10.8 ± 1.8 | 99.6 ± 1.9 | 1.7 ± 0.1 | 0.8 ± 0.2 |
| Ethylenethiourea | 4-A | 200 | 0/50 | 8.5 ± 3.1 | 39/41 | 10.3 ± 2.6 | 95.9 ± 12.0 | 1.8 ± 0.2 | 0.6 ± 0.1 |
| Sodium selenite | 4-A | 14 | 22/50 | 4.5 ± 3.6* | 10/25* | 10.0 ± 3.4 | 87.6 ± 19.9* | 1.4 ± 0.1* | 0.4 ± 0.3* |
| Control | 4-A | Water | 0/50 | 7.5 ± 1.9 | 34/38 | 10.3 ± 3.3 | 98.6 ± 4.2 | 1.7 ± 0.2 | 0.5 ± 0.2 |
| Ethylenediamine | 4-B | 400 | 1/50 | 6.5 ± 2.5 | 26/28 | 10.0 ± 2.0 | 98.1 ± 4.7 | 1.6 ± 0.2* | 0.5 ± 0.2* |
| Ethylene glycol | 4-B | 11,090[b] | 5/50 | 1.2 ± 1.2* | 15/37* | 2.0 ± 1.9* | 37.0 ± 41.5* | 1.4 ± 0.2* | 0.2 ± 0.2* |
| Diethylene glycol | 4-B | 11,180[b] | 2/50 | 6.8 ± 2.5 | 33/36 | 9.5 ± 2.3 | 97.0 ± 17.4 | 1.6 ± 0.2 | 0.6 ± 0.2 |
| Benzyl alcohol | 4-B | 750 | 19/50 | 6.2 ± 3.6* | 21/22 | 10.0 ± 1.9 | 98.4 ± 7.3 | 1.6 ± 0.2* | 0.5 ± 0.2* |
| Control | 4-B | Water | 0/50 | 7.9 ± 2.3 | 29/29 | 9.2 ± 2.7 | 99.6 ± 2.1 | 1.7 ± 0.2 | 0.7 ± 0.2 |
| Trimellitic anhydride | 4-C | 550 | 1/50 | 7.0 ± 2.3 | 39/41 | 10.6 ± 2.4 | 99.2 ± 3.8 | 1.7 ± 0.1 | 0.7 ± 0.2 |
| Nitrofurazone | 4-C | 100 | 1/50 | 7.8 ± 2.0 | 28/35* | 11.0 ± 1.8 | 99.6 ± 1.9 | 1.6 ± 0.1* | 0.6 ± 0.2 |
| Diethylene glycol dibutyl ether | 4-C | 2,000 | 4/50 | 7.7 ± 1.7 | 38/40 | 11.1 ± 2.0 | 99.5 ± 2.2 | 1.7 ± 0.1 | 0.6 ± 0.2 |
| Control | 4-C | Corn oil | 0/50 | 6.6 ± 2.8 | 45/45 | 10.7 ± 2.6 | 99.8 ± 1.1 | 1.7 ± 0.2 | 0.6 ± 0.2 |

