BIOMETRICS 42, 73–84
March 1986

Maximum Likelihood Estimation for Cytogenetic Dose–Response Curves

E. L. Frome

Mathematical Sciences Section, Engineering Physics and Mathematics Division, Oak Ridge National Laboratory, P. O. Box Y, Oak Ridge, Tennessee 37831, U.S.A.

and

R. J. DuFrain

Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, Tennessee 37831, U.S.A.

SUMMARY

In vitro dose–response curves are used to describe the relation between chromosome aberrations and radiation dose for human lymphocytes. The lymphocytes are exposed to low-LET radiation, and the resulting dicentric chromosome aberrations follow the Poisson distribution. The expected yield depends on both the magnitude and the temporal distribution of the dose. A general dose–response model that describes this relation has been presented by Kellerer and Rossi (1972, *Current Topics on Radiation Research Quarterly* **8**, 85–158; 1978, *Radiation Research* **75**, 471–488) using the theory of dual radiation action. Two special cases of practical interest are split-dose and continuous exposure experiments, and the resulting dose–time–response models are intrinsically nonlinear in the parameters. A general-purpose maximum likelihood estimation procedure is described, and estimation for the nonlinear models is illustrated with numerical examples from both experimental designs. Poisson regression analysis is used for estimation, hypothesis testing, and regression diagnostics. Results are discussed in the context of exposure assessment procedures for both acute and chronic human radiation exposure.

1. Introduction

In recent years there has been considerable interest in evaluating the influence of the magnitude and temporal distribution of low linear energy transfer (LET) radiation on biological systems. An extensive review of studies on a wide spectrum of species and experimental systems is given in NCRP Report No. 64—*Influence of Dose and Its Distribution in Time on Dose–Response Relationships for Low-LET Radiations* (1980). Throughout the report the linear–quadratic (LQ) model

$$\lambda(d) = \alpha d + \beta d^2 \tag{1.1}$$

is used to describe the effect of absorbed dose d on a specific biologic endpoint. The LQ model and its more general form (1.2) are also discussed in the latest report of the Committee on the Biological Effects of Ionizing Radiations of the National Academy of Science (BEIR III, 1980, Chap. 2). It is pointed out that the LQ model is a convenient empirical model for complicated endpoints in complex systems. For "simple" cellular systems the LQ model has been extensively used in the evaluation of radiobiologic data.

We will consider studies that focus on specific lesions in the chromosomes of somatic cells as the endpoint of interest. Most of the early quantitative studies of chromosome

Key words: Dual radiation action; Generalized linear function; Iterative weighted least squares; Maximum likelihood estimation; Nonlinear models; Poisson regression; Regression diagnostics.

aberrations used plant cells [see Savage (1975) for a recent review], but starting in the 1960s and continuing on to the present, this line of research has shifted more to the use of animal cells. Recent work in human cytogenetic dosimetry used cultured peripheral blood lymphocytes to quantitatively assess the effect of low-LET radiation on chromosome damage. This approach provides an effective method for the evaluation of one type of radiation damage in man. Numerous studies have demonstrated that chromosome alterations induced in lymphocytes after *in vitro* exposure to low-LET radiation are both qualitatively and quantitatively similar to alterations observed after *in vivo* exposure. Dose–response curves obtained from carefully controlled *in vitro* studies are used to estimate the dose for exposed individuals. This is the basis for the indirect evaluation of the effects of both acute and chronic human radiation exposure. These methods are currently used to provide dose estimates for radiation accident management (see DuFrain et al., 1980; Frome and DuFrain, 1978). It has also been proposed that they be used for the indirect assessment of the long-term biologic effects of chronic exposure to radiation and other clastogens in human populations—see Evans et al. (1979), Savage (1979), and Holden (1982).

In Section 2 we will describe a maximum likelihood (ML) estimation procedure that can be used to estimate the parameters from an *in vitro* experiment. We assume that (i) the dependent variable y (the number of chromosome aberrations) follows the Poisson distribution and (ii) that a regression function that describes the relation between the expected value of y and the radiation exposure is specified. The role of the Poisson distribution in describing the dispersion of the number of dicentric chromosome aberrations has been discussed by Edwards, Lloyd, and Purrott (1979) and by Merkle (1981). The index of dispersion can be used as a monitoring test for Poisson variation—see Fisher (1950) and Frome (1982). Frome, Kutner, and Beauchamp (1973) have discussed testing for heterogeneity of variance and goodness of fit in a regression context.

Two examples are presented to illustrate both linear and nonlinear analysis, using both empirically and theoretically derived models. In the first example we present results that were obtained using a "linear model" approach to evaluate the effect of dose and dose rate on aberration yield. This initial analysis is straightforward and was designed to test the hypothesis that the coefficient of the d^2 term "depends" on dose rate. Although this initial analysis is technically correct, we were led to reject this approach as being both inappropriate and misleading on biologic grounds (see Section 4). We then present a more appropriate analysis that uses a nonlinear regression function. The nonlinear model is derived from the theory of dual radiation action (DRA) described by Kellerer and Rossi (1972), hereinafter referred to as KR. A second example is presented using data obtained from a dose-fractionation experiment and a nonlinear regression function predicted by the DRA theory.

