Random Litter Size Procedures for Developmental Toxicity Studies

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Summary

This paper introduces procedures for analyzing data from a developmental study in which litter size is considered to be random. Many commonly used approaches condition on litter size when estimating marginal response rates. The effect of conditioning on litter size is explored while methods of estimating marginal response rates in the presence of intralitter correlation and random litter size are developed and compared. Procedures are discussed which allow for a test for dose effects on litter size as well as for binary endpoints.

Keywords: Correlated data; Dose-response; Litter size

1 Introduction

Developmental experiments are designed to measure toxic and developmental effects of hazardous materials. In a typical study, females are randomly assigned to dose groups and then exposed to fixed levels of a toxin. Effects such as fetal death, resorption, and malformation are then measured on the offspring as binary events. Typically, the proportional risk is measured with respect to number of implantation sites or number of live births, either of these quantities being termed litter size. Depending on properties of the toxic material and when exposure occurs, either of these quantities could be effected by the exposure, thus contributing to overall risk associated with the toxin.

Variation in random litter size will cause extra-variation in the data when compared to data with fixed litter size. In addition to the extra-variation due to the influence of litter size variation, the tendency for littermates to respond more similarly than non-littermates results in extra-variation as well. The litter effect is well documented in the literature (Donner, 1993; Kupper, *et al*, 1986; Ryan, 1992; and Williams,1975). Most procedures used for analyzing data of this type account for the overdispersion due to the litter effect but fail to consider the variation due to litter size. These procedures include the beta-binomial model (Williams, 1975), GEE procedures (Lipsitz, Laird and Harrington, 1991), and non-parametric procedures (Gladen, 1979).

There are two issues which arise when modeling data of this type in the presence of random litter sizes: the effect of conditioning on litter size in the estimation of mean response and the risk associated with a dose effect on the litter sizes. The former has a direct effect on the determination of adverse risk while the latter has implications in the study of infertility. Several authors have attempted to address the effects of litter size in a developmental study. Rai and Van Ryzin (1985) propose a model where risk is assumed to be a function of both litter size and dose level. However Rai and Van Ryzin do not provide for the correlation among litter mates in their analysis, nor do they provide a means for associating litter size with exposure level. Commenting on this procedure, Williams (1987) suggests a model incorporating the intralitter correlation while modeling response as a function of dose level and litter size, and also accounting for interaction between dose level and litter size. More recently,

Bowman and George (1995) construct maximum likelihood estimates of the parameters of a model based on an assumption of exchangeability between litter mates. This estimation procedure does not condition on litter size, but assumes a multinomial distribution for the litter sizes.

In this paper, procedures are given which may be used to test for dose effects on litter size; a semiparametric estimation procedure is introduced to estimate response rates for various parametric litter size assumptions, and a procedure is discussed for estimating the amount of over-dispersion in the data that is attributable to the variation in litter size.

Two procedures are used to test for dose effects in the litter size. The first method assumes the concept of the "hypothetical fetus" discussed by Dunson (1997). As applied to this procedure, it is assumed that there is a maximum litter size attainable by the species being tested. Then a balanced exchangeable binary model (George and Bowman, 1995) is used to model litter size as a function of dose.

The second test for dose effect makes use of GEE's to model jointly litter size and number of positive responses per litter as functions of dose. The approach is similar to that of Bowman, Chen, and George (1995). The procedure provides for the correlation between littermates and quantifies the proportion of over-dispersion in the data which is due to the variation in litter size.

Semiparametric estimates are derived from the saturated exchangeable binary model of Bowman and George (1995) by replacing the assumption of a multinomial litter size distribution with other parametric distributions. It is often difficult to specify a parametric distribution which provides adequate fit to the litter sizes. Rai and Van Ryzin (1985) use a Poisson distribution, but Williams (1987) points out that litter size is often underdispersed relative to the Poisson. McCaughran and Arnold (1976) fit several parametric models to litter sizes from developmental studies and conclude that many common discrete distributions do not model litter size well. One reason for the lack of fit is that litter sizes tend to cluster tightly about the mode. In addition, it is often the case that litter sizes appear bi-modal. Several models are investigated in this paper in an attempt to better model the litter sizes, including the exchangeable binary (EB) model previously discussed, truncated versions of binomial

and Poisson distributions, and simple mixtures of truncated distributions. Semiparametric estimates of mean response using the EB model are computed for each litter size model.

2 Generalized Estimating Equations

Consider an experiment with g dose levels, $d_1, \ldots, d_g$. At the ith dose level there are m_i females. In the jth litter of the ith dose group there are X_{ij} positive responses out of a litter of size N_{ij} for $j = 1, \ldots, m_i$ and $i = 1, \ldots, g$. Assume that the moments of the litter size random variable are given by $E(N_{ij}) = \lambda_i$ and $Var(N_{ij}) = \tau v(\lambda_i)$, where τ is an unknown constant and $v(\lambda_i)$ is a known function of λ_i. For example, if the N_{ij} follow a Poisson distribution, then $v(\lambda_i) = \lambda_i$ and $\tau = 1$.