*(continued)*

**TABLE II. Test Results (Mean ± SD; NA = not available) by Experimental Laboratory and Block (continued)**

| Chemical name | Lab block[a] | Dose (mg/kg/d) | Maternal response variables: No. dead/ treated | Maternal response variables: Weight change (g) | Maternal response variables: Viable litters | Neonatal response variables: Liveborn per litter | Neonatal response variables: Percentage survival | Neonatal response variables: g per pup: Birth weight | Neonatal response variables: g per pup: Weight gain |
|---|---|---|---|---|---|---|---|---|---|
| Decalin | 5-A | 2,700 | 7/48 | 7.5 ± 2.6* | 23/24 | 9.9 ± 2.3 | 100 | 1.6 ± 0.1 | 1.0 ± 0.2 |
| Trioctanoin | 5-A | 4,750[b] | 3/50 | 5.2 ± 3.3 | 35/37 | 9.2 ± 2.9 | 93.9 ± 19.4 | 1.5 ± 0.2 | 0.9 ± 0.3 |
| Allyl chloride | 5-A | 500 | 25/50 | 6.3 ± 1.5 | 5/7 | 9.2 ± 2.5 | 80.0 ± 44.7 | 1.3 ± 0.3 | 0.9 ± 0.2 |
| Control | 5-A | Corn oil | 0/50 | 6.1 ± 3.7 | 37/39 | 9.4 ± 2.7 | 97.1 ± 17.1 | 1.5 ± 0.2 | 0.9 ± 0.3 |
| Tergitol NP-10 | 5-B | 600 | 0/49 | 4.6 ± 3.0 | 25/29 | 9.2 ± 2.9 | 96.0 ± 20.0 | 1.6 ± 0.2 | 0.5 ± 0.1 |
| Triton X-100 | 5-B | 800 | 1/50 | 5.2 ± 2.3 | 33/34 | 9.6 ± 2.0 | 99.3 ± 3.9 | 1.5 ± 0.1 | 0.5 ± 0.1 |
| Control | 5-B | Corn oil | 1/50 | 5.0 ± 1.9 | 31/32 | 9.0 ± 2.4 | 98.5 ± 5.0 | 1.5 ± 0.1 | 0.5 ± 0.2 |
| Tween 60 | 5-C | 5,200 | 0/50 | 5.0 ± 3.4 | 34/34 | 10.5 ± 2.7 | 98.7 ± 4.3 | 1.5 ± 0.1 | 0.3 ± 0.2* |
| 2-Ethylthioethanol | 5-C | 1,200 | 0/50 | 5.9 ± 2.5 | 31/32 | 10.1 ± 2.3 | 97.7 ± 5.2 | 1.4 ± 0.1 | 0.5 ± 0.2 |
| 2-Ethoxyethanethiol | 5-C | 175 | 0/50 | 5.5 ± 3.1 | 22/22 | 10.4 ± 1.6 | 99.6 ± 4.5 | 1.4 ± 0.2 | 0.4 ± 0.1 |
| 2-Ethylthioethanethiol | 5-C | 125 | 0/50 | 4.6 ± 2.7 | 27/29 | 9.1 ± 3.2 | 92.3 ± 20.2 | 1.4 ± 0.2 | 0.5 ± 0.2 |
| 1,3-Dichloro-5,5-dimethylhydantoin | 5-C | 500 | 9/50 | 5.4 ± 2.9 | 24/24 | 9.0 ± 2.8 | 93.7 ± 18.0 | 1.5 ± 0.1 | 0.4 ± 0.2 |
| Disulfiram | 5-C | 4,900 | 4/50 | 3.6 ± 2.9 | 22/24 | 9.3 ± 3.6 | 85.7 ± 38.4 | 1.5 ± 0.1 | 0.4 ± 0.2 |
| Control | 5-C | Corn oil | 0/50 | 4.6 ± 3.4 | 34/37 | 9.1 ± 3.9 | 98.4 ± 16.0 | 1.5 ± 0.3 | 0.5 ± 0.2 |
| Ethylene glycol monomethyl ether acrylate | 5-D | 650 | 15/50 | NA | 0/14* | 0 | NA | NA | NA |
| Ethylene glycol monomethyl ether acetate | 5-D | 1,225 | 0/49 | NA | 0/31* | 0 | NA | NA | NA |
| Ethylene glycol monopropyl ether | 5-D | 2,000 | 1/49 | 5.7 ± 2.5 | 28/31 | 8.5 ± 2.9 | 83.9 ± 28.0 | 1.5 ± 0.1 | 0.5 ± 0.1 |
| Propylene glycol 3-ethyl ether | 5-D | 3,000 | 13/50 | 5.5 ± 2.4 | 21/21 | 7.7 ± 3.3* | 90.0 ± 21.4 | 1.4 ± 0.3 | 0.5 ± 0.2 |
| Control | 5-D | Water | 0/50 | 6.9 ± 3.0 | 25/28 | 10.0 ± 2.9 | 96.1 ± 10.4 | 1.5 ± 0.2 | 0.4 ± 0.3 |