The DRA theory uses concepts from microdosimetry to provide a quantitative characterization of the effect of various temporal distributions of absorbed dose on the production of chromosome aberrations (CAs). It is postulated that elementary lesions are produced at a rate that is proportional to the square of the specific energy that is deposited in certain "critical sites." The general form of the dose–effect model that is appropriate here (see KR, §5.4) is

$$\lambda(d, t) = \kappa[\gamma d + g(t, \tau)d^2], \tag{1.2}$$

where d denotes dose, t is time, and $\lambda(d, t)$ is the yield of elementary lesions. The parameter κ is a biophysical proportionality constant that reflects the target sensitivity for the biologic system (lymphocyte). The parameter γ depends on the radiation quality and can be related to the specific energy transferred from the radiation field to the critical site. Kellerer and Rossi (1978) interpret γ as an average of specific energy produced in individual events in the site. The linear and quadratic terms in (1.2) are often interpreted as intratrack and intertrack effects, respectively (Edwards and Lloyd, 1980). The coefficient of the d^2 term

in (1.2) is referred to by KR as the "reduction function," and describes the "interactions" between dose elements given at different times. If irradiation takes place over an interval of time, say 0 to t, at a constant rate (d/t), then the reduction function is

$$g(t, \tau) = \frac{2\tau}{t}\left[1 - \frac{\tau}{t}(1 - e^{-t/\tau})\right]. \tag{1.3}$$

If the dose d is given in two parts (d_1 and d_2) separated by a time interval t, then

$$g(t, \tau) = 1 - 2f(1 - f)(1 - e^{-t/\tau}), \tag{1.4}$$

where $f = d_1/d$. Substitution of (1.3) and (1.4) into (1.2) gives the appropriate dose–response curve for the continuous exposure and split-dose experiments, respectively. In both situations, the parameter τ represents the average "recovery time" and (1.3) and (1.4) were derived under the assumption that recovery takes place at a constant rate over the interval 0 to t. The resulting models are intrinsically nonlinear in the parameters, and the appropriate statistical analysis is based on the general maximum likelihood estimation procedure described in Section 2. Note that as $t \to 0$ in both (1.3) and (1.4), $g(t, \tau) \to 1$. Consequently, for the limiting acute exposure situation, $\lambda(d) \approx \kappa(\gamma d + d^2)$, which is equivalent to the LQ model (1.1). The parametrization in (1.1) has traditionally been used as a matter of computational convenience, and consequently the estimates of α and β can be viewed as "computational artifacts." Note that for the continuous exposure, split-dose, and acute exposure experiments, the parameters of interest are the same, i.e., κ, γ, and τ. In the acute exposure experiments, one assumes that $t \ll \tau$, so that $g(t, \tau) \approx 1$ for all values of d, and τ cannot be estimated.

In summary, current biologic knowledge (obtained from theory and experimental studies) predicts that dicentric CA yields will follow the Poisson distribution, and that dose–time–response relations for acute, chronic, and fractionated exposures can be described by the regression functions (1.1)–(1.4). The statistical methods presented in this paper are concerned with analytic procedures for rejecting these conjecture-based regression functions. Thus, the goodness-of-fit test provides a probabilistic basis for evaluating the falsifiability of these proposed models (see Dolby, 1982). When the regression function cannot be rejected, the ML estimation procedure can be used for inference on the parameters of biologic interest. This, at the very least, provides an effective means for summarizing data from two different experimental designs over a wide range of experimental conditions. We have analyzed most of the available data from both split-dose and continuous exposure experiments that have been reported in the cytogenetic literature. Our evaluation of results of these analyses (Frome and DuFrain, work as yet unpublished) indicate that these data are consistent with the regression functions obtained from the DRA theory. The results presented here illustrate how nonlinear regression functions (that are derived from global conjectures) can be used to summarize data obtained in cytogenetic studies. Poisson regression provides a useful and effective methodology for an in-depth evaluation of results from individual experiments, and also provides a basis for combining the results from different studies. Whatever the true nature of the biophysical events that cause damage and repair of material in the chromosomes, it appears that the regression functions (1.3) and (1.4) provide a useful way of describing experimental data. Equivalent expressions have been obtained by Thames (1985) using another theoretical approach to modeling the biological effects of protracted and fractionated radiation exposure. He considered a kinetic repair–misrepair model (Tobias et al., 1980) and showed that it is equivalent to an empirical model (Oliver, 1964) that has been developed to describe experimental data from cell survival studies. These results can be related to those presented in this paper by noting that the probability that a cell will be undamaged (i.e., have no chromosome aberrations) is equal to $\exp[-\lambda(d, t)]$.