Let random variable X_{ijk} be equal to 1 if the kth offspring in the jth litter of the ith dose group exhibits a positive response (death or malformation) and 0 otherwise for $k = 1, \ldots, N_{ij}$, $j = 1, \ldots, m_i$ and $i = 1, \ldots, g$. Assume

$$E(X_{ijk} \mid N_{ij} = n_{ij}) = \mu_i \tag{1}$$

$$Var(X_{ijk} \mid N_{ij} = n_{ij}) = \mu_i(1 - \mu_i) \tag{2}$$

$$Corr(X_{ijk}, X_{ijl} \mid N_{ij} = n_{ij}) = \phi_i. \tag{3}$$

Here μ_i is the conditional mean response at dose level i, and ϕ_i is the intralitter correlation. Let $X_{ij} = \sum_{k=1}^{N_{ij}} X_{ijk}$, then

$$E(X_{ij}) = E[E(X_{ij} \mid N_{ij} = n_{ij})] = \mu_i \lambda_i \tag{4}$$

and

$$Var(X_{ij}) = E(Var(X_{ij} \mid N_{ij} = n_{ij})) + Var(E(X_{ij} \mid N_{ij} = n_{ij})) \tag{5}$$

$$= \lambda_i \mu_i (1 - \mu_i) + \phi_i \mu_i (1 - \mu_i)(Var(N_{ij}) + \lambda_i^2 - \lambda_i) + \mu_i^2 Var(N_{ij}) \tag{6}$$

To determine the effect of conditioning on litter size in the variance of X_{ij}, note that the extra term in $Var(X_{ij})$ due to the randomness of N_{ij} is given by

$$(\phi_i \mu_i (1 - \mu_i) + \mu_i^2) Var(N_{ij}), \tag{7}$$

which is positive if ϕ_i is greater than or equal to zero.

To model both the number of positive responses and the litter size using generalized estimating equations, let $\boldsymbol{X} = (X_{11}, \ldots, X_{gm_g})$ be the random vector of the sum of positive responses per litter. Let

$$E(\boldsymbol{X}) = \boldsymbol{\theta} = (\mu_1\lambda_1, \ldots, \mu_g\lambda_g), \tag{8}$$

$$\boldsymbol{V_X} = Cov(\boldsymbol{X}) = diag(Var(X_{11}), \ldots, Var(X_{gm_g})), \tag{9}$$

Where $Var(X_{ij})$ is as given in (6). We relate the mean response to exposure level by assuming a dose response function on the parameter $\boldsymbol{\theta}$, the vector of unconditional mean response. Assuming a link function for $\boldsymbol{\theta}$ with parameters $\boldsymbol{\beta}$, let $\boldsymbol{D_X}$ be the matrix whose ij element is $\frac{\partial\theta_i}{\partial\beta_j}$. An estimating equation for $\boldsymbol{\beta}$ is given by

$$\boldsymbol{U_X}(\boldsymbol{X}, \boldsymbol{\theta}) = \boldsymbol{D'_X}\boldsymbol{V_X^{-1}}(\boldsymbol{X} - \boldsymbol{\theta}) = \boldsymbol{0}. \tag{10}$$

The asymptotic variance of estimates of $\boldsymbol{\theta}$ is given by

$$V(\boldsymbol{\theta}) = (\boldsymbol{D'_X}\boldsymbol{V_X^{-1}}\boldsymbol{D_X})^{-1}. \tag{11}$$

A similar estimating equation for $\boldsymbol{\lambda}$, the vector of mean litter size, is developed by letting $\boldsymbol{N} = (N_{11}, \ldots, N_{gm_g})$ be the random vector of litter size. Then the mean vector and covariance matrix of $\boldsymbol{N}$ are given by

$$E(\boldsymbol{N}) = \boldsymbol{\lambda} = (\lambda_1, \ldots, \lambda_g) \tag{12}$$

$$Var(\boldsymbol{N}) = \boldsymbol{V_N}. \tag{13}$$

where $\boldsymbol{V_N} = diag(Var(N_{11}), \ldots, Var(N_{gm_g}))$. This is similar to the moment structure assumed for $\boldsymbol{N}$ by Kim and Taylor (1994). Assume some link function for $\boldsymbol{\lambda}$ with parameters $\boldsymbol{\alpha}$ linking litter size with dose. Let $\boldsymbol{D_N}$ be the matrix whose ij element is given by $\frac{\partial\lambda_i}{\partial\alpha_j}$. An estimating equation for $\boldsymbol{\alpha}$ may be written as

$$\boldsymbol{U_N}(\boldsymbol{N}, \boldsymbol{\alpha}) = \boldsymbol{D'_N}\boldsymbol{V_N^{-1}}(\boldsymbol{N} - \boldsymbol{\lambda}) = \boldsymbol{0}. \tag{14}$$

