Experimental Designs for Developmental Toxicity Experiments to Estimate the Benchmark Dose

Karen Y. Fung[1*], Daniel Krewski[2], Robert Smythe[3]

[1] Dept. of Mathematics & Statistics, University of Windsor, Windsor, Ontario, Canada N9B 3P4

[2] Dept. of Epidemiology & Community Medicine, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5

[3] Dept. of Statistics, Oregon State University, Corvallis, Oregon, U.S.A. 97331

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Corresponding author: Karen Fung
E-mail address: kfung@uwindsor.ca
Telephone: (519) 253-4232, x3022
Fax: (519) 971-3649

ABSTRACT

Background: In a previous paper, we developed optimal experimental designs for the estimation of the benchmark dose in developmental toxicity experiments using joint Weibull dose-response models to represent the dose response relationships for prenatal death and fetal malformations. In this article, we explore the effect of the number of implants and the degree of intralitter correlation on these optimal designs. Efficiencies of fixed 3 and 4-dose suboptimal designs which do not require prior information on the nature of the dose-response relationships for prenatal death and developmental toxicity are also evaluated. Finally, we also develop optimal experimental designs for a series of developmental toxicity studies, and use these results to develop general recommendations on efficient designs that may be used in practice.

Methods: Optimal experimental designs are specified in terms of (1) the number of doses, (2) the dose levels, and (3) the fraction of animals allocated to each dose. Numerical search routines were used to find optimal designs for experiments with equal or unequal number of implants, and varying degrees of intralitter correlation.

Results: The optimal designs were found to be quite robust against variation in the number of implants and the degree of intralitter correlation. The optimal designs included three doses, corresponding to the number of parameters to be estimated in the Weibull dose-response model. The optimal doses generally included an unexposed control, a group at the maximum tolerated dose, and a dose above the benchmark dose (ED_{05}). Given the optimal dose levels, a design with a 1:2:1 allocation ratio was more efficient than a design with (1:1:1) allocation.

Conclusions: When no prior information about the dose-response relationship for developmental toxicity is available, our results suggest that a 3-dose design which includes a maximum tolerated dose, an unexposed control group, and a middle dose equals to approximately half the maximum dose and a 3:6:1 allocation ratio may be reasonably efficient for estimating the benchmark dose. In practice, however, additional doses may be desirable to define the shape of the dose-response curve in more detail, protect against the loss of an entire dose group, or evaluate goodness-of-fit. Nonetheless, our optimal designs provide a basis against which the efficiency of other designs may be evaluated.

KEY WORDS: Benchmark dose; developmental toxicity; prenatal death; fetal malformation; Weibull dose-response model; optimal design.

1. Introduction

Developmental toxicity studies are conducted to detect agents capable of causing developmental anomalies, such as prenatal death, structural anomalies, or growth alterations in offspring of exposed mothers. Typically, groups of 20-30 mated female rodents are exposed to the test agent at 1 of 3-4 concentrations, in addition to an unexposed control group, during major organogenesis. The highest dose is targeted at producing minimal maternal toxicity, defined as marginal body weight loss and not more than 10% death.

The benchmark dose, defined as a lower confidence limit on the dose associated with an excess risk of 5%, is a useful indicator of developmental toxicity. However,

experimental designs for estimating the benchmark dose have received only limited attention to date (Kavlock et al., '96, Fung et al. '98, Krewski et al., '99).

An important advance in developmental toxicity risk assessment is the development of joint dose response models to describe prenatal death and fetal malformation rates (Ryan, '92, Catalano et al., '93, Krewski and Zhu, '94, Zhu and Fung, '96). These models can be used to estimate the benchmark dose for both of these endpoints simultaneously, as well as for overall toxicity. In a previous article (Krewski et al., 2000), we developed optimal designs for estimation of benchmark doses using joint Weibull models for prenatal death and fetal malformation. Since these Weibull models each involve three parameters, the optimal designs for estimating the benchmark dose (BMD) for prenatal death and fetal malformation involve 3 dose groups. Because of the orthogonality of the estimating equations used to fit the joint Weibull models, the optimal design for overall toxicity also involves only 3 dose levels. Based on the two examples considered in our previous paper (Krewski et al., 2000), the optimal designs include both the maximum tolerated dose and an unexposed control group, with a low dose above or close to the BMD. Since the optimal designs for prenatal death, fetal malformation and overall toxicity appear to be comparable, it is possible to construct a single 3 dose design that is reasonably efficient for estimating the BMD for all 3 endpoints.

This paper extends our previous investigation by studying the effect that the number of implants and the degree of intralitter correlation have on the optimal designs. The efficiencies of suboptimal designs that have been used in past practice are also calculated. Finally, we derive experimental designs for the series of developmental toxicity studies considered previously by Krewski and Zhu ('95). These latter results are used to

develop general design guidelines that may be useful in the absence of prior information on the nature of the dose-response relationships for prenatal death and fetal malformation.