2. Maximum Likelihood Estimation

Let y_i denote the number of dicentric CAs observed at the ith set of experimental conditions, i.e., dose d_i and time t_i for $i = 1, \ldots, n$. The y_i's are assumed to be independent and to follow the Poisson distribution with expectation

$$\mu_i = c_i \lambda(\mathbf{X}_i, \boldsymbol{\beta}),$$

where c_i denotes the total number of cells scored (in units of 100 cells). The regression function $\lambda(\mathbf{X}, \boldsymbol{\beta})$ describes the relation between the expected CA yield, the ith set of predictor variables $\mathbf{X}_i = (x_{i1}, x_{i2}, \ldots, x_{im})$, and the p-dimensional vector of unknown parameters $\boldsymbol{\beta}$. The kernel of the log-likelihood function is

$$L(\boldsymbol{\beta}) = \sum_{i=1}^{n} \{ y_i \log[c_i \lambda(\mathbf{X}_i, \boldsymbol{\beta})] - c_i \lambda(\mathbf{X}_i, \boldsymbol{\beta})\}. \tag{2.1}$$

A convenient computational approach to ML estimation is obtained by using iteratively reweighted least squares (IRLS). Let $\bar{y}_i = y_i/c_i$ denote the observed CA yield per 100 cells scored and consider the following weighted sum of squares:

$$S(\boldsymbol{\beta}) = \sum_i w_i[\bar{y}_i - \lambda(\mathbf{X}_i, \boldsymbol{\beta})]^2, \tag{2.2}$$

where w_i denotes a weight that is inversely proportional to the variance of $\bar{y}_i$. Since $\lambda(\mathbf{X}_i, \boldsymbol{\beta})$ is, in general, nonlinear in the parameters, an iterative procedure is required to obtain an estimate of $\boldsymbol{\beta}$. The equivalence of the resulting IRLS procedure and ML estimation for general Poisson regression models was demonstrated by Frome et al. (1973). Further details required for the analysis in Section 3 are given in the Appendix. The results of the IRLS are an ML estimate $\hat{\boldsymbol{\beta}}$, the estimated parameter covariance matrix, the deviance, and the basic "building blocks" required for regression diagnostics [see Pregibon (1981) and Frome (1983)]. The deviance, $D(\mathbf{y}, \hat{\boldsymbol{\mu}}) = \sum d_i^2$, is used to construct an ANOVA table for Poisson regression models, where the "deviance residuals" are ($i = 1, \ldots, n$)

$$d_i = \operatorname{sgn}(y_i - \mu_i)\{2[y_i \log(y_i/\hat{\mu}_i) - (y_i - \hat{\mu}_i)]\}^{1/2}, \tag{2.3}$$

and $\hat{\mu}_i = c_i \lambda(\mathbf{X}_i, \hat{\boldsymbol{\beta}})$. This measure of residual variation was proposed by Nelder and Wedderburn (1972) and is minus twice the ratio of the log-likelihood function of the model defined by $\lambda(\mathbf{X}_i, \boldsymbol{\beta})$ relative to the complete model in which there is one parameter for each value of i. The simplest (or minimal) model of interest in this situation is given by $\lambda_i = \beta x_i$, where x_i is the radiation dose. The ML estimate of β is $\hat{\boldsymbol{\beta}} = \sum_i y_i / \sum_i x_i c_i$, and the deviance for the minimal model is

$$D[\mathbf{y}, \hat{\boldsymbol{\mu}}(1)] = 2 \sum_i y_i \log[y_i/(c_i \hat{\boldsymbol{\beta}} x_i)].$$

Following the approach described by Efron (1978) for the binomial distribution, we then fit an increasing sequence of models for the explanatory vector $\boldsymbol{\mu}$, and the value of the deviance for each model is recorded in the Poisson ANOVA table. The procedure is illustrated in the next section for a sequence of models that are linear in the parameters.

3. Examples

3.1 *Continuous Exposure Experiment—Example* 1

The data in Table 1 (Purrott and Reeder, 1976) were obtained from an experiment (using gamma radiation from a cesium-137 source) that was designed to investigate the effect of dose rate on CA yield. According to theoretical predictions from microdosimetry, the LQ

Table 1
Number of dicentrics (y) and cells scored for continuous exposure experiment

Dose rate Gy/hr	Dose (Grays) 1.0 Cells	1.0 y	2.5 Cells	2.5 y	5.0 Cells	5.0 y
0.1	478	25	328	52	210	100
0.25	1907	102	185	51	138	113
0.5	2258	149	342	100	160	144
1.0	2329	160	310	100	120	106
1.5	1238	75	278	107	90	111
2.0	1491	100	259	107	100	132
2.5	1518	99	249	102	313	419
3.0	764	50	298	110	182	225
4.0	1367	100	243	107	144	206

Source: Purrott and Reeder (1976).