The variance of the estimates of $\boldsymbol{\alpha}$ is given by

$$V(\boldsymbol{\lambda}) = (\boldsymbol{D'_N}\boldsymbol{V_N^{-1}}\boldsymbol{D_N})^{-1}. \tag{15}$$

Estimates for $\boldsymbol{\beta}$ and $\boldsymbol{\alpha}$ may be obtaining using an alternating Newton-Rhapson iterative procedure by starting with initial estimates of β_0 and α_0. Conditioning on α_0, a new $\hat{\beta}$ is obtained. Conditioning on the most recent estimate of β, a new $\hat{\alpha}$ is obtained. This process is repeated until both estimates are within tolerance. The intralitter correlation in the ith dose group ϕ_i is estimated with a moment estimator within each iteration.

3 Semi-Parametric Estimates of Mean Response

Bowman and George (1995) develop non-parametric estimates of mean response using an exchangeable binary model. In general let $X_1, \ldots, X_n$ be a set of exchangeable binary random variables, so that $X_i = 1$ with probability λ_1 and $X_i = 0$ with probability $1 - \lambda_1$, where $\lambda_k = P(X_1 = 1, \ldots, X_k = 1)$. Since the X_i's are exchangeable, the probability λ_k will be the same for any set of k of the X_i's. Now let $Y = \sum_{i=1}^{n} X_i$. Then

$$P(Y = r) = \binom{n}{r} \sum_{j=0}^{n-r} (-1)^j \binom{n-r}{j} \lambda_{r+j}. \tag{16}$$

This is the exchangeable binary (EB) model discussed by George and Bowman (1995). Now suppose the data consist of M clusters of exchangeable random variables where cluster size is random . In this case, the parameters λ_r can be written as

$$\lambda_r = \sum_{n=r}^{K} \sum_{j=0}^{n-r} \binom{n-r}{j} p_{(n-j,n)} q_n, \tag{17}$$

where q_n is the probability that cluster size equals n, K is the maximum possible cluster size, and

$$p_{(r,n)} = P(X_1 = 1, \ldots, X_r = 1, X_{r+1} = 0, \ldots, X_n = 0 \mid clustersize = n). \tag{18}$$

Using this representation, Bowman and George obtain the following expression for non-parametric estimates of λ_r,

$$\hat{\lambda}_r = \frac{1}{M} \sum_{n=r}^{K} \sum_{j=0}^{n-r} \frac{\binom{n-r}{j}}{\binom{n}{j}} A_{n-j,n}, \tag{19}$$

where $A_{r,n}$ is the number of clusters of size n which have r positive responses. If we define m_n to be the number of clusters having cluster size equal to n, then the non-parametric estimates

given in equation 19 are based on the assumption that $(m_1, \ldots, m_K)$ follow a multinomial distribution with parameters $(M; q_1, \ldots, q_K)$ and that given m_n, $A_{r,n}$ for $r = 0, \ldots, n$ follow a multinomial distribution with parameters $(m_n; p_{0,n}, \binom{n}{1}p_{1,n}, \ldots, p_{n,n})$.

To extend the results of Bowman and George (1995), semi-parametric estimates of the marginal probabilities may be obtained by altering the assumption of a multinomial distribution for cluster size. Instead, assume that cluster size N is a random variable with a specified probability distribution $f(n)$ for $n = 1, \ldots, K$. Then a semi-parametric estimate of λ_r is given by

$$\hat{\lambda}_r = \sum_{n=r}^{K} \sum_{j=0}^{n-r} \frac{\binom{n-r}{j}}{\binom{n}{j}} \frac{A_{n-j,n}}{m_n} \hat{f}(n), \tag{20}$$

where $\hat{f}(n)$ is the estimated value of the assumed probability distribution at $N = n$.

In general the estimated variance of the semi-parametric estimates has the form

$$\begin{aligned}
\hat{Var}(\hat{\lambda}_r) = & \\
& \sum_{n=r}^{K} \sum_{j=0}^{n-r} \frac{\binom{n-r}{j}^2}{\binom{n}{j}^2} \frac{A_{n-j,n}^2}{{m_n}^2} \hat{Var}(\hat{f}_n) + \\
& \underbrace{\sum_{n=r}^{K} \sum_{l=r}^{K}}_{n \neq l} \hat{Cov}(\hat{f}(n), \hat{f}(l)) \left(\sum_{j=0}^{n-r} \frac{\binom{n-r}{j}}{\binom{n}{j}} \frac{A_{n-j,n}}{m_n} \right) \left(\sum_{j=0}^{l-r} \frac{\binom{l-r}{j}}{\binom{l}{j}} \frac{A_{l-j,l}}{m_l} \right) + \\
& \sum_{n=r}^{K} \hat{f}(n) \left(\sum_{j=0}^{n-r} \frac{\binom{n-r}{j}^2}{\binom{n}{j}^2} \frac{A_{n-j,n}}{m_n} - \left(\sum_{j=0}^{n-r} \frac{\binom{n-r}{j}}{\binom{n}{j}} \frac{A_{n-j,n}}{m_n} \right)^2 \right).
\end{aligned} \tag{21}$$