| Diethyl phthalate | 6-A | 4,500 | 2/50 | 7.1 ± 2.2 | 31/33 | 9.5 ± 2.5 | 95.7 ± 16.5 | 1.6 ± 0.2 | 0.4 ± 0.2 |
| Di(n-butyl) phthalate | 6-A | 2,500 | 5/49 | NA | 0/26* | 0 | NA | NA | NA |
| Di(i-butyl) phthalate | 6-A | 4,000 | 27/50 | NA | 0/17* | 0 | NA | NA | NA |
| Control | 6-A | Corn oil | 0/50 | 7.5 ± 3.2 | 32/33 | 9.5 ± 2.0 | 99.4 ± 2.5 | 1.7 ± 0.1 | 0.5 ± 0.2 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Dimethyl methyl phosphonate | 6-B | 4,175 | 1/50 | 8.2 ± 2.0 | 32/36 | 9.4 ± 2.5 | 97.8 ± 6.9 | 1.5 ± 0.1* | 0.5 ± 0.2 |
| Tris(2-chloroethyl) phosphate | 6-B | 940 | 0/49 | 7.2 ± 2.4* | 35/37 | 10.1 ± 2.5 | 98.0 ± 5.0 | 1.6 ± 0.1 | 0.4 ± 0.2 |
| N-Isopropyl-N′-phenyl-p-phenylenediamine | 6-B | 800 | 48/50 | NA | 0/2 | 0 | NA | NA | NA |
| Control | 6-B | Corn oil | 0/50 | 8.2 ± 2.3 | 37/39 | 9.0 ± 3.1 | 98.4 ± 6.8 | 1.6 ± 0.2 | 0.5 ± 0.2 |
| D-Phenylalanine | 6-C | 5,000 | 1/50 | 7.3 ± 2.7 | 34/39 | 9.9 ± 2.9 | 98.2 ± 6.2 | 1.6 ± 0.2 | 0.4 ± 0.1 |
| Probenecid | 6-C | 1,200 | 0/47 | 7.2 ± 2.9* | 33/39 | 10.1 ± 2.4 | 90.7 ± 23.7 | 1.5 ± 0.1 | 0.3 ± 0.2 |
| Cinnamaldehyde | 6-C | 1,200 | 0/49 | 7.4 ± 2.4 | 34/34 | 9.4 ± 2.5 | 95.9 ± 8.3 | 1.6 ± 0.1 | 0.4 ± 0.3 |
| Control | 6-C | Corn oil | 0/50 | 7.8 ± 2.1 | 39/46 | 9.5 ± 2.6 | 94.8 ± 18.8 | 1.6 ± 0.2 | 0.5 ± 0.7 |
| Di(i-decyl) phthalate | 6-D | 9,650[b] | 0/50 | 8.0 ± 4.0 | 31/34 | 9.8 ± 2.4 | 99.5 ± 3.7 | 1.6 ± 0.1 | 0.6 ± 0.1 |
| Di(2-ethylhexyl) phthalate | 6-D | 9,860[b] | 0/48 | 4.9 ± 1.0 | 2/32* | 6.5 | 95.5 | 1.5 | 0.5 |
| 2-Ethyl-l-hexanol | 6-D | 1,525 | 17/49 | 3.9 ± 3.2* | 11/20* | 6.8 ± 3.4* | 73.4 ± 32.2* | 1.4 ± 0.2* | 0.3 ± 0.2* |
| Control | 6-D | Corn oil | 0/50 | 7.0 ± 2.5 | 33/34 | 9.9 ± 2.4 | 98.2 ± 8.8 | 1.6 ± 0.1 | 0.6 ± 0.1 |
| Mono(2-ethylhexyl) phthalate | 6-E | 545 | 6/49 | 9.9 ± 1.7 | 2/33* | 9.0 | 83.8 | 1.7 | 0.8 |
| Di(n-hexyl) phthalate | 6-E | 9,900[b] | 1/48 | NA | 0/34* | 0 | NA | NA | NA |
| Di(n-octyl) phthalate | 6-E | 9,780[b] | 0/50 | 9.6 ± 2.4 | 39/40 | 10.2 ± 2.8* | 97.2 ± 16.0 | 1.7 ± 0.1 | 0.6 ± 0.1* |
| Control | 6-E | Corn oil | 0/50 | 10.5 ± 2.2 | 34/38 | 11.5 ± 1.7 | 99.1 ± 4.0 | 1.6 ± 0.1 | 0.7 ± 0.2 |
| Trimellitic anhydride | 7-A | 800 | 1/47 | 7.1 ± 2.7* | 29/32 | 8.6 ± 3.6 | 96.9 ± 9.6 | 1.5 ± 0.2 | 0.9 ± 0.4 |
| Diethylene glycol monobutyl ether | 7-A | 2,050 | 12/48 | 9.0 ± 4.5 | 19/22 | 8.8 ± 3.4 | 100 | 1.6 ± 0.1 | 1.0 ± 0.2 |
| Toluene | 7-A | 3,000 | 3/49 | 7.0 ± 3.9 | 22/26 | 8.4 ± 3.1 | 89.8 ± 23.4 | 1.4 ± 0.1 | 1.0 ± 0.4 |
| Dimethyl phthalate | 7-A | 5,000 | 12/43 | 7.5 ± 2.8 | 17/19 | 8.5 ± 3.3 | 90.0 ± 25.3 | 1.5 ± 0.2 | 1.0 ± 0.2 |
| Control | 7-A | Corn oil | 0/46 | 9.0 ± 3.4 | 31/34 | 9.2 ± 3.3 | 98.3 ± 4.8 | 1.5 ± 0.1 | 0.9 ± 0.2 |