Table 2
Poisson ANOVA *for the data in Table* 1

Regression model	Number of parameters	Deviance	df
1. αd_i	1	1075.30	26
2. $\alpha d_i + \beta d_i^2$	2	228.00	25
3. $\alpha d_i + \beta_j d_i^2$	10	21.52	17
4. $\alpha_j d_i + \beta_j d_i^2$	18	11.10	9
5. Complete	27	0.00	0

model (1.1) should be appropriate for this situation. The coefficient α of the linear term describes the formation of dicentrics from a single track, and β describes the induction of dicentrics by two tracks. Thus, the two-break asymmetric exchange (dicentric) is believed to be the result of these two phenomena, and the frequency of dicentrics is described by a second-degree polynomial in dose. The validity of the LQ model is based on the assumption that the absorbed dose is delivered to a "critical site" in a short period of time, i.e., at a high dose rate. The purpose of the study by Purrott and Reeder was to test the hypothesis that the effect of decreasing the dose rate would be to decrease the contribution of the dose-squared term, without changing the linear term. Model 4 (see Table 2) corresponds to the most general case in which both the linear and quadratic coefficients are allowed to vary with dose rate, i.e., $\lambda_{jk} = \alpha_j d_k + \beta_j d_k^2$, where j identifies the dose rate group. For each of the models in Table 2 the regression function is linear in the parameters, and the procedure described in Section 2 was used to obtain the Poisson ANOVA.

A test statistic for the hypothesis $\beta_1 = \beta_2 = \cdots = \beta_9$ is obtained using the difference of the deviance, $D[\mathbf{y}, \hat{\boldsymbol{\mu}}(2)] - D[\mathbf{y}, \hat{\boldsymbol{\mu}}(3)] = 206.48$. This test statistic has an asymptotic chi-square distribution with 8 degrees of freedom (df) if the more restrictive hypothesis is true. Consequently, we reject the hypothesis that the coefficient of the quadratic term is independent of dose rate. An alternative approach is to test the goodness of fit of Model 3. The deviance for this model is 21.52 with 17 df, indicating that Model 3 cannot be rejected.

Ad hoc model for Example 1 If the ML estimates of the quadratic coefficients obtained from Model 3 are plotted against the log of the dose rate, it appears that the $\hat{\beta}_j$'s increase linearly with log dose rate, and this can be described by the following regression function:

$$\lambda_{jk} = \alpha d_k + [(\theta_1 + \theta_2 \log(r_j)]d_k^2.$$

Table 3
Results for the ad hoc regression function for the data in Table 1

Parameter	Estimate	Standard deviation
α	2.86	0.305
θ_1	3.80	0.141
θ_2	2.26	0.144

Table 4
Results for the DRA model for the data in Table 1

Parameter	Estimate	Standard deviation
κ	5.44	0.208
γ	0.269	0.0677
τ	7.40	0.857

The ith row of the model matrix for this ad hoc model is $\mathbf{X}_i = (d_i, d_i^2, d_i^2 \log r_i)$, where r_i is the dose rate (column 1 of Table 1) and d_i is the dose for the ith set of experimental conditions. The ML estimates and estimated standard errors for this model are given in Table 3. The value of the deviance is 29.95 with 24 df, indicating that this ad hoc model cannot be rejected for these data. This model provides a good description of the effect of dose rate on dicentric yield, i.e., the quadratic component increases with the log of dose rate, and the linear component is independent of dose rate.

Dual radiation action model The ad hoc model described in the previous section can be used as an empirical description of cytogenetic dose–response curves for this experiment. The parameters in this model do not have a clear interpretation in terms of the quantitative effects of ionizing radiation. The DRA theory (see Section 1) leads to the dose effect model (1.2), and for a continuous exposure experiment the function $g(t, \tau)$—originally proposed by Lea (1955)—is given by (1.3). Using (1.3) in (1.2), we obtain

$$\lambda(\mathbf{X}_i, \boldsymbol{\beta}) = \kappa\left(\gamma d_i + \frac{2\tau}{t_i}\{1 - \tau[1 - \exp(-t_i/\tau)]/t_i\}d_i^2\right), \tag{3.1}$$

where d is the absorbed dose and t is the duration of exposure at a constant dose rate. The parameters γ, κ, and τ can be related to the radiation quality, target sensitivity, and the recovery process (see Sections 1 and 4).

The ML estimates of the parameters in (3.1) for the data in Table 1 were obtained using the IRLS procedure described in Section 2. Since the DRA model is nonlinear in the parameters, the partial derivatives of (3.1) with respect to the parameters are needed (see the Appendix). The ML estimates and their standard deviations are given in Table 4. The deviance for this model is 28.58 with 24 df ($P = .236$), indicating that the DRA model cannot be rejected. The standardized residuals in Table 5a are used to identify outlying observations, and in this example there is one large negative residual. The diagonal terms from the $\mathbf{H}$ matrix (see the Appendix) in Table 5b are used to identify extreme points in the model (design) space. There are several large h-values (greater than $2p/n = 0.22$) in Column 3, and two of these are in the first two rows, i.e., at the highest dose and the lowest dose rate (see Section 4).