The covariance between semi-parametric estimates is given by

$$\begin{aligned}
\hat{Cov}(\hat{\lambda}_r, \hat{\lambda}_l) = & \\
& \frac{-1}{M} \sum_{n=r}^{K} \frac{f(n)}{m_n^2} \sum_{j=0}^{n-r} \sum_{i=0}^{n-l} \frac{\binom{n-r}{j}}{\binom{n}{j}} \frac{\binom{n-l}{i}}{\binom{n}{i}} A_{n-j,n} A_{n-i,n} + \\
& \sum_{n=r}^{K} \sum_{n'=l}^{K} \frac{Cov(\hat{f}(n), \hat{f}(n'))}{m_n m_{n'}} \left(\sum_{j=0}^{n-r} \frac{\binom{n-r}{j}}{\binom{n}{j}} A_{n-j,n} \right) \left(\sum_{i=0}^{n'-l} \frac{\binom{n'-l}{i}}{\binom{n'}{i}} A_{n'-i,n'} \right).
\end{aligned} \tag{22}$$

4 Litter Size Distributions

While the semi-parametric estimates developed in the previous section are applicable to any clustered exchangeable random variables, the focus of this paper is the random litter sizes which appear in developmental studies. As previously mentioned, several authors have discussed modeling litter size. Rai and Van Ryzin (1985) use a Poisson distribution to model litter size, while Williams (1987) states that often litter size data is underdispersed relative to the Poisson. McCaughran and Arnold (1976) attempt to fit several litter size models and conclude that none of the common distributions tried consistently provide adequate fit to litter size data. The poor fit may be atributed to two characteristics of random litter size which make it difficult to model. First, the data seem to cluster tightly about the mode, resulting in underdispersion relative to many common distributions, and in addition, the data often appear to be bi-modal.

In order to obtain semi-parametric estimates of mean response, it first becomes necessary to adequately model the litter size to obtain good estimates of $f(n)$. Three different types of distributions are discussed here. First to address the issue of clustering about the mode, truncated Poisson and binomial distributions are considered. The issue of bi-modality is addressed by considering mixtures of truncated binomial and Poisson distributions. A third approach is suggested by modeling litter size with a parametric exchangeable binary model. (George and Bowman, 1995).

4.1 Truncated distributions

Let N be the random variable of litter size. Define random variables U_x and U_n to be the maximum and minimum possible litter sizes under consideration respectively. These parameters will typically be unknown and must be estimated from the data.

4.1.1 Truncated Binomial

The likelihood for the truncated binomial distribution is given by

$$P(N=n) = f(n; U_x, U_n, p) = \frac{\binom{U_x}{n} p^n (1-p)^{U_x - n}}{1 - F(U_n - 1)} \quad n = U_n, \ldots, U_x. \tag{23}$$

where $F(x) = \sum_{i=0}^{x} \binom{U_x}{i} p^i (1-p)^{U_x - i}$. Let $N_1, \ldots, N_m$ be the sample of observed litter sizes

and $N_{(1)} \leq \ldots \leq N_{(m)}$ be the ordered sample values. Estimate U_n by $N_{(1)}$ and U_x by $N_{(m)}$. Conditioned on these values a maximum likelihood estimate of p may be obtained using an iterative procedure.

4.1.2 Truncated Poisson

The likelihood for the truncated Poisson model is given by

$$P(N = n) = f(n; U_x, U_n, \lambda) = \frac{\lambda^n e^{-\lambda}}{n! \left[F(U_x) - F(U_n)\right]} \quad n = U_n, \ldots, U_x, \tag{24}$$

where $F(x) = \sum_{i=0}^{x} \frac{\lambda^i e^{-\lambda}}{i!}$. As with the truncated binomial, parameters U_x and U_n are estimated by $N_{(m)}$ and $N_{(1)}$ respectively, and λ is estimated by maximum likelihood using an iterative procedure.