*$p < .05$ relative to concurrent control.

[a]Test laboratories: 1 = Borriston Laboratories, Inc. [8]; 2 = Bioassay Systems Corporation [10]; 3 = Minority Enterprise Service Associates [5]; 4 = Inveresk Research International, Ltd. [6]; 5 = Borriston Laboratories, Inc. [9]; 6 = Hazleton Laboratories America, Inc. [7]; 7 = NIOSH.

[b]Undiluted chemical, 10 ml/kg/d; 5 ml/kg/d in block 5-A.

ether, block 2-B) adversely affected four of five indices of reproductive success. Ethyl-substituted ethylene glycol ethers were also positive, but the ethyl-substituted diethylene glycol ethers were negative. Propyl- and butyl-substituted derivatives were generally negative, although results were not clear-cut for ethylene glycol monobutyl ether, which increased the proportion of failed pregnancies (fewer viable litters) but affected no other measure of reproductive success. None of the sulfur-substituted chemicals produced evidence of developmental toxicity, although challenge might have been low in view of the absence of maternal deaths and no effect on body weight gain. However, all three of these sulfur-substituted chemicals were much more toxic than the corresponding glycol ether, ethylene glycol monoethyl ether, and dose-response curves for lethality were very sharp in the dose-finding study. Thus, a small increase in dose would be expected to produce a large increase in maternal toxicity.

A class study was also conducted using phthalic acid esters (Table V). For di(2-ethylhexyl) phthalate, both initial metabolic products were also included: mono(2-ethylhexyl) phthalate and 2-ethylhexanol. Dramatically positive results were seen with the diesters having intermediate chain lengths: n-butyl, i-butyl, and n-hexyl. The shorter (methyl and ethyl) and longer (n-octyl and i-decyl) diesters were generally negative, although litter size and neonatal weight gain were both reduced in the di(n-octyl) phthalate group relative to its concurrent control. Examination of the data for that block (6-E) reveals that control values were exceptionally high. Although both

**TABLE III. Results of Multiple Tests of Single Chemicals**

| | | Maternal response variables | | | Neonatal response variables | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | g per pup | |
| Lab-block | Dose (mg/kg/d) | Percentage maternal mortality | Weight change | Viable litters | Liveborn per litter | Percentage survival | Birth weight | Weight gain |
| Ethylenethiourea | | | | | | | | |
| 1-A | 100 | 0 | –[a] | – | – | – | – | – |
| 4-A | 200 | 0 | – | – | – | – | – | – |
| 2-A | 300 | 0 | – | – | +[b] | – | + | – |
| 3-A | 600 | 0 | – | + | – | – | – | – |
| Sodium selenite | | | | | | | | |
| 1-A | 3.5 | 0 | – | – | – | – | – | – |
| 2-A | 5 | 0 | – | – | – | – | – | – |
| 3-A | 7 | 2 | – | – | – | – | – | – |
| 4-A | 14 | 44 | + | + | – | + | + | + |
| Diethylene glycol monobutyl ether | | | | | | | | |
| 1-A | 500 | 0 | – | – | – | – | – | – |
| 7-A | 2,050 | 25 | – | – | – | – | – | – |
| Dimethyl phthalate | | | | | | | | |
| 3-C | 3,500 | 0 | – | – | – | – | – | – |
| 7-A | 5,000 | 28 | – | – | – | – | – | – |
| Trimellitic anhydride | | | | | | | | |
| 4-C | 550 | 2 | – | – | – | – | – | – |
| 7-A | 800 | 2 | + | – | – | – | – | – |
| Toluene | | | | | | | | |
| 2-C | 2,350 | 2 | – | – | – | – | – | – |
| 7-A | 3,000 | 6 | – | – | – | – | – | – |

[a]No statistically significant treatment effect (from Table II).
[b]Statistically significant treatment effect.