Table 5
Regression diagnostics for data in Table 1 *using the nonlinear model* (3.1)

(a) Standardized residuals $u_i = (y_i - \hat{\mu}_i)/(\hat{\mu}_i)^{1/2}$			(b) Diagonal terms from the **H** matrix ($p/n = 0.111$)		
0.127	−0.929	1.35	0.056	0.164	0.406
−1.23	0.315	1.19	0.143	0.038	0.239
0.291	−0.627	−1.05	0.155	0.036	0.157
0.383	−0.563	−2.92	0.161	0.035	0.080
−0.927	0.914	−0.140	0.086	0.037	0.062
−0.111	1.48	0.247	0.105	0.038	0.075
−0.423	1.26	0.315	0.107	0.039	0.251
−0.293	0.144	−1.17	0.054	0.049	0.154
0.670	1.88	0.732	0.097	0.043	0.132

3.2 *Split-dose Experiment—Example* 2

Schmid, Bauchinger, and Mergenthaler (1976) undertook a study to investigate the time-dependent quadratic component of the LQ model using a split-dose technique. Two experiments were carried out using 250 kV X-rays for the *in vitro* exposure of human peripheral lymphocytes. The purpose of the first experiment was to determine the coefficients for the LQ model (see Table 6a). In the second experiment the lymphocytes were irradiated with a dose of 3.4 grays split into two equal fractions separated by intervals of from 50 minutes up to 6 hours—see Table 6b. They assumed that the primary damage induced by the first dose fraction decreases at a constant rate and obtained the following for the "interval-dependent" yield:

$$u_t = \tfrac{1}{2}\beta e^{-t/\tau} d^2.$$

The interval-dependent yield was estimated by subtracting the yield at $d = 1.7$ with $t = 0$ from the observed yield obtained for each value of t with $d = 3.4$. The resulting values were then used to estimate the parameter τ.

The DRA theory predicts that the coefficient of the d^2 term will be given by (1.4) for this split-dose experiment (see KR, §5). Using (1.4) with $f = \frac{1}{2}$ in (1.2), we obtain the dose–time–response function

$$\lambda(\mathbf{X}_i, \boldsymbol{\beta}) = \kappa\{\gamma d_i + \tfrac{1}{2}[1 + \exp(-t_i/\tau)]\, d_i^2\}, \tag{3.2}$$

where $\mathbf{X}_i = (d_i, t_i)$ and $\boldsymbol{\beta} = (\kappa, \gamma, \tau)'$. Consequently, we can combine the data in Tables 6a and 6b and use (3.2) to obtain ML estimates of κ, γ, and τ. The ML estimates and their standard deviations are given in Table 7a. The corresponding estimates of κ, γ, and τ obtained using the results given by Schmid et al. (1976) are 5.4, 1.5, and 1.8, respectively. Using their estimates in (3.2) gives 22.56 for the deviance (the deviance for the ML estimates is 18.45). The Poisson ANOVA table for the data in Table 6 is given in Table 7b. The difference in the deviance values on rows 2 and 3 is 96.7 with 1 df. This is a test statistic for the hypothesis $\tau = 0$; i.e., the simple LQ model is rejected. The next-to-last line in Table 7b corresponds to the "pure error" sum of squares in the usual ANOVA table (note that there are two replications for each value of d in Table 6a). A goodness-of-fit test for the nonlinear model (3.2) is obtained from the deviance values on lines 3 and 4 in Table 7b, and the value of the resulting likelihood ratio statistic is 15.8 with 22 df, indicating that (3.2) cannot be rejected.

Table 6
(a) Dicentric yields for acute exposure experiment ($t = 0$ and $c = 1$)

d, dose (Grays)								
.25	.50	1.0	1.5	2.0	2.5	3.0	3.5	4.0
3	5	9	30	37	54	74	77	128
1	4	12	27	41	57	70	84	123

(b) Dicentric yield for split-dose experiment ($d = 3.4$ Gy)[a]

Interval (hours)	Cells	Dicentrics
0	500	135[a]
0	600	540
.83	500	417
1.00	500	393
1.17	300	238
1.33	200	150
1.50	300	214
1.67	500	354
1.83	200	141
2.00	400	277
2.50	300	200
3.00	200	122
3.33	200	127
4.00	200	104
5.00	200	107
6.00	200	104

[a] Dose = 3.4 Gy for all except the first row, where $d = 1.7$ Gy.
Source: Schmid et al. (1976).

Table 7
Results for split-dose data in Table 6
(a) ML Estimates

	κ	γ	τ
Acute only	5.49	1.37	—
Acute and split-dose	6.23	0.88	2.15
(Standard deviation)	(0.49)	(0.28)	(0.42)

(b) Poisson ANOVA

Regression function	df	Deviance
αd	33	162.2
$\alpha d + \beta d^2$	32	115.2
DRA (3.2)	31	18.45
Each (d, t)	9	2.6
Complete	0	0.0

4. Discussion

The results in Section 3 show how Poisson regression methods can be used in the analysis of cytogenetic dose–response curves. In our original analysis of the data in Table 1 (Frome and DuFrain, unpublished work), our objective was to show how to use linear regression (with Poisson weights) to obtain a Poisson ANOVA table. The deviance values were then used to construct a likelihood ratio test statistic for the hypothesis of interest as specified