4.2 Mixture of Truncated Models

One method of improving the model fit in certain situations is to consider mixture models. Let $\pi_1(n; \theta_1)$ and $\pi_2(n; \theta_2)$ be two distributions for litter size N indexed by parameters θ_1 and θ_2 respectively. Define U_{x_1} and U_{n_1} to be the maximum and minimum values that litter size can take in the range covered by π_1. Similarly define U_{x_2} and U_{n_2}. Let

$$F_i(x; \theta_i) = \sum_{j=0}^{x} \pi_i(j; \theta_i). \tag{25}$$

Then a general model for the mixture of two truncated distributions with mixing probability α is

$$f(n) = \frac{\alpha \pi_1(n; \theta_1)}{F_1(U_{x_1}; \theta_1) - F_1(U_{n_1} - 1; \theta_1)} + \frac{(1 - \alpha)\pi_2(n; \theta_2)}{F_2(U_{x_2}; \theta_2) - F_2(U_{n_2} - 1; \theta_2)} \tag{26}$$

As simplifying assumptions, let $U_x = U_{x_1} = U_{x_2}$ and $U_n = U_{n_1} = U_{n_2}$, so that both π_1 and π_2 are non-zero in the same range. The parameters U_x and U_n may be estimated as before by $N_{(m)}$ and $N_{(1)}$ respectively. As a further simplification, assume that π_1 and π_2 are from the same family of distributions.

4.2.1 Mixture of Truncated Poissons

If both π_1 and π_2 are Poisson distributions indexed by parameters θ_1 and θ_2 respectively, then conditioned on the maximum and minimum litter sizes, the mixture distribution becomes

$$f(n; \alpha, \theta_1, \theta_2) = \alpha \left(\frac{\theta_1^n e^{-\theta_1}}{n! \left[F_1(U_x) - F_1(U_n - 1)\right]} \right) + (1 - \alpha) \left(\frac{\theta_2^n e^{-\theta_2}}{n! \left[F_2(U_x) - F_2(U_n - 1)\right]} \right). \tag{27}$$

for $F_j(x) = \sum_{i=0}^{x} \frac{\theta_j^i e^{-\theta_j}}{i!}$. Since the purpose of the mixture model is to better account for the effects of bi-modality, reasonable estimates of θ_1 and θ_2 may be obtained by using the two largest sample modes. Specifically, if the two largest modes occur in the data at points n_1 and n_2, then θ_1 may be estimated by $\frac{n_1+1}{2}$ and θ_2 may be estimated by $\frac{n_2+1}{2}$. In the event that one mode occurs at adjacent data points n_i and $n_i + 1$, then θ_i is estimated by $n_i + 1$.

To estimate α, let m_1 be the number of litters having size equal to the first mode n_1 and m_2 be the number of litters having size equal to n_2. Then let $\hat{\alpha} = \frac{m_1}{m_1+m_2}$. Note that if there is only one mode in the sample, then $\hat{\alpha}$ becomes equal to 1 and the mixture simplifies to the truncated Poisson model discussed in Section 4.1.1.

4.2.2 Mixture of Truncated Binomials

The corresponding distribution conditioned on maximum and minimum litter size when π_1 and π_2 are both binomial distributions is

$$f(n : \alpha, \theta_1, \theta_2) = \alpha \left(\frac{\binom{U_x}{n} \theta_1^n (1-\theta_1)^{U_x - n}}{1 - F_1(U_n - 1)} \right) + (1-\alpha) \left(\frac{\binom{U_x}{n} \theta_2^n (1-\theta_2)^{U_x - n}}{1 - F_2(U_n - 1)} \right). \qquad (28)$$

where $F_j(x) = \sum_{i=0}^{x} \binom{U_x}{i} \theta_j^i (1-\theta_j)^{U_x - i}$. Reasonable estimates for θ_1 and θ_2 may be obtained as with the Poisson model by estimating θ_1 and θ_2 so that sample modes correspond with the modes of π_1 and π_2. Specifically, if modes occur in the data at litter sizes n_1 and n_2, estimate θ_1 and θ_2 respectively by $\frac{n_1}{U_x}$ and $\frac{n_2}{U_x}$ unless both n_i and $n_i + 1$ are modes, in which case estimate θ_i by $\frac{n_i+n_i+1}{U_x}$. The mixing probability α is estimated as before by $\frac{m_1}{m_1+m_2}$.

In both mixture models it is difficult to obtain variances of the parameter estimates. Since estimates of the variance and covariances of the estimates $\hat{f}(n)$ are required to obtain the variance of semi-parametric estimates of mean response, these values may be easily estimated using a bootstrap procedure.

4.3 Exchangeable Binary Model

Suppose that for an animal under consideration there exists a hypothetical maximum litter size K as previously discussed. Then let the individual random variables $X_{i1}, \ldots, X_{i,K}$ of a hypothetical litter be defined as $X_{ij} = 1$ if the jth pup is implanted and 0 otherwise. Then $\sum_{j=1}^{K} X_{ij}$ is equal to the ith litter size N_i. Further, $X_{i1}, \ldots, X_{i,K}$ form a set of exchangeable

binary random variables. For these random variables, define

$$\lambda_k = P(X_{i1} = 1, \ldots, X_{ik} = 1). \tag{29}$$

The probability of observing a litter size n_i may then be found using the exchangeable binary (EB) model given in Section 3. The parameters λ_k of the EB model may be modeled as functions of covariates, including dose, using the parametric procedure discussed in George and Bowman (1995). George and Bowman (1995) use the folded logistic model,

$$\lambda_k = \frac{2}{1 + (k+1)^{\beta_1 + \beta_2 d_i}}. \tag{30}$$

where d_i is dose level in the ith dose group. The parameters β_1 and β_2 may be estimated by maximum likelihood using an iterative procedure.