2.1 Statistical Models for Developmental Toxicity

Data from developmental toxicity studies can be conveniently summarized in the form of multinomial counts for the j^{th} animal at the i^{th} dose level d_i ($i = 1,\ldots, t$; $j = 1,\ldots, n_i$), including the total number of implants m_{ij}, the number of prenatal death r_{ij}, the number of live offspring s_{ij}, and the number of malformed live offspring y_{ij}. The doses are ordered such that $0 = d_1 < d_2 < \ldots < d_t = D$, where the maximum tolerated dose D is chosen to elicit only minimal maternal toxicity.

Following the notation adopted by Krewski et al. (2000), we let π_1 be the probability of any malformation in a live fetus, π_2 the probability of a prenatal death, and ϕ_i the intralitter correlation (constrained to be the same for both malformation and prenatal death) within the i^{th} treatment group. Letting $z_{ij} = (y_{ij}, r_{ij})^T$, the mean and covariance of the observations are

$$E(z_{ij} \mid m_{ij}) = m_{ij}\begin{bmatrix}\mu_1 \\ \mu_2\end{bmatrix} = m_{ij}\begin{bmatrix}\pi_1(1-\pi_2) \\ \pi_2\end{bmatrix}$$

and

$$Cov(z_{ij} \mid m_{ij}) = m_{ij}[1+(m_{ij}-1)\phi_i]\begin{bmatrix}\mu_1(1-\mu_1) & -\mu_1\mu_2 \\ -\mu_1\mu_2 & \mu_2(1-\mu_2)\end{bmatrix}$$

respectively, where $\mu_1 = \pi_1(1-\pi_2)$, $\mu_2 = \pi_2$ and $\max\{-(m_{ij}-1)^{-1}\} < \phi_i < 1$. This covariance structure corresponds to the variance of the extended Dirichlet-trinomial distribution; when ϕ_i =0, it reduces to that of a trinomial distribution.

Weibull models

$$\pi_i(d) = 1 - \exp(-a_i - b_i d^{\gamma_i}) \qquad (i = 1, 2)$$

($a_i > 0, b_i > 0, \gamma_i > 0$) are used to describe the dose-response relationships for fetal malformation and prenatal death respectively. The dose-response relationship for overall toxicity is then described by

$$\pi_3(d) = 1 - [1 - \pi_1(d)][1 - \pi_2(d)] \ .$$

Specifically, $\pi_3(d)$ gives the probability of either a death or a malformation occurring at dose d.

2.2 The Effective Dose and the Benchmark Dose

The effective dose ED_α that induces α x 100% extra risk is defined by the equation

$$\frac{\pi(ED_\alpha) - \pi(0)}{1 - \pi(0)} = \alpha$$

($0 < \alpha < 1$), where $\pi(d)$ represents the probability of a response at dose d. Under the flexible Weibull models used here, the ED_α for both malformations and prenatal death is of the form

$$ED_\alpha = \{-\ln(1-\alpha)/b\}^{1/\gamma} .$$

In large samples, the variance of the estimator $\hat{ED}_\alpha$ of ED_α based on the fitted model $\hat{\pi}$ is approximated by

$$Var(\hat{ED}_\alpha) \approx D^T Cov(\theta) D ,$$

where $D = \partial ED_\alpha / \partial \theta$, with $\theta = (a, b, \gamma)^T$. The benchmark dose for either of these two endpoints can then be calculated as

$$BMD_\alpha = \hat{ED}_\alpha - 1.645[Var(\hat{ED}_\alpha)]^{1/2} \ ,$$

where 1.645 corresponds to the upper 95th percentile of the standard normal distribution, yielding a lower 95% confidence limit on the $\hat{ED}_\alpha$.

The effective dose defined for overall toxicity is obtained by setting

$$\alpha = [\pi_3(ED_\alpha) - \pi_3(0)]/[1 - \pi_3(0)]$$

$$= 1 - \exp\{-b_1 ED_\alpha^{\gamma_1} - b_2 ED_\alpha^{\gamma_2}\}.$$

This gives the equation

$$-\ln(1-\alpha) = b_1 ED_\alpha^{\gamma_1} + b_2 ED_\alpha^{\gamma_2}$$

which can be solved iteratively for ED_α. The variance of ED_α is

$$Var(ED_\alpha) = \{b_1\gamma_1 ED_\alpha^{\gamma_1 - 1} + b_2 ED_\alpha^{\gamma_2 - 1}\}^{-2} \Psi^T V_1 \Psi$$

where

$$\Psi^T = (ED_\alpha^{\gamma_1}, b_1 ED_\alpha^{\gamma_1} \ln(ED_\alpha), ED_\alpha^{\gamma_2}, b_2 ED_\alpha^{\gamma_2} \ln(ED_\alpha))$$

and V_1 is the asymptotic covariance matrix of (b_1, γ_1, b_2, γ_2).

Numerical search routines can then be used to find the dose levels and allocation of animals to each level that minimize the variance of the estimated ED_α.

In estimating the model parameters, the correlation parameter ϕ is usually estimated by an *ad hoc* procedure; separately from the first order model parameters. Since the correlation generally depends on the dose level, we estimate a different value of ϕ within each treated group. In constructing the optimal designs, we assume that all parameters including ϕ are known. Following Krewski et al. (2000), we assume that the number of implants, m, depends only on the dose level and not on the individual litter, i.e., $m_{ij} = m_i$ for j = 1,…,n_i for simplicity.