Table 8
Additional data for continuous exposure experiment in Table 1

Dose	Dose rate (Gy/hr)	Cells	Dicentrics
5.0	0.15	204	157
2.5	0.15	225	50
2.5	0.05	540	100
1.0	0.05	1401	50
1.0	0.05	574	25
1.0	0.019	629	25

Table 9
Values of the deviance for continuous exposure study

Regression model	Table 1, $n = 27$	Table 1 + Table 8, $n = 33$
$d + d^2 + d^2 \log t$	24.54	35.00
$d + d^2 + d^2 \log r$	29.95	41.96
DRA (3.1)	28.58	50.37

by Purrott and Reeder (1976). In order to simplify the analysis, only those data with three doses at each dose rate were included. There were six additional data points at the low dose rates (see Table 8), and these data were also excluded from our later analysis using the DRA model (see Frome and DuFrain, 1982). This was done partially to ensure comparability with the earlier analysis and partly on biological grounds since the stability of the unstimulated G_0 lymphocyte maintained in culture for long time intervals can be questioned. The values of the deviance for the ad hoc model, the DRA model, and a third model,

$$\lambda(\mathbf{X}_i, \boldsymbol{\beta}) = \beta_1 d + \beta_2 d^2 + \beta_3(d^2 \log t),$$

are given in Table 9. When all of the data are included, both of the empirical linear models provide better fits for the complete set of data. Both of these models can be rejected, however, on biological grounds since they do not lead to reasonable results in the limiting situations of interest, i.e., as $t \to 0$ and as $t \to \infty$. Much of the lack of fit for the DRA model comes from the data at the lowest dose rates, and, as we noted earlier, there are reasons to question these data. Further support for the DRA paradigm is indicated in the second example. The nonlinear dose–time–response function (3.2) for the split-dose experiment is also predicted by the DRA theory. The important point is that *both* local regression functions are deduced from the DRA paradigm. The experimental data are used to challenge these models, not to generate them. The goodness-of-fit test statistic can be used to attempt to reject the dose–time–response functions and thereby show that the theory is false.

It is apparent that both of these studies were motivated by the DRA theory, and consequently we feel that the use of the appropriate model for these and related experiments is of prime importance in furthering our understanding of the effects of the temporal distribution of low-LET radiation on the yield of dicentric aberrations. Under similar experimental conditions the results from both continuous exposure and split-dose experiments should be comparable for the human lymphocyte data. The parameter γ is related to radiation quality but the values of κ and τ should be the same for normal human lymphocytes. We propose that future research efforts should focus on experiments that are designed to test for lack of fit of the regression function (1.2), with particular emphasis on the time-dependent component. If either (3.1) or (3.2) can be rejected, then a more complex model could be obtained, for example, by assuming a more general form for the recovery

process. This would provide evidence against the DRA theory or any other theory that leads to the same dose–response models. The purpose of this paper is to describe the ML estimation, hypothesis testing, and regression diagnostic procedures that can be used for any appropriate regression functions for CAs that follow the Poisson distribution. The two examples illustrate the effectiveness of Poisson regression methods in cytogenetic data analysis.

Acknowledgements

This research was supported by the Office of Health and Environmental Research, U.S. Department of Energy under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc., and under contract DE-AC05-76OR00033 with Oak Ridge Associated Universities. The authors wish to thank Drs J. J. Beauchamp, D. G. Gosslee, L. G. Littlefield, C. C. Lushbaugh, M. D. Morris, J. Preston, and J. A. Nelder for reviewing an earlier version of this manuscript, and to Dawn Human for help with manuscript preparation. The critical comments provided by the associate editor and referees were appreciated and have been taken into account in the revision of this paper.

Résumé

Des courbes de réponses in vitro sont utilisées pour décrire la relation entre aberrations chromosomiques et doses d'irradiations dans le cas de lymphocytes humains.

Les lymphocytes sont exposés à de faibles radiations L.E.T. Les aberrations chromosomiques décentriques qui en résultent obéissent à une loi de Poisson. L'espérance de leur nombre dépend à la fois de l'amplitude et de la répartition de la dose dans le temps.

Un modèle général de réponses aux doses décrivant cette relation a été proposé par Kellerer et Rossi (1972, *Current Topics on Radiation Research Quarterly* **8,** 85–158; 1978, *Radiation Research* **75,** 471–488) à l'aide de la théorie de l'effet dual de radiation. Deux cas particuliers d'intérêt pratique sont d'une part celui des expérimentations en doses fragmentées et d'autre part celui des expérimentations en expositions continues. Les modèles correspondants de réponses aux doses sont intrinsèquement non linéaires.

Une procédure générale d'estimation par maximum de vraisemblance est décrite, et des exemples d'estimations dans le cas de modèles non linéaires sont présentés à partir des deux plans d'expériences précédents. La régression poissonienne est utilisée pour l'estimation, les tests d'hypothèses et toutes les interprétations.

Les résultats sont discutés dans le contexte de procédures de répartition d'expositions dans les cas d'irradiations intenses et d'irradiations chroniques chez des humains.

References

Baker, R. J. and Nelder, J. A. (1978). *Generalized Linear Interactive Modeling* (*GLIM*), *Release* 3. Oxford: Numerical Algorithms Group.