Modeling litter size using an EB model with dose covariates exploits the exchangeability of animals within the hypothetical litter, and further provides a means for testing for a dose effect on litter size. A test of $\beta_2 = 0$ in the above model is equivalent to a test for a dose effect on litter size.

5 Illustration

The procedures discussed in previous sections for estimating mean response in developmental studies when litter size is random are illustrated using results from a developmental toxicity study in mice conducted by the National Toxicology Program. The experiment involved the chemical triethylene glycol dimethyl ether (TGDM) (George et al., 1987) and consisted of a control group and three dose groups where outcomes of fetal death, malformations, and fetal weight were measured. For illustrative purposes we consider only the outcome of fetal death here. In this case, the random variable we will call litter size is equal to the number of implantation sites. Table 1 summarizes the incidence of fetal death and the litter size distribution (number of implantations) for the TGDM data.

5.1 Generalized Estimating Equations

In order to illustrate the GEE procedure developed in Section 2, two different variance functions are assumed and compared for the litter size distribution. Following Rai and Van

Ryzin (1985), a Poisson variance function is assumed. Recall N_{ij} is the litter size in the jth litter of the ith dose group and X_{ij} is the number of positive responses there. Then with Poisson variance, $Var(N_{ij}) = \lambda_i$ and $Var(X_{ij}) = \lambda_i\mu_i + \phi_i\lambda_i^2(1-\mu_i)$, and the extra variation due to litter size, hereafter labeled $ExVar$, is given by $ExVar = [\phi_i\mu_i(1-\mu_i) + \mu_i^2]\,\lambda_i$.

The results of McCaughran and Arnold (1976) and Williams (1987) previously mentioned suggest that the Poisson variance may be too large in some cases. In light of this, a second variance function, log-Poisson, is considered. In this case the $Var(N_{ij}) = log(\lambda_i)$ and $Var(X_{ij}) = \lambda_i\mu_i(1-\mu_i)\,[1+\phi_i(\lambda_i-1)] + ExVar$ for $ExVar = [\mu_i^2 + \phi_i\mu_i(1-\mu_i)]\,log(\lambda_i)$.

An exponential link function $\lambda_i = e^{\alpha_1+\alpha_2 d_i}$ is assumed for the expected litter size, and a logistic link function $log\left(\frac{\theta_i}{1-\theta_i}\right) = \beta_1 + \beta_2 d_i$ is assumed for the expected number of mean responses. The dosages are scales by a factor of 1/100 in the estimation procedure.

Using the generalized estimating equations derived in Section 2, estimates of $\boldsymbol{\alpha}$ and $\boldsymbol{\beta}$ were obtained. The two variance assumptions for the litter size had little effect on the estimates of these parameters, the only difference being in their variances. The estimates obtained were $\hat{\beta}_1$ = -2.6994, $\hat{\beta}_2$ = .07919. The standard error of these estimates for Poisson variation were .1967 and .0432, and for log-Poisson variation were .1911 and .0416. The estimates for $\boldsymbol{\alpha}$ for both variance assumptions were $\hat{\alpha}_1$ = 2.4918 (.04316) and $\hat{\alpha}_2$ = -.00587 (.00755). The standard errors (given in parenthesis) were the same for both variance assumptions. Pearson residuals were computed for each variance assumption to determine which provided better fit to the data. For the number of positive responses, the Pearson residual $(\frac{(X-\theta)}{Var(X)})$ for the assumption of Poisson variation in litter size was 109.77, and the corresponding value for log-poisson variation was 117.37. The number of litters in the study was 107, hence both models fit the positive responses relatively well. This suggests that estimation of mean response may be robust to the misspecification of the variance of the litter size distribution. The Pearson residuals for the litter size itself were 68.25 for Poisson variation and 325.81 for log-Poisson. It is clear that the log-Poisson model does not fit the data. The resulting estimates of mean response are shown in Table 4, and estimates of the intralitter correlation are compared with the other procedures in Table 5.

A test for dose effect on the death rate is equivalent to a test of $\beta_2 = 0$. For this data, there

is a significant dose effect on death. A test for a dose effect on litter size is conducted by testing $\alpha_2 = 0$. Using the asymptotic normality of these estimates, the test is not significant, indicating that for this data there is no effect of dose on littersize.