3. Effect of Number of Implants and Intralitter Correlation on Optimal Designs

In this section, we investigate the impact of the number of implants on the optimal design for the benchmark dose for prenatal death. Similar results are expected for the fetal malformations given alive pups. Since the effective dose, ED_{05}, and its variance are scale invariant, the optimal design is also scale invariant. Hence, we scaled the maximum dose to 1.0 in the following examples.

Tables 1 gives the optimal designs for equal or unequal numbers of implants m_i in combination with equal or unequal ϕ_i based on the Weibull model parameters of the ethylene glycol diethyl ether (EGDE, Table 1a) and ethylene glycol (EG, Table 1b) for prenatal death given in our previous paper (Krewski et al., 2000). Again, we assume a constant number m=8 of implants per litter across all dose groups in the EDGE experiment and m=14 for all litters in the EG experiment.

Following Bowman et al. ('95), a logistic-type function

$$\phi_i = \frac{2}{1+\exp(\alpha_1 + \alpha_2 d_i)} - 1 \ .$$

is used to describe the relationship between the intralitter correlation and dose. This model was fitted to each data set by standard nonlinear regression analysis, and the corresponding ϕ_i's were used in finding the optimal design. Numerical search routines are used to find the optimal designs that minimize the variance of the effective dose ED_{05}.

The results in Tables 1a and 1b show that when m_i and ϕ_i are both equal in all three dose groups, the optimal designs are identical for all values of the correlation coefficient ϕ considered; however, the variance of the estimates of ED_{05} increases as the degree of intralitter correlation ϕ_i increases. Slightly different optimal designs are obtained when

either m_i or ϕ_i differ across dose groups. Again, when we restricted the highest dose d_3 to be at most 1 (the maximum tolerated dose), we found that all optimal doses include $d_1=0$ and $d_3=1$. The middle dose is slightly higher than ED_{05} in Table 1a and comparable to the ED_{05} in Table 1b. Based on two examples, the optimal designs appear to be quite robust against variation in the values of m_i and ϕ_i across treatment groups.

4. Efficiencies of Suboptimal Designs

4.1 3-Dose Designs

Determination of an optimal design requires some knowledge about the shape of the dose response curve (or the dose-response model parameters). In practice, this information may not be available, precluding the determination of the optimal design. In this case, the investigator will need to be satisfied with a suboptimal design.

Here, we investigate some fixed 3-dose designs and evaluate their efficiencies relative to the optimal designs for the two data sets considered previously. The efficiencies of fixed suboptimal designs for 3 endpoints (prenatal death, fetal malformation, and overall toxicity), conditional on m, are given for ethylene glycol diethyl ether (EGDE, Table 2a) and ethylene glycol (EG, Table 2b). Designs for malformation conditional on the number of live births are also given in Table 2. Seven different designs are presented, with design 1 being the optimal design. Designs 2 and 3 represent the situations when the doses are fixed at the optimal levels, but the allocations are equal (design 2) or in the ratio 1:2:1 (design 3). The next three designs have fixed allocations at the optimal levels $d_1=0$ and $d_3=1$, but varying middle dose d_2: design 4 places d_2 at the ED_{05}, design 5 has d_2 below the ED_{05}, and design 6 has d_2 higher than ED_{05}. Design 7, with halving of doses and equal allocation,

is the one that is used most often in practice. The response probability at each dose is also presented in Table 2.

These results indicate that for all cases with fixed optimal doses, a design with a 1:2:1 allocation ratio produces higher efficiency than a design with equal allocation. For fixed optimal allocation ratios, designs in which the ED_{05} is used as the middle dose are not particularly efficient, unless ED_{05} is right at the optimal dose (EG death). When the middle dose is lower than ED_{05}, the efficiency is usually low, whereas doses higher than ED_{05} produce higher efficiencies. Correspondingly, it is desirable to have two doses higher than ED_{05} rather than only one. Note that the commonly used design 7 does not yield high efficiency in all cases considered here.

4.2 4-Dose Designs

Risk assessment strategies for non-carcinogenic health endpoints have traditionally focused on the determination of the no-observed-adverse-effect-level (NOAEL) with designs formulated to detect significant pairwise differences in response probabilities. This strategy, in conjunction with practical considerations, has resulted in the use of designs containing 4 dose groups with about 20 litters in each group. The high dose is targeted at the maximum tolerated dose, and the lower doses set by either progressively halving the higher dose, or by the desire to ensure that no adverse effects are observed at the lowest experimental dose. With this in mind, we selected several commonly used 4-dose designs to explore the impact of placement of doses and sample allocation on the variance of the estimated ED_{05}. Again, all designs investigated here have $d_1=0$ and $d_4=1$.

Design 1 involves progressively halving the doses, with an equal allocation of animals to each dose; design 2 uses the same doses with an allocation ratio of 1:2:2:1. We

also considered designs 3 and 4 in which the optimum dose for death and malformation are both included: design 3 has equal allocation ratio and design 4 has allocation ratio of 1:2:2:1.