**TABLE IV. Results of Glycol Ethers Class Study**

| | | | Maternal response variables | | | Neonatal response variables | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | g per pup | |
| Chemical name | Lab-Block | Dose (mg/kg/d) | Percentage maternal mortality | Weight change | Viable litters | Liveborn per litter | Percentage survival | Birth weight | Weight gain |
| Ethylene glycol | 4-B | 11,090[a] | 10 | +[b] | + | + | + | + | + |
| Monomethyl ether | 2-B | 1,400 | 14 | NA[c] | + | NA | NA | NA | NA |
| Monomethyl ether acetate | 5-D | 1,225 | 0 | NA | + | NA | NA | NA | NA |
| Monomethyl ether acrylate | 5-D | 650 | 30 | NA | + | NA | NA | NA | NA |
| Dimethyl ether | 3-B | 2,000 | 26 | NA | + | NA | NA | NA | NA |
| Monoethyl ether | 2-B | 3,605 | 10 | NA | + | NA | NA | NA | NA |
| Diethyl ether | 1-B | 2,955 | 10 | + | + | + | + | + | –[d] |
| Monopropyl ether | 5-D | 2,000 | 2 | – | – | – | – | – | – |
| Monobutyl ether | 2-B | 1,180 | 20 | + | + | – | – | – | – |
| Diethylene glycol | 4-B | 11,180[a] | 4 | – | – | – | – | – | – |
| Monomethyl ether | 2-B | 4,000 | 10 | + | + | + | + | – | + |
| Dimethyl ether | 3-B | 3,000 | 40 | NA | + | NA | NA | NA | NA |
| Monoethyl ether | 1-B | 5,500 | 14 | + | – | – | – | – | – |
| Diethyl ether | 3-A | 3,000 | 0 | + | – | – | – | – | – |
| Monobutyl ether | 1-A | 500 | 0 | – | – | – | – | – | – |
| Monobutyl ether | 7-A | 2,050 | 25 | – | – | – | – | – | – |
| Dibutyl ether | 4-C | 2,000 | 8 | – | – | – | – | – | – |
| Triethylene glycol | 1-B | 11,270[a] | 4 | + | – | – | – | – | – |
| Dimethyl ether | 3-B | 3,500 | 4 | NA | + | NA | NA | NA | NA |
| Propylene glycol 3-ethyl ether | 5-D | 3,000 | 26 | – | – | + | – | – | – |
| 2-Ethylthioethanol | 5-C | 1,200 | 0 | – | – | – | – | – | – |
| 2-Ethoxyethanethiol | 5-C | 175 | 0 | – | – | – | – | – | – |
| 2-Ethylthioethanethiol | 5-C | 125 | 0 | – | – | – | – | – | – |

[a]Administered as undiluted chemical, 10 ml/kg/day.
[b]Statistically significant treatment effect.
[c]Not evaluated statistically done to small sample size or no live pups.
[d]No statistically significant treatment effect (from Table II).