Table 2 gives values of the variance of X_{ij} and values of $ExVar$, the extra-variation attributable to the variation in litter size. Table 2 also shows the percentage of the extra-variation (over what we expect from a binomial distribution with fixed litter size) which is attributable to litter size for both variance assumptions (Poisson and log-Poisson). This table demonstrates that the proportion of the total extra-variation which is due to variation in litter size depends upon the assumed form of $Var(N_{ij})$. The results also indicate that a large portion of the overdispersion may in fact be due to variation inherent in the litter sizes rather than solely due to the intralitter correlation.

5.2 Semi-Parametric Estimates

The procedures developed in Section 3 are illustrated for the TGDM data. The random litter size for this data is number of implantation sites and the positive response of interest, as before, is fetal death. Before the semi-parametric estimates are computed, it is necessary to fit the litter size distribution with an appropriate model.

5.2.1 Litter Size Distributions

Each of the distributions for random litter size discussed in Section 3 are fit to the TGDM data. For the exchangeable model, a folded logistic function is used to model litter size as a function of dose. In addition, the dosages are scaled so that the modeled dose is equal to $log(1+dose/100)$. Table 3 shows the resulting loglikelihood values and chi-square numbers for each litter size distribution, as well as for non-truncated Poisson and binomial distributions for comparison. This table shows that the loglikelihood values are very similar for all of the models, with a big gain occurring in the binomial model when a mixture is assumed and a big gain occurring in the Poisson family when the distribution is truncated. The smallest loglikelihood occurs for the parametric exchangeable binary model, which is not surprising as this is the only one which uses a single model for all dose levels.

The total number of litters in this study is 107. Comparing this to the chi-square values, it is

apparent that only the mixture models and the parametric EB model provide adequate fit to the TGDM litter size data. Both mixture models fit the data better than the GEE procedures discussed in the previous section. The EB model may be preferred in this situation since it also provides a means of testing for dose effect on litter size. For the TGDM data, the estimate of the parameter relating marginal response to dose level was 0.1829 with a standard deviation of 0.2584. In agreement with the GEE results, dose apparently has no effect on litter size for this data, although the observed mean litter size decreases with dose.

5.2.2 Semi-Parametric estimates

The semi-parametric estimates of Section 3 for death rate were computed for the TGDM data using each of the litter size distributions previously discussed. Table 4 shows the resulting estimate of λ_1, the mean response rate for death, for each dose level and for each assumption about the litter size distribution. Included in the table for comparison are the non-parametric estimates of Bowman and George (1995) where litter size is assumed to follow a multinomial distribution, the GEE estimates, obtained in the previous section for the assumption of Poisson variance, and the observed death rates. The estimates of mean response λ_1 are seen to vary with the litter size assumption, especially in the control group and the third dose group. The GEE estimates are the only ones which increase monotonically with dose since the GEE estimates use a parametric model for all dose groups while separate estimates are computed within each dose group for the semi-parametric procedure.

Table 5 shows the estimated values of the intralitter correlation parameter ϕ_i for each litter size distribution, including the GEE estimates. The estimation of ϕ also seems to depend on the method used, whether semi-parametric or GEE, and on the litter size model, especially in the lower dose groups. One drawback of the semi-parametric estimates is the simplifying assumptions necessary to obtain estimates of the variance of the estimates; namely assuming that the expected value of $\frac{1}{\hat{f}(n)}$ is approximately equal to $\frac{1}{E(\hat{f}(n))}$, can result in some estimates of the variance of the correlation being negative. This is also a problem for the variance of the correlation estimates for the non-parametric estimation procedure of Bowman and George (1995).

Table 6 compares actual vs expected numbers for both types of procedures and all litter

assumptions. From this table it is clear that for an appropriate choice for the litter size distribution, the semi-parametric estimates provide improved estimation over the non parametric procedure. The GEE estimates as well appear to give good results in terms of expected numbers of deaths for this data.

6 Discussion

The methods developed here for incorporation of random litter size into the estimation of mean response provide means for testing for a dose effect on litter sizes. Such a test is particularly useful in evaluating effects of toxins on fertility. In some developmental studies, the random variable of litter size is measured as implantation sites while other studies measure live births. Particularly in studies involving live births, there is often a significant dose effect on litter size. In studies where implantation sites are measured, there is often an observed reduction in average litter size with increasing dose as with the TGDM data illustrated here, but this effect is often not significant using the procedures given. The extent to which litter size is effected by exposure is also related to the point at which exposure occurs - whether before or after implantation.