Efficiencies of these designs relative to the optimal 3-dose design are given in Table 3. For EGDE, designs 1 and 2 generally do not yield very high efficiencies when compared with the optimal 3-dose design, although the unequal 1:2:2:1 allocation ratio is slightly better than equal allocation. When we included the optimum doses for death and malformation in the 4-dose designs, the efficiencies were much higher, with the highest efficiency achieved with overall toxicity. For EG, designs 3 and 4 do not perform as well as designs 1 and 2 for malformation and overall toxicity. Thus, including the optimum doses for death and malformation in a design does not necessarily guarantee high efficiency. Note that whereas the optimum doses for malformation and death are quite distant for EG, they are quite close for EGDE.

5. Optimal Designs for a Series of Developmental Toxicity Studies

5.1 Developmental Toxicity Data

In this section, we develop optimal experimental designs for the series of developmental toxicity studies conducted under the U.S. National Toxicity Program, considered previously by Krewski and Zhu (‘95). These studies typically involve groups of 20-30 female animals exposed to one of four or five doses of compounds, with an unexposed control. Table 4 gives an overview of the original designs for the 11 developmental toxicity studies. The reader is referred to Krewski and Zhu (‘95) for the graphical displays of the rates of fetal malformation, prenatal death, and overall toxicity.

This series of experiments was chosen for analysis because it represents a variety of situations in which the dose-response relationship may be convex, sigmoidal or irregular in shape. It is also possible that a dose-response relationship may or may not be evident in either fetal malformation or prenatal death. The objective of this analysis is to develop some general design guidelines that may be useful in the absence of prior information on the nature of the dose-response relationships.

5.2 Optimal Designs

Optimal designs for estimating the effective dose for prenatal death, fetal malformation and overall toxicity conditional on the number of implants for these 11 developmental toxicity studies are presented in Table 5. For these studies to be comparable, we again scaled all maximum doses to 1. Optimal designs were not constructed for prenatal death and overall toxicity for study #6 because of the lack of a clear dose-response relationship.

Table 5 shows that in 7 out of the 10 studies considered (omitting study #6), the effective dose (ED_{05}) for prenatal death is the highest among the three endpoints considered. On the other hand, the effective dose for overall toxicity is the lowest in all the studies. This occurs because the ED_{05} for overall toxicity takes into account both prenatal death and fetal malformation. The optimal middle dose, d_2, is the highest for prenatal death in all but studies #7 and #9, and lowest for fetal malformation except in studies #3, #7, and #9. The average optimal middle dose d_2, presented at the end of Table 5, is approximately 0.5 for fetal malformation and overall toxicity, and slightly higher (d_2 = 0.64) for prenatal death. On average, roughly 60% of the animals are assigned to the middle dose, with 10% at the maximum tolerated dose and 30% at the control. Since these

averages do not differ markedly from individual studies, a 3:6:1 allocation could be considered for use in practice in the absence of prior information on the nature of the dose-response relationship.

6. Conclusions

In this article, we have examined optimal designs for the estimation of effective dose (ED_{05}) for prenatal death, fetal malformation and overall toxicity, conditional on the number of implants. In general, the ED_{05} for overall toxicity is of primary importance for risk assessment purposes, since it takes into account both prenatal death and fetal malformation. When no prior information about the dose-response relationship is available for prenatal death and fetal malformation, our results suggest that a 3-dose design which includes a maximum tolerated dose, an unexposed control group, and the middle dose equal to 50%-60% of the maximum dose with a allocation ratio of 3:6:1 may be reasonably efficient in practice.

However, as discussed in our previous study (Krewski et al., 2000), there are often reasons to prefer to use a suboptimal design with more than 3 doses. Although the results for the 4-dose designs considered here suggest some loss in efficiency, the use of an additional dose both protects against the loss of an entire treatment group, and provides more information on the slope of the underlying dose-response curve .

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Table 1a
Optimal Designs for the BMD Conditional on the Number of Implants m
for Prenatal Death due to Exposure to EGDE*