**TABLE V. Results of Phthalic Acid Ester Class Study**

| Chemical name | Lab-block | Dose (mg/kg/d) | Maternal response variables: Percentage maternal mortality | Weight change | Viable litters | Neonatal response variables: Liveborn per litter | Percentage survival | g per pup: Birth weight | Weight gain |
|---|---|---|---|---|---|---|---|---|---|
| Phthalates | | | | | | | | | |
| Dimethyl | 3-C | 3,500 | 0 | –[a] | – | – | – | – | – |
| Dimethyl | 7-A | 5,000 | 28 | – | – | – | – | – | – |
| Diethyl | 6-A | 4,500 | 4 | – | – | – | – | – | – |
| Di(n-butyl) | 6-A | 2,500 | 10 | NA[b] | +[c] | NA | NA | NA | NA |
| Di(i-butyl) | 6-A | 4,000 | 54 | NA | + | NA | NA | NA | NA |
| Di(n-hexyl) | 6-E | 9,900[d] | 2 | NA | + | NA | NA | NA | NA |
| Di(2-ethylhexyl) | 6-D | 9,860[d] | 0 | NA | + | NA | NA | NA | NA |
| Mono(2-ethylhexyl) | 6-E | 545 | 12 | NA | + | NA | NA | NA | NA |
| Di(n-octyl) | 6-E | 9,780[d] | 0 | – | – | + | – | – | + |
| Di(i-decyl) | 6-D | 9,650[d] | 0 | – | – | – | – | – | – |
| 2-Ethyl-1-hexanol | 6-D | 1,525 | 35 | + | + | + | + | + | + |

[a]No statistically significant treatment effect (from Table II).
[b]Not evaluated statistically due to small sample size or no live pups.
[c]Statistically significant treatment effect.
[d]Administered as undiluted chemical, 10 ml/kg/day.

**TABLE VI. Classification of Screening Outcomes and Conventional Test Results**

| Category of response | Conventional test results[a] | | | |
|---|---|---|---|---|
| | Mouse | Rat | Rabbit | Hamster |
| Reduced No. of viable litters, litter size, or viability | | | | |
| Aniline, p-nitro | | | | |
| Ethylenethiourea | − [25] | + [25] | | + [25] |
| 2-Ethyl-1-hexanol | | | | |
| Glycol, ethylene | + [15] | + [15] | | |
| Glycol, ethylene, monomethyl ether | + [3] | + [19] | | |
| Glycol, ethylene, monomethyl ether acetate | | | | |
| Glycol, ethylene, monomethyl ether acrylate | | | | |
| Glycol, ethylene, dimethyl ether | + [20] | | | |
| Glycol, ethylene, monoethyl ether | | + [4] | + [4] | |
| Glycol, ethylene, diethyl ether | | | | |
| Glycol, ethylene, monobutyl ether | | − [23] | − [23] | |
| Glycol, diethylene, monomethyl ether | | + [17] | | |
| Glycol, diethylene, dimethyl ether | + [18] | | | |
| Glycol, triethylene, dimethyl ether | + [16] | | | |
| Glycol, propylene, 3-ethyl ether | | | | |
| Naphthalene | | − [26] | | |
| Nitrofurazone | + [27] | | | |
| Phthalate, di(n-butyl) | + [28] | ? [29] | | |
| Phthalate, di(i-butyl) | | ? [29] | | |
| Phthalate, di(n-hexyl) | | | | |
| Phthalate, di(n-octyl) | | ? [29] | | |
| Phthalate, di(2-ethylhexyl) | + [28,30] | ? [29] | − [25] | |
| Phthalate, mono(2-ethylhexyl) | + [30] | F [31] | − [32] | |
| Sodium selenite | F [1,2] | − [25] | − [25] | − [25] |
| Toluene, 2,4-diamino | | | | |
| Reduced birth weight, weight gain | | | | |
| Aniline | | −[25] | | |
| Benzyl alcohol | | | | |
| Dimethyl methyl phosphonate | | | | |
| Tween 60 | | | | |
| No effect | | | | |
| Allyl chloride | | − [33] | − [33] | |
| Aniline, N,N-dimethyl | | | | |
| Cinnamaldehyde | | | | |
| Decalin | | | | |
| 1,3-Dichloro-5,5-dimethylhydantoin | | | | |
| Disulfiram | | − [25] | | − [34] |
| 2-Ethylthioethanol | | | | |
| 2-Ethoxyethanethiol | | | | |
| 2-Ethylthioethanethiol | | | | |
| Glycol, ethylene, monopropyl ether | | | | |
| Glycol, diethylene | | | | |