The generalized estimating equations derived in Section 2 also provide a means of gauging the effect of conditioning on litter size in the estimation of mean response. In many experiments a sizeable portion of the observed overdispersion in the data is due to variation in litter size rather than in intralitter correlation as shown in Table 2 for the TGDM data. Comparison of estimates of the extra variation due to litter size and correlation show an inverse relationship. Most of the extra variation appears to be accounted for by the correlation parameter when the litter size is assumed to be non-random. This affects the estimate of the variance of the number of positive responses per litter. Table 5 shows that the choice of litter size distribution may also have a great effect on estimates of mean response, particularly in low doses where interest is typically focused. The semi-parametric estimates in general tend to be more efficient than the non-parametric estimates of Bowman and George (1995) except when there is a very poor fit between the observed values and the assumed litter size distribution.

The semi-parametric estimates developed in Section 3 may be particularly useful when ob-

served response rates are not monotonic. In these situations it is often difficult to obtain good fit to the data with simple parametric models. However the semi-parametric estimates do not suffer this limitation.

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Table 1. Frequency Distribution of Mice According to the Number of Implantation Sites and Fetal Deaths Following Exposure to TGDM, George, et.al., 1987

Dose	No. Dead	No. Implantation Sites																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
0	0											4	5	2	2			
	1	1											2	1		2	2	
	2													2	1	1		
	3												1				1	
250	0									1	1	3	2	3	1			
	1										2	3	4		2			
	2				1					1		1						
	4														1			
500	0					1						2	1	4	2	1		
	1						1				1	1	2	2				
	2										1		1		1	1	1	
	3			1	1													
	11											1						
1000	0				1			1			1	3	4	2		2		
	1										1	2	3					
	2													2		1		
	3													1				
	4												1					1
	7							1										
	11											1						

Table 2. Summary of Extra Variation Due to Litter Size

Poisson Variance Dose Group	Binomial Variance	$\hat{Var}(X_{ij})$	Total Extra Variation	$\hat{ExVar}$	Percent Due to ExVar
1	0.7135	0.8945	0.1810	0.7245	100%
2	0.8338	0.8566	0.0228	0.8299	100%
3	0.9689	4.7331	3.7642	1.2814	34%
4	1.2818	6.0345	4.7527	1.6823	35%
log-Poisson Variance 1	0.7134	0.8477	0.1342	0.0122	9%
2	0.8338	0.8054	-.0284	0.0134	–
3	0.9691	4.4097	3.4406	0.0859	2%
4	1.2825	5.5699	4.2873	0.1261	3%

Table 3. Loglikelihood and Chi-Square Values for Implantation Site Distribution fit for TGDM Data

	Binomial	Truncated Binomial	Mixture Trunc. Bin.	Poisson	Truncated Poisson	Mixture Trunc. Poiss.	Parametric EB
LLH	-276	-276	-240	-270	-252	-251	-280
χ^2	4.1 E7	4.1 E7	94	991	3735	70	120

Table 4. Semi-Parametric and GEE estimates of Mean Response

Estimation	Dose Group			
Procedure	1	2	3	4
Observed Proportions	.0645	.0709	.1149	.1254
Non-Parametric	.1271 (.0711)	.0823 (.0684)	.1614 (.1763)	.1347 (.2271)
GEE	.0630 (.0124)	.0758 (.0538)	.0908 (.1271)	.1292 (.3615)
Semiparametric Estimates				
Truncated Binomial	.0803 (.0654)	.0547 (.6586)	.1084 (.2706)	.0861 (.7596)
Mixture Binomial	.1374 (.2829)	.0740 (.2125)	.1370 (.3406)	.0889 (.2600)
Truncated Poisson	.0750 (.0530)	.0572 (.0944)	.0944 (.1558)	.0866 (.1720)
Mixture Poisson	.1066 (.0490)	.0671 (.0613)	.1370 (.1520)	.0887 (.1769)
Exchangeable	.0606 (.0045)	.0759 (.0034)	.1691 (.0060)	.0785 (.0039)

Table 5. Semi-parametric and GEE Estimates of Correlation

Dose Group	Non Parametric	GEE	Truncated Binomial	Mixture Binomial	Truncated Poisson	Mixture Poisson	Exchangeable
1	.1664	.0154	.3341	.1402	.2817	.1445	.2314
2	.0523	-.0046	-.0089	.0711	.0317	.0984	.1798
3	.5369	.3225	.4443	.5171	.3545	.4790	.6384
4	.5210	.3121	.4438	.5410	.4983	.5644	.5116

Table 6. Actual and Expected Number of Deaths for Semi-Parametric and GEE Estimates

Estimation Procedure	Dose Group 1	2	3	4	Total
Actual	22	21	34	41	118
Non Parametric	43.35	24.35	47.77	44.05	159.52
GEE	21.49	22.42	26.89	42.27	113.07
Semiparametric					
Truncated Binomial	27.37	16.19	32.10	28.16	103.82
Mixture Binomial	46.86	21.92	41.36	29.08	139.22
Truncated Poisson	25.55	16.94	27.94	28.31	98.74
Mixture Poisson	36.35	19.87	40.55	28.99	125.76
Exchangeable	20.68	22.47	50.07	25.66	118.88