Number of Implants m(i)	Intralitter Correlation $\phi(i)$	Optimal Doses			Optimal Allocation			Var($\widehat{ED}_{05}$)
		d_1	d_2	d_3	δ_1	δ_2	δ_3	
8,8,8	0.0,0.0,0.0	0.00	0.687	1.00	0.40	0.55	0.06	0.00812
	0.1,0.1,0.1	0.00	0.687	1.00	0.40	0.55	0.06	0.01381
	0.3,0.3,0.3	0.00	0.687	1.00	0.40	0.55	0.06	0.02518
	0.5,0.5,0.5	0.00	0.687	1.00	0.40	0.55	0.06	0.03709
	0.7,0.7,0.7	0.00	0.687	1.00	0.40	0.55	0.06	0.04862
	0.9,0.9,0.9	0.00	0.687	1.00	0.40	0.55	0.06	0.05930
8,8,8	0.2,0.3,0.4	0.00	0.676	1.00	0.37	0.57	0.06	0.02309
	0.4,0.3,0.2	0.00	0.697	1.00	0.41	0.53	0.06	0.02696
	0.3,0.2,0.4	0.00	0.682	1.00	0.43	0.51	0.06	0.02226
	0.2,0.4,0.3	0.00	0.682	1.00	0.35	0.60	0.05	0.02573
	0.3,0.4,0.2	0.00	0.692	1.00	0.37	0.58	0.05	0.02785
	0.4,0.2,0.3	0.00	0.692	1.00	0.44	0.49	0.06	0.02401
10,8,6	0.0,0.0,0.0	0.00	0.675	1.00	0.38	0.57	0.06	0.00758
	0.1,0.1,0.1	0.00	0.681	1.00	0.39	0.56	0.06	0.01334
	0.3,0.3,0.3	0.00	0.684	1.00	0.39	0.55	0.06	0.02483
	0.5,0.5,0.5	0.00	0.685	1.00	0.39	0.55	0.06	0.03631
	0.7,0.7,0.7	0.00	0.686	1.00	0.40	0.55	0.06	0.04778
	0.9,0.9,0.9	0.00	0.687	1.00	0.40	0.55	0.06	0.05926
10,8,6	0.2,0.3,0.4	0.00	0.674	1.00	0.37	0.58	0.06	0.02257
	0.4,0.3,0.2	0.00	0.694	1.00	0.41	0.53	0.06	0.02676
	0.2,0.4,0.3	0.00	0.678	1.00	0.34	0.61	0.05	0.02522
	0.3,0.4,0.2	0.00	0.689	1.00	0.37	0.58	0.05	0.02753
	0.3,0.2,0.4	0.00	0.680	1.00	0.42	0.52	0.06	0.02190
	0.4,0.2,0.3	0.00	0.689	1.00	0.44	0.50	0.06	0.02378

*Weibull model parameter a=0.133, b=0.272, γ=3.334 based on EGDE data set for prenatal death given by Krewski et al., 1996. (ED_{05} =0.6063)

Table 1b
Optimal Designs for the BMD Conditional on the Number of Implants m
for Prenatal Death due to Exposure to EG*

Number of Implants m(i)	Intralitter Correlation $\phi(i)$	Optimal Doses			Optimal Allocation			Var($\hat{ED}_{05}$)
		d_1	d_2	d_3	δ_1	δ_2	δ_3	
14,14,14	0.0,0.0,0.0	0.00	0.710	1.00	0.41	0.59	0.00	0.00290
	0.1,0.1,0.1	0.00	0.710	1.00	0.41	0.59	0.00	0.00667
	0.3,0.3,0.3	0.00	0.710	1.00	0.41	0.59	0.00	0.01422
	0.5,0.5,0.5	0.00	0.710	1.00	0.41	0.59	0.00	0.02176
	0.7,0.7,0.7	0.00	0.710	1.00	0.41	0.59	0.00	0.02930
	0.9,0.9,0.9	0.00	0.710	1.00	0.41	0.59	0.00	0.03685
14,14,14	0.2,0.3,0.4	0.00	0.710	1.00	0.38	0.62	0.00	0.01258
	0.4,0.3,0.2	0.00	0.712	1.00	0.44	0.56	0.00	0.01573
	0.3,0.2,0.4	0.00	0.710	1.00	0.45	0.55	0.00	0.01194
	0.2,0.4,0.3	0.00	0.710	1.00	0.35	0.65	0.00	0.01461
	0.3,0.4,0.2	0.00	0.710	1.00	0.39	0.61	0.00	0.01637
	0.4,0.2,0.3	0.00	0.710	1.00	0.48	0.52	0.00	0.01333
16,14,12	0.0,0.0,0.0	0.00	0.710	1.00	0.40	0.60	0.00	0.00275
	0.1,0.1,0.1	0.00	0.710	1.00	0.41	0.59	0.00	0.00654
	0.3,0.3,0.3	0.00	0.710	1.00	0.41	0.59	0.00	0.01411
	0.5,0.5,0.5	0.00	0.710	1.00	0.41	0.59	0.00	0.02168
	0.7,0.7,0.7	0.00	0.710	1.00	0.41	0.59	0.00	0.02926
	0.9,0.9,0.9	0.00	0.710	1.00	0.41	0.59	0.00	0.03683
16,14,12	0.2,0.3,0.4	0.00	0.710	1.00	0.38	0.63	0.00	0.01245
	0.4,0.3,0.2	0.00	0.710	1.00	0.41	0.53	0.00	0.01564
	0.2,0.4,0.3	0.00	0.710	1.00	0.34	0.61	0.00	0.01446
	0.3,0.4,0.2	0.00	0.710	1.00	0.37	0.58	0.00	0.01625
	0.3,0.2,0.4	0.00	0.710	1.00	0.42	0.52	0.00	0.01184
	0.4,0.2,0.3	0.00	0.710	1.00	0.44	0.50	0.00	0.01325

*Weibull model parameter a=0.055, b=0.183, γ=3.718 based on EG data set for prenatal death given by Krewski et al., 1996. (ED_{05} =0.710)

Table 2a
Efficiencies of Selected Three-dosed Designs for Estimating
the ED_{05} for the Compound EGDE