*(continued)*

**TABLE VI. Classification of Screening Outcomes and Conventional Test Results (continued)**

| Category of response | Conventional test results[a] | | | |
|---|---|---|---|---|
| | Mouse | Rat | Rabbit | Hamster |
| No effect | | | | |
| Glycol, diethylene, monoethyl ether | | − [21] | | |
| Glycol, diethylene, diethyl ether | | | | |
| Glycol, diethylene, monobutyl ether | | − [35] | − [35] | |
| Glycol, diethylene, dibutyl ether | | | | |
| Glycol, triethylene | − [25] | − [25] | − [25] | − [25] |
| p-Nitrophenol | | | | |
| Phthalate, dimethyl | | ? [29] | | |
| Phthalate, diethyl | | ? [29] | | |
| Phthalate, di(i-decyl) | | | | |
| D-Phenylalanine | | | | |
| Probenecid | | | − [25] | |
| Tergitol NP-10 | | | | |
| Toluene | + [14] | − [25] | | |
| Toluene, 2,4-dinitro | | − [25] | | |
| Trimellitic anhydride | | | | |
| Trioctanoin | − [25] | | | |
| Tris(2-chloroethyl) phosphate | | | | |
| Triton X-100 | | | | |
| Not classified (inadequate maternal survival) | | | | |
| N-Isopropyl-N′-phenyl-p-phenylenediamine | | | | |

[a]Results and reference number for standard teratology test: +, Teratogenic; −, not teratogenic; ?, Equivocal or contradictory results; F, fetotoxic (intrauterine death or growth retardation without malformations).

**TABLE VII. Correspondence of Current Test Results With Conventional Test Data in Mice**

| Current results | Conventional results | | | |
|---|---|---|---|---|
| | +[a] | F[b] | −[c] | Total |
| Reduced litter size and/or viability | 9 | 1 | 1 | 11 |
| Reduced birth weight and/or weight gain | 0 | 0 | 0 | 0 |
| No effect | 1 | 0 | 2 | 3 |
| Total | 10 | 1 | 3 | 14 |

[a]Teratogenic.
[b]Fetotoxic (intrauterine death or growth retardation without malformations).
[c]Not teratogenic.

the number of liveborn and the neonatal weight gain in the di(n-octyl) phthalate group were significantly less than in the control, both of those di(n-octyl) phthalate means were higher than was observed in most control groups. The statistical significance here may have resulted more from an exceptional concurrent control group than from chemical toxicity. Di(n-octyl) and di(2-ethylhexyl) phthalate have the same molecular weight, were administered at essentially the same dose, and neither produced maternal mortality, but the reproductive outcomes differed drastically, with di(2-ethylhexyl) phthalate reducing viable litters to only two of 32 pregnant mice. Both metabolic

products of di(2-ethylhexyl) phthalate were clearly positive, as well as being much more acutely toxic to the treated females.

Two more limited class studies were also included in this series of chemicals. Aniline, p-nitroaniline, and N,N-dimethylaniline were tested in one experimental block (1-C). The latter chemical was negative, while both aniline and p-nitroaniline produced some evidence of developmental toxocity. Unexpectedly high maternal mortality in the p-nitroaniline group, however, complicated evaluation of those results. Toluene, 2,4-dinitrotoluene, and 2,4-diaminotoluene were tested in another experimental block (2-C, with toluene retested in block 7-A), with positive results associated only with 2,4-diaminotoluene. Those positive results, too, are complicated by unexpectedly high maternal mortality. In contrast, results for 2,4-dinitrotoluene were clearly negative since that group had comparable maternal mortality but showed no adverse effects on reproductive indices.

## DISCUSSION

In the original publication of this assay [1,2], there was not a standard protocol for the number of treatments given (either one or five), for the duration of dosage (gestation days 8, 11, 12, or 8 to 12), or for the route of administration (oral, intraperitoneal, and intramuscular were used). Treatment days, duration, and route were selected for each chemical based on experience or published information on sensitive periods. For the present studies, gavage on days 6–13 of gestation was universally employed. Water and corn oil were selected as vehicles since practically all chemicals can be delivered in one or the other. Standardization of route, duration, and timing of treatment was necessary since, with unknown chemicals, there is no basis for selection of an optimal combination. Furthermore, consistent protocols are required if a number of chemicals are to be prioritized on the basis of results from this preliminary test.