Becker, R. A. and Chambers, J. M. (1984). *S*: *An Interactive Environment for Data Analysis and Graphics*. Belmont, California: Wadsworth.

BEIR III (1980). *The Effects on Populations of Exposure to Low Levels of Ionizing Radiation*: 1980. Washington D.C.: National Academy Press.

Charnes, A., Frome, E. L., and Yu, P. L. (1976). The equivalence of generalized least squares and maximum likelihood estimation in the exponential family. *Journal of the American Statistical Association* **71,** 169–172.

Dolby, G. R. (1982). The role of statistics in the methodology of science. *Biometrics* **38,** 1069–1083.

DuFrain, R. J., Littlefield, L. G., Joiner, E. E., and Frome, E. L. (1980). *In vitro* human cytogenetic dose–response systems. In *The Medical Basis for Radiation Accident Preparedness*, K. F. Hubner and S. S. Fry (eds), 357–374. New York: Elsevier North-Holland.

Edwards, A. A. and Lloyd, D. C. (1980). On the prediction of dose rate effects for dicentric production in human lymphocytes by X- and γ-rays. *International Journal of Radiation Biology* **37,** 89–92.

Edwards, A. A., Lloyd, D. C., and Purrott, R. C. (1979). Radiation-induced chromosome aberrations and the Poisson distribution. *Radiation and Environment Biophysics* **16,** 89–100.

Efron, B. (1978). Regression and ANOVA with zero–one data: Measures of residual variation. *Journal of the American Statistical Association* **73,** 113–121.

Evans, H. J., Buckton, G. E., Hamilton, G. E., and Carothers. A. (1979). Radiation-induced chromosome aberrations in nuclear dockyard workers. *Nature* **277,** 531–534.

Fisher, R. A. (1950). The significance of deviations from expectation in a Poisson series. *Biometrics* **6,** 17–24.

Frome, E. L. (1981). Poisson regression. *The American Statistician* **35,** 262–263.

Frome, E. L. (1982). Fisher's exact variance test for the Poisson distribution. *Applied Statistics* **31,** 67–71.

Frome, E. L. (1983). The analysis of rates using Poisson regression models. *Biometrics* **39,** 665–674.

Frome, E. L. (1984). Response to Nelder's reaction on Poisson rate analysis. *Biometrics* **40,** 1160–1162.

Frome, E. L. (1985). Regression methods for binomial and Poisson distributed data. In *Proceedings of American Association of Physicists in Medicine First Midyear Topical Symposium on Multiple Regression Analysis, Applications in the Health Sciences,* D. Herbert and R. Meyers (eds), 84–123. New York: The American Institute of Physics.

Frome, E. L. and Beauchamp, J. J. (1968). Maximum likelihood estimation of survival curve parameters. *Biometrics* **24,** 595–605.

Frome, E. L. and Checkoway, H. (1985). Use of Poisson regression models in estimating incidence rates and ratios. *American Journal of Epidemiology* **121,** 309–323.

Frome, E. L. and DuFrain, R. J. (1978). Estimation of the amount of exposure to an environmental clastogen using human cytogenetic dosimetry. In *Proceedings of the* 1978 *DOE Statistical Symposium* (Albuquerque, New Mexico), H. T. Davis, R. R. Prairie, and T. Truett (eds), 169–191. Oak Ridge, Tennessee: Oak Ridge National Laboratory.

Frome, E. L. and DuFrain, R. J. (1982). Analysis of cytogenetic dose–response data using a model derived from the theory of dual radiation action. *Biometrics* **38,** 1117.

Frome, E. L., Kutner, M. H., and Beauchamp, J. J. (1973). Regression analysis of Poisson-distributed data. *Journal of the American Statistical Association* **68,** 935–940.

Frome, E. L. and MaClain, R. (1984). A macro for fitting nonlinear models to Poisson distributed data. In *Proceedings of Ninth Annual SAS Users Group International Conference,* 883–888. Cary, North Carolina: SAS Institute, Inc.

Goodnight, J. H. and Sall, J. P. (1982). The NLIN procedure. *SAS Users Guide: Statistics.* Cary, North Carolina: SAS Institute, Inc.

Holden, C. (1982). Looking at genes in the workplace. *Science* **217,** 336–337.

Kellerer, A. M. and Rossi, H. H. (1972). The theory of dual radiation action. *Current Topics on Radiation Research Quarterly* **8,** 85–158.

Kellerer, A. M. and Rossi, H. H. (1978). A generalized formulation of dual radiation action. *Radiation Research* **75,** 471–488.

Lea, D. E. (1955). *Action of Radiations on Living Cells,* 2nd edition. Cambridge: The University Press.

McCullagh, P. and Nelder, J. A. (1983). *Generalized Linear Models.* New York: Chapman and Hall.

Merkle, W. (1981). Poisson goodness-of-fit tests for radiation-induced chromosome aberrations. *International Journal of Radiation Biology* **40,** 685–692.