Endpoint	Design	Doses	Allocation	Response Probabilities	Efficiency of design
Malform | m[1]	1	0, 0.62, 1	0.30, 0.59, 0.11	0.061, 0.189, 0.532	1.000
	2	0, 0.62, 1	0.33, 0.33, 0.33	0.061, 0.189, 0.532	0.733
	3	0, 0.62, 1	0.25, 0.50, 0.25	0.061, 0.189, 0.532	0.910
	4	0, 0.46, 1	0.30, 0.59, 0.11	0.061, 0.108, 0.532	0.520
	5	0, 0.40, 1	0.30, 0.59, 0.11	0.061, 0.090, 0.532	0.303
	6	0, 0.50, 1	0.30, 0.59, 0.11	0.061, 0.122, 0.532	0.684
	7	0, 0.50, 1	0.33, 0.33, 0.33	0.061, 0.122, 0.532	0.501
Death[2]	1	0, 0.70, 1	0.41, 0.53, 0.06	0.125, 0.194, 0.333	1.000
	2	0, 0.70, 1	0.33, 0.33, 0.33	0.125, 0.194, 0.333	0.728
	3	0, 0.70, 1	0.25, 0.50, 0.25	0.125, 0.194, 0.333	0.808
	4	0, 0.61, 1	0.41, 0.53, 0.06	0.125, 0.169, 0.333	0.815
	5	0, 0.50, 1	0.41, 0.53, 0.06	0.125, 0.148, 0.333	0.427
	6	0, 0.75, 1	0.41, 0.53, 0.06	0.125, 0.211, 0.333	0.894
	7	0, 0.50, 1	0.33, 0.33, 0.33	0.125, 0.148, 0.333	0.307
Overall toxicity	1	0, 0.65, 1	0.33, 0.54, 0.13	0.178, 0.345, 0.688	1.000
	2	0, 0.65, 1	0.33, 0.33, 0.33	0.178, 0.345, 0.688	0.780
	3	0, 0.65, 1	0.25, 0.50 0.25	0.178, 0.345, 0.688	0.915
	4	0, 0.42, 1	0.33, 0.54, 0.13	0.178, 0.220, 0.688	0.293
	5	0, 0.35, 1	0.33, 0.54, 0.13	0.178, 0.201, 0.688	0.131
	6	0, 0.50, 1	0.33, 0.54, 0.13	0.178, 0.252, 0.688	0.571
	7	0, 0.50, 1	0.33, 0.33, 0.33	0.178, 0.252, 0.688	0.439
Malform | s[3]	1	0, 0.62, 1	0.31, 0.58, 0.11	0.057, 0.195, 0.558	1.000
	2	0, 0.62, 1	0.33, 0.33, 0.33	0.057, 0.195, 0.558	0.733
	3	0, 0.62, 1	0.25, 0.50, 0.25	0.057, 0.195, 0.558	0.897
	4	0, 0.44, 1	0.31, 0.58, 0.11	0.057, 0.104, 0.558	0.445
	5	0, 0.35, 1	0.31, 0.58, 0.11	0.057, 0.080, 0.558	0.177
	6	0, 0.50, 1	0.31, 0.58, 0.11	0.057, 0.128, 0.558	0.678
	7	0, 0.50, 1	0.33, 0.33, 0.33	0.057, 0.128, 0.558	0.500

[1] Weibull parameters for malformation conditional on m are: a_1=0.063, b_1=0.696, γ_1=3.368, ED_{05}=0.461.
[2] Weibull parameters for prenatal death conditional on m are: a_2=0.133, b_2=0.272, γ_2=3.334, ED_{05}=0.606.
[3] Weibull parameters for malformation conditional on s are: a=0.059, b=0.758, γ=0.568, ED_{05}=0.441.

Table 2b
Efficiencies of Selected Three-dosed Design for Estimating the ED_{05} for EG

Endpoint	Design	Doses	Allocation	Response Probabilities	Efficiency of design
Malform | m[1]	1	0, 0.31, 1	0.17, 0.75, 0.08	0.013, 0.098, 0.711	1.000
	2	0, 0.31, 1	0.33, 0.33, 0.33	0.013, 0.098, 0.711	0.543
	3	0, 0.31, 1	0.25, 0.50, 0.25	0.013, 0.098, 0.711	0.796
	4	0, 0.24, 1	0.17, 0.75, 0.08	0.013, 0.062, 0.711	0.812
	5	0, 0.20, 1	0.17, 0.75, 0.08	0.013, 0.046, 0.711	0.579
	6	0, 0.35 1	0.17, 0.75, 0.08	0.013, 0.092, 0.711	0.926
	7	0, 0.50, 1	0.33, 0.33, 0.33	0.019, 0.240, 0.711	0.369
Death[2]	1	0, 0.71, 1	0.27, 0.72, 0.01	0.054, 0.101, 0.212	1.000
	2	0, 0.71, 1	0.33, 0.33, 0.33	0.054, 0.101, 0.212	0.537
	3	0, 0.71, 1	0.25, 0.50, 0.25	0.054, 0.101, 0.212	0.743
	4	0, 0.71, 1	0.27, 0.72, 0.01	0.054, 0.101, 0.212	1.000
	5	0, 0.60, 1	0.27, 0.72, 0.01	0.054, 0.079, 0.212	0.349
	6	0, 0.75, 1	0.27, 0.72, 0.01	0.054, 0.126, 0.212	0.644
	7	0, 0.50, 1	0.33, 0.33, 0.33	0.054, 0.067, 0.212	0.270
Overall toxicity	1	0, 0.37, 1	0.18, 0.68, 0.14	0.066, 0.186, 0.772	1.000
	2	0, 0.37, 1	0.33, 0.33, 0.33	0.066, 0.186, 0.772	0.617
	3	0, 0.37, 1	0.25, 0.50, 0.25	0.066, 0.186, 0.772	0.879
	4	0, 0.24, 1	0.18, 0.68, 0.14	0.066, 0.113, 0.772	0.359
	5	0, 0.20, 1	0.18, 0.68, 0.14	0.066, 0.097, 0.772	0.153
	6	0, 0.35, 1	0.18, 0.68, 0.14	0.066, 0.173, 0.772	0.983
	7	0, 0.50, 1	0.33, 0.33, 0.33	0.066, 0.290, 0.772	0.470
Malform | s[3]	1	0, 0.32, 1	0.17, 0.75, 0.08	0.009, 0.107, 0.725	1.000
	2	0, 0.32, 1	0.33, 0.33, 0.33	0.009, 0.107, 0.725	0.554
	3	0, 0.32, 1	0.25, 0.50, 0.25	0.009, 0.107, 0.725	0.816
	4	0, 0.20, 1	0.17, 0.75, 0.08	0.009, 0.058, 0.725	0.771
	5	0, 0.40, 1	0.17, 0.75, 0.08	0.009, 0.045, 0.725	0.600
	6	0, 0.50, 1	0.17, 0.75, 0.08	0.009, 0.165, 0.725	0.824
	7	0, 0.50, 1	0.33, 0.33, 0.33	0.009, 0.251, 0.725	0.417

[1] Weibull parameters for malformation conditional on m are: a_1=0.013, b_1=1.228, γ_1=2.233, ED_{05}=0.241.
[2] Weibull parameters for prenatal death conditional on m are: a_2=0.055, b_2=0.183, γ_2=3.718, ED_{05}=0.710.
[3] Weibull parameters for malformation conditional on s are: a=0.0087, b=1.2822, γ=2.1960, ED_{05}=0.241.

Table 3

Efficiencies of Selected 4-dose designs for estimating the ED_{05}

Compound (endpoint)	Design	Doses	Allocation	Efficiency of design
EGDE	1	0, 0.25, 0.50, 1	0.25, 0.25, 0.25, 0.25	0.396
(malform | m)	2	0, 0.25, 0.50, 1	0.17, 0.33, 0.33, 0.17	0.446
	3	0, 0.63, 0.70, 1	0.25, 0.25, 0.25, 0.25	0.889
ED_{05} =0.46	4	0, 0.63, 0.70, 1	0.17, 0.33, 0.33, 0.17	0.860
EGDE	1	0, 0.25, 0.50, 1	0.25, 0.25, 0.25, 0.25	0.263
(death)	2	0, 0.25, 0.50, 1	0.17, 0.33, 0.33, 0.17	0.291
	3	0, 0.63, 0.70, 1	0.25, 0.25, 0.25, 0.25	0.734
ED_{05} =0.61	4	0, 0.63, 0.70, 1	0.17, 0.33, 0.33, 0.17	0.633
EGDE	1	0, 0.25, 0.50, 1	0.25, 0.25, 0.25, 0.25	0.361
(overall	2	0, 0.25, 0.50, 1	0.17, 0.33, 0.33 ,0.17	0.401
toxicity)	3	0, 0.63, 0.70, 1	0.25, 0.25, 0.25, 0.25	0.914
ED_{05}=0.42	4	0, 0.63, 0.70, 1	0.17, 0.33, 0.33, 0.17	0.827
EG	1	0, 0.25, 0.50, 1	0.25, 0.25, 0.25, 0.25	0.613
(malform | m)	2	0, 0.25, 0.50, 1	0.17, 0.33, 0.33, 0.17	0.688
	3	0, 0.33, 0.71, 1	0.25, 0.25, 0.25, 0.25	0.464
ED_{05} =0.24	4	0, 0.33, 0.71, 1	0.17, 0.33, 0.33, 0.17	0.532
EG	1	0, 0.25, 0.50, 1	0.25, 0.25, 0.25, 0.25	0.210
(death)	2	0, 0.25, 0.50, 1	0.17, 0.33, 0.33, 0.17	0.237
	3	0, 0.33, 0.71, 1	0.25, 0.25, 0.25, 0.25	0.417
ED_{05} =0.71	4	0, 0.33, 0.71, 1	0.17, 0.33, 0.33, 0.17	0.490
EG	1	0, 0.25, 0.50, 1	0.25, 0.25, 0.25, 0.25	0.787
(overall	2	0, 0.25, 0.50, 1	0.17, 0.33, 0.33, 0.17	0.894
toxicity)	3	0, 0.33, 0.71, 1	0.25, 0.25, 0.25, 0.25	0.606
ED_{05}=0.24	4	0, 0.33, 0.71, 1	0.17, 0.33, 0.33, 0.17	0.701