Following the classification used by Chernoff and Kavlock [1,2], Table VI groups the chemicals tested here into three classes: those that had no effect, those that reduced birth weight or neonatal weight gain, and those that reduced litter size or survival of pups. Reduced proportion of pregnant females that produced a viable litter was not reported previously, but is included here with reduced litter size and viability. For comparison, Chernoff and Kavlock [1,2] also categorized chemicals they tested according to results of conventional teratology tests in mice. Conventional tests in various species were found for 29 of the 60 chemicals tested here (some of those tests were conducted in response to high priority determinations here). Those conventional result are also summarized in Table VI. Only 14 of those chemicals had conventional teratology studies in mice.

The overall correlation between results from this test and those from conventional teratology studies in mice is summarized in Table VII. As seen in this simple tabulation, nine of ten teratogens and the fetotoxin produced significant effects on viable litters, litter size, or neonatal viability, while two of three nonteratogens produced no adverse effect. The selection of doses here (the predicted maternal LD10) should have weighted the system toward producing signs of developmental toxicity. Nevertheless, for these three nonteratogens, the false-positive rate was only one in three. A greater concern is the failure to produce adverse reproductive outcomes with a presumptive teratogen (toluene), for a 10% false-negative rate.

Toluene was clearly negative in two separate evaluations, producing no evidence of developmental toxicity in the presence of 2% (at 2,350 mg/kg/day) and 6% maternal mortality (at 3,000 mg/kg/day). Increased intrauterine death and reduced fetal body weight were reported [14] in the same strain of mouse orally dosed three times daily with 0.5 or 1.0 ml/kg (approximately 1,500 and 3,000 mg/kg/day) on gestation days 6–15. Malformations (cleft palate) were seen after 3,000 mg/kg/day. Those effects might be expected to be expressed in this test system as reduced litter size, reduced birth weight, and impaired neonatal survival. However, a volatile compound like toluene may be quickly eliminated in expired breath. Thus, fractionated oral treatments [14] may produce higher average body burdens than the same dose administered once daily.

Results of the present tests with glycol ethers are consistent with the known teratogenicity of these chemicals. Ethylene glycol was positive here, and subsequent conventional tests demonstrated that it is teratogenic in mice and rats [15]. All of the methyl derivatives were positive here, and all except two (ethylene glycol monomethyl ether acetate and acrylate) are reported to be teratogenic in mice and/or rats [3,16–20]. Ethyl-substituted derivatives of ethylene glycol were positive, but not ethyl-substituted derivatives of diethylene glycol. These results are consistent with reports that ethylene glycol monoethyl ether is teratogenic in rats and rabbits [4], while diethylene glycol monoethyl ether is not teratogenic in rats [21]. Negative results for propyl- and butyl-substituted glycol ethers also are consistent with reports that ethylene glycol monopropyl ether acetate [22] and ethylene glycol monobutyl ether [23] produce fetotoxicity in the presence of maternal toxicity, but not teratogenic responses. The molecular structures of propylene glycol 3-ethyl ether and ethylene glycol monopropyl ether differ only in having the primary alcohol group located on the propyl or ethyl terminus, respectively. Unlike ethylene glycol monopropyl ether, propylene glycol 3-ethyl ether affected one reproductive endpoint (average litter size was reduced), but maternal mortality was considerably higher and no other indices were adversely affected.

Table VI essentially divides these chemicals into two equal-sized groups: one set of 30 with some evidence of developmental toxicity and another set of 29 without evidence (N-isopropyl-N′-phenyl-p-phenylenediamine is unclassified due to almost total maternal mortality). Further prioritization of those with evidence of developmental toxicity would involve consideration of factors such as extent and patterns of usage, as well as detailed evaluation of which indices of response were affected, eg, maternal toxicity, viable litters, etc. In an accompanying paper [24], a scoring system is proposed which yields numerical scores weighted by maternal mortality and each of the five reproductive endpoints reported (viable litters, live litter size, percent survival, birth weight, and neonatal weight gain).

No assay is infallible, and this one is no exception. Results from this test should not be used to label a chemical as safe or unsafe, as teratogenic or nonteratogenic. Neither a high nor a low priority ranking in this test should, by itself, determine the testing future of a chemical. On the other hand, this test also should not be a required preliminary to conventional testing. Scientific judgement and consideration of extent and patterns of usage should also contribute to a final testing decision. Significant human exposure to some chemicals will justify testing on that basis alone. Nevertheless, this assay has value as a preliminary test which can help to establish priorities for conventional testing.

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