Hierarchical Modelling Approach for Risk Assessment in Developmental Toxicity Studies.

C. Faes[1], H. Geys[1], M. Aerts[1] and G. Molenberghs[1]

[1] Center for Statistics, Limburgs Universitair Centrum, Universitaire Campus, B-3590 Diepenbeek, Belgium. e-mail: christel.faes@luc.ac.be

Abstract: Within the past decade, there has been an increasing interest in the problem of joint analysis of clustered multiple outcome data, motivated by developmental toxicity applications (Fitzmaurice and Laird 1995, Gueorguieva and Agresti 2001, Molenberghs and Ryan 1999, Regan and Catalano 1999, Aerts et al. 2002). So far however, one has tackled the challenges in this setting only partly each time making different restricting assumptions (e.g. restriction to viable fetuses only). Ideally, a model should take the complete correlated hierarchical structure of the data into account. A hierarchical bayesian method will be discussed in this context. Once a suitable model is selected, it can serve as basis for quantitative risk assessment.

Keywords: Toxicology, Benchmark Dose, Hierarchical Model

1 Introduction

Lately, society has become increasingly concerned about problems related to fertility and pregnancy, birth defects and developmental abnormalities. Questions are raised about the potential risk of chemical compounds and other environmental agents on the development of fetuses. Consequently, regulatory agencies such as the U.S. Environmental Protection Agency and the Food and Drug Administration have given increased priority to reproductive and developmental toxicity research, in order to investigate the causes of these problems and to assess the potential adverse effects of exposure on the developing fetuses.
However, because of ethical reasons, reliable epidemiological information of adverse effects on fetal development may often be limited or unavailable. As an alternative, laboratory experiments in small mammalian species can be conducted in advance of human exposure (Williams and Ryan 1996). In developmental toxicity studies with a Segment II design, pregnant animals are exposed during the period of major organogenesis and structural development to a compound of interest. Dose levels for this design typically consist of a control group and three or four exposed groups, each with 20

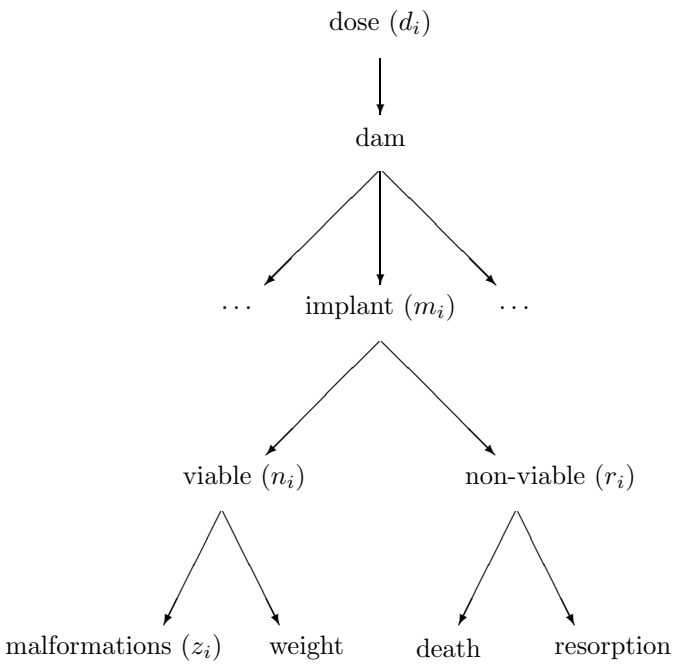

FIGURE 1. Data Structure of Developmental Toxicity Studies

to 30 pregnant animals. The dams are sacrificed just prior to normal delivery, at which time the uterus is removed and the contents are thoroughly examined for the occurrence of defects. The viable fetuses are measured for birth weight and examined carefully for the presence of malformation.

The analysis of developmental toxicity data raises a number of challenges (Molenberghs et al. 1998). Since deleterious events can occur at several points in development, an interesting aspect lies in the staging or hierarchy of possible adverse fetal outcomes (Williams and Ryan 1996). Figure 1 illustrates the data structure. A toxic insult early in gestation may result in a resorbed fetus. If the implant survives being absorbed, the developing fetus is at risk of fetal death. If the fetus survives the entire gestation period, growth reduction such as low birth weight may occur. The fetus may also exhibit one or more types of malformation. Ultimately, a model should take into account this hierarchical structure. In addition, because of genetic similarity and the same treatment conditions, offsprings of the same mother behave more alike than those of another mother, i.e., the litter effect. Thus, responses on different fetuses within a cluster are likely to be correlated.

2 Risk Assessment

The primary goal of these studies is to determine a safe level of exposure. Recent techniques for risk assessment in this area are based on fitting dose-response models and estimating the dose corresponding to a certain increase in risk of an adverse effect over background, i.e. benchmark dose (Crump 1984).
In case of multiple outcomes, the outcomes are often examined individually, using appropriate methods to account for the correlation, and regulation of exposure is based on the most sensitive outcome. It has been found, however, that a clear pattern of correlation exists between all the outcomes (Ryan et al. 1991), so that risk assessment based on a joint model may be more appropriate. The model must both incorporate the correlation between the outcomes, as well as the correlation due to clustering. Estimation of the risk, will be illustrated in Section 4.

3 Modelling Approach

Until now, most models have looked only to a small part of the hierarchical structure, and assumed that the response distribution for the malformation outcomes and weight outcomes is independent of the cluster size. The analysis of developmental toxicity data has usually been conducted on the number of viable fetuses only. In other models, the litter-size was included as a covariate in modelling these response probabilities (Williams 1987, Rai and Van Ryzin 1985, Catalano and Ryan 1992). Some attempts have already been made towards a joint model for death and malformation outcomes (Chen 1993). Kuk (2002) proposed a model for fetal response in developmental toxicity studies when the number of implants is dose-dependent.
We propose a Bayesian hierarchical modelling framework for the joint analysis of fetal death and malformation/weight among the viable fetuses. In a first step, we construct a model for the joint analysis of death and malformation. In a later step, we will extend this approach to include the weight of the viable fetuses.
Let N denote the total number of dams, and hence litters, in the study. For the ith dam ($i = 1, \ldots, N$), let m_i be the number of implants. Let r_i indicate the number of fetal deaths in cluster i. The number of viable fetuses, i.e., the litter size, is $n_i \equiv m_i - r_i$. The number of malformed fetuses of a dam is denoted z_i.
A joint model for the possible adverse fetal outcomes is developed using the underlying hierarchy of the data. In the first stage, a toxic insult may result in a fetal death. This effect of dose d_i on cluster i with m_i implants can be described using the distribution $f(r_i|m_i, d_i)$. We assume that

$$r_i \sim Binomial(p_{dth,i}, m_i)$$

with $p_{dth,i}$ the probability of a death fetus in litter i, depending on the dose. In the second stage, the fetuses that survived the entire gestation period are at risk of malformation. The effect of malformation of dose d_i on cluster i with n_i viable fetuses can be described using the distribution $f(z_i|n_i, d_i)$. We assume that

$$z_i \sim Binomial(p_{mal,i}, n_i)$$

with $p_{mal,i}$ the probability of a malformed fetus in litter i, depending on dose d_i. A joint model for the number of deaths and the number of malformations can be assessed by jointly modelling both stages.
To account for the litter effect, we assume a hierarchical model in which the probability of an adverse event in each litter come from a prior distribution. We assume the malformation and death probability p_i of any fetus in litter i to come from a beta distribution with mean π_i, i.e.,

$$\begin{aligned} p_{dth,i} &\sim Beta(a_{1i}, b_{1i}) & \pi_{dth,i} &= \frac{a_{1i}}{a_{1i} + b_{1i}} \\ p_{mal,i} &\sim Beta(a_{2i}, b_{2i}) & \pi_{mal,i} &= \frac{a_{2i}}{a_{2i} + b_{2i}} \end{aligned}$$

Both the malformation and death probability are affected by dose, and can be modelled using appropriate link functions. We assume

$$\begin{aligned} \text{logit}(\pi_{dth,i}) &= \alpha_0 + \alpha_d d_i \\ \text{logit}(\pi_{mal,i}) &= \beta_0 + (\alpha_d + \beta_d) d_i, \end{aligned}$$

with a common parameter for the dose effect.
In a last step, we specify hyperprior distributions on the regression parameters $\alpha_0, \alpha_d, \beta_0$ and β_d. The hyperpriors chosen for this analysis were $N(0, 10^6)$. We expect these priors to have minimal influence on the final conclusions of our analysis.

4 Data Analysis

This article is motivated by the analysis of developmental toxicity of Ethylene Glycol (EG) in mice. EG is a high-volume industrial chemical with diverse applications. For instance, it can be used as an antifreeze, as a solvent in the paint and plastics industries, as a softener in cellophane, etc. The potential reproductive toxicity of EG has been evaluated in several laboratories. Price et al. (1985) for example, describe a study in which timed-pregnant CD-1 mice were dosed by gavage with EG in distilled water. Dosing occurred during the period of organogenesis and structural development of the fetuses (gestational days 8 through 15). Table 1 shows the rate of malformed litters for each dose group and suggests clear dose-related trends for the rate of malformation. The mean litter size is also tabulated, and shows a decrease with dose.

TABLE 1. Summary Data from an EG Experiment in Mice

Dose (mg/kg/day)	Dams	Live	Litter Size (mean)	Malformations (%)
0	25	297	11.9	4.0
750	24	276	11.5	66.7
1500	22	229	10.4	81.8
3000	23	226	9.8	95.7

TABLE 2. Risk Assessment for EG Study in Mice.

Model	$q = 0.01$	$q = 0.05$
Joint	103	447
Malf	142	563
Death	340	1493

We define the combined risk due to a toxic effect as the probability that a fetus is death or a viable fetus is malformed. This risk can be expressed as

$$\begin{aligned} r(d) &= \text{P(death fetus} \mid d) + \text{P(viable fetus} \mid d) \times \text{P(malformed} \mid \text{viable}, d) \\ &= \pi_{dth} + (1 - \pi_{dth})\pi_{mal}. \end{aligned}$$

The benchmark dose is defined as the level of exposure corresponding to an acceptably small excess risk (q) over background, i.e., the dose satisfying

$$r^*(d) = \frac{r(d) - r(0)}{1 - r(0)} = q.$$

Table 2 shows the benchmark doses corresponding to the 1% and 5% excess risk. We also added the corresponding quantities, calculated from univariate risks (only malformation, or only death). The joint model yields more conservative doses.

References

Aerts, M., Geys, H., Molenberghs, G. and Ryan, L.M. (2002). *Topics in Modelling of Clustered Data*. Chapmann and Hall.

Ryan, L.M., Catalano, P.J., Kimmel, C.A., Kimmel, G.L. (1991). Relationship between Fetal Weight and Malformation in Developmental Toxicity Studies. *Teratology*, **44**, 215-223.

Williams, P.L., Ryan, L.M. (1996). Dose-Response Models for Developmental Toxicology. In: *Handbook of Developmental Toxicology of R.D. Hood (ed.)*, 636-666, New York: CRC Press.