Table 4

Overview of Experimental Designs for 11 Developmental Toxicity Studies Conducted Under the U.S. National Toxicology Program

Study No.	Compound	Species	Dose Units	Dose Levels (dams per dose group)
1	Ethylene glycol (EG)	Mice	mg/kg	0, 750, 1500, 3000 (25, 24, 23, 23)
2	EG	Rats	mg/kg	0, 1250, 2500, 5000 (28, 28, 29, 27)
3	EG diethyl ether (EGDE)	Mice	mg/kg	0, 50, 150, 500, 1000 (23, 24, 22, 23, 23)
4	EGDE	Rabbits	mg/kg	0, 25, 50, 100 (26, 22, 24, 24)
5	Sulfamethezine (SM)	Rabbits	mg/kg	0, 600, 1200, 1500, 1800 (24, 26, 25, 28, 23)
6	SM	Rats	mg/kg	0, 545, 685, 865 (26, 22, 24, 24)
7	Nitrofurazone (NF)	Rabbits	mg/kg	0, 5, 10, 15, 20 (25, 23, 27, 22, 24)
8	Triethylene glycol dimethyl ether (TGDM)	Rabbits	mg/kg	0, 75, 125, 175, 250 (25, 22, 25, 23, 23)
9	Analine (2A)	Rats	mg/kg	0, 10, 30, 100, 200 (22, 21, 24, 22, 25)
10	Diethylhexalphthalate (DEPH)	Mice	% in diet	0, 0.025, 0.05, 0.1, 0.15 (30, 26, 26, 24, 25)
11	Diethylene glycol dimethyl ether (DYME)	Mice	mg/kg	0, 62.5, 125, 250, 500 (21, 20, 24, 23, 23)

Table 5
Optimal Designs for Estimating the ED_{05} for Prenatal Death, Fetal Malformation and Overall Toxicity Conditional on the Number of Implants

Study	Endpoint	Optimal Dose			Optimal Allocation			Effective Dose
		d_1	d_2	d_3	δ_1	δ_2	δ_3	ED_{05}
1	Prenatal Death	0	0.56	1	0.37	0.63	0.00	0.555
	Fetal Malformation	0	0.26	1	0.21	0.66	0.13	0.142
	Overall Toxicity	0	0.31	1	0.23	0.64	0.13	0.131
2	Prenatal Death	0	0.71	1	0.27	0.73	0.00	0.710
	Fetal Malformation	0	0.31	1	0.17	0.75	0.08	0.241
	Overall Toxicity	0	0.37	1	0.17	0.69	0.14	0.239
3	Prenatal Death	0	0.59	1	0.38	0.55	0.07	0.440
	Fetal Malformation	0	0.47	1	0.15	0.60	0.25	0.325
	Overall Toxicity	0	0.32	1	0.37	0.61	0.02	0.282
4	Prenatal Death	0	0.69	1	0.41	0.53	0.06	0.606
	Fetal Malformation	0	0.63	1	0.30	0.58	0.12	0.461
	Overall Toxicity	0	0.65	1	0.33	0.54	0.13	0.417
5	Prenatal Death	0	0.81	1	0.34	0.61	0.05	0.767
	Fetal Malformation	0	0.43	1	0.31	0.56	0.13	1.435
	Overall Toxicity	0	0.77	1	0.36	0.59	0.05	0.735
6	Prenatal Death	a			a			a
	Fetal Malformation	0	0.81	1	0.32	0.66	0.02	0.810
	Overall Toxicity	a			a			0.808

7	Prenatal Death	0	0.84	1	0.40	0.56	0.04	0.811
	Fetal Malformation	0	0.97	1	0.35	0.63	0.02	0.971
	Overall Toxicity	0	0.84	1	0.39	0.54	0.07	0.785
8	Prenatal Death	0	0.59	1	0.30	0.60	0.10	0.438
	Fetal Malformation	0	0.43	1	0.25	0.62	0.13	0.284
	Overall Toxicity	0	0.47	1	0.25	0.58	0.17	0.259
9	Prenatal Death	0	0.51	1	0.40	0.60	0.00	0.511
	Fetal Malformation	0	0.72	1	0.26	0.72	0.02	0.709
	Overall Toxicity	0	0.50	1	0.39	0.59	0.02	0.474
10	Prenatal Death	0	0.54	1	0.25	0.60	0.15	0.320
	Fetal Malformation	0	0.33	1	0.21	0.68	0.11	0.263
	Overall Toxicity	0	0.41	1	0.22	0.55	0.23	0.226
11	Prenatal Death	0	0.61	1	0.30	0.60	0.10	0.464
	Fetal Malformation	0	0.37	1	0.21	0.73	0.06	0.321
	Overall Toxicity	0	0.50	1	0.23	0.58	0.19	0.298
Average	Prenatal Death	0	0.64	1	0.34	0.60	0.06	
	Fetal Malformation	0	0.52	1	0.25	0.65	0.10	
	Overall Toxicity	0	0.51	1	0.29	0.59	0.12	

[a] An optimal design was not calculated because of the lack of a dose-response relationship for prenatal death.