NCRP Report No. 64 (1980). *Influences of Dose and Its Distribution in Time on Dose–Response Relationships for Low-LET Radiations.* Washington, D.C.: National Council on Radiation Protection and Measurements.

Nelder, J. A. and Wedderburn, R. W. M. (1972). Generalized linear models. *Journal of the Royal Statistical Society, Series A* **135,** 370–384.

Oliver, R. (1964). A comparison of the effects of acute and protracted gamma-radiation on the growth of seedlings of *Vicia Fuba. International Journal of Radiation Biology* **8,** 475–488.

Pregibon, D. (1981). Logistic regression diagnostics. *The Annals of Statistics* **9,** 705–724.

Purrott, R. J. and Reeder, E. (1976). The effect of changes in dose rate on the yield of chromosome aberrations in human lymphocytes exposed to gamma radiation. *Mutation Research* **35,** 437–444.

Savage, J. R. K. (1975). Radiation-induced chromosomal aberrations in the plant tradescantia: Dose–response curves. *Radiation Botany* **15,** 87–140.

Savage, J. R. K. (1979). Chromosomal aberrations at very low radiation dose rates. *Nature* **277,** 512–513.

Schmid, E., Bauchinger, M., and Mergenthaler, W. (1976). Analysis of time relationships for the interaction of X-ray-induced primary breaks in the formation of dicentric chromosomes. *International Journal of Radiation Biology* **30,** 339–346.

Thames, H. D. (1985). An incomplete repair model for fractionated and continuous irradiation. *International Journal of Radiation Biology* **47,** 319–339.

Tobias, C. A., Blakely, E. A., Ngo, R. Q. H., and Yang, T. C. H. (1980). The repair–misrepair model of cell survival. In *Radiation Biology in Cancer Research*, R. E. Meyn and H. R. Withers (eds), 195–230. New York: Raven Press.

Received May 1983; *revised February and December* 1985.

Appendix

Maximum Likelihood Estimation Using Weighted Least Squares

Frome et al. (1973) have shown that for the general Poisson regression model the ML estimate of the parameter vector $\boldsymbol{\beta}$ can be obtained using a properly weighted iterative least squares procedure. The resulting iterative procedure is equivalent to using the method of scoring to obtain a root of the likelihood equations. On iteration $k + 1$, this leads to the following system of p linear equations:

$$(\mathbf{P}'\mathbf{W}\mathbf{P})\boldsymbol{\delta}^k = \mathbf{P}'\mathbf{W}[\bar{\mathbf{y}} - \boldsymbol{\lambda}^k], \tag{A.1}$$

where $\mathbf{W}$ is diagonal with $w_i = c_i/\lambda(\mathbf{X}_i, \boldsymbol{\beta}^k)$, $\mathbf{P}$ is an n by p matrix of partial derivatives, and $\bar{\mathbf{y}}$ and $\boldsymbol{\lambda}^k$ are n-dimensional vectors with elements y_i and $\lambda(\mathbf{X}_i, \boldsymbol{\beta}^k)$, respectively. The elements of the ith row of the matrix $\mathbf{P}$ are $\partial\lambda(\mathbf{X}_i, \boldsymbol{\beta})/\partial\beta_j$, $j = 1, \ldots, p$. Each of the quantities in (A.1) that involves $\boldsymbol{\beta}$ is evaluated at the current value, $\boldsymbol{\beta}^k$, and an estimate of the "correction vector" $\boldsymbol{\delta}^k$ is obtained and used to compute a revised estimate $\boldsymbol{\beta}^{k+1} = \boldsymbol{\beta}^k + \boldsymbol{\delta}^k$. This iterative procedure (Gauss–Newton method) continues until some convergence criteria are satisfied. This can be viewed (on each iteration) as a weighted linear regression of the residuals $\bar{y}_i - \lambda(\mathbf{X}_i, \boldsymbol{\beta}^k)$ on the p-dimensional row vector of "predictor variables" $\mathbf{P}_i$ evaluated at $\boldsymbol{\beta}^k$ (see Frome, 1983, 1984). Consequently, any statistical package that supports IRLS can be used to obtain ML estimates of the β_i. The estimate of the asymptotic covariance is obtained by inverting the matrix $\mathbf{P}'\mathbf{W}\mathbf{P}$ evaluated at $\hat{\boldsymbol{\beta}}$. The basic "building blocks" that are required for regression diagnostics are standardized residuals and the diagonal terms from the matrix

$$\mathbf{H} = \mathbf{W}^{1/2}\mathbf{P}(\mathbf{P}'\mathbf{W}\mathbf{P})^{-1}\mathbf{P}'\mathbf{W}^{1/2}$$

evaluated at the ML estimate $\hat{\boldsymbol{\beta}}$ (see Frome, 1983).

A special situation of considerable practical interest occurs when the regression function is a generalized linear function (GLF); i.e., $\lambda(\mathbf{X}_i, \boldsymbol{\beta}) = g(\sum_i \beta_i x_{ij})$, where g is a nonnegative monotonic differentiable function. The statistical package GLIM (Baker and Nelder, 1978) is especially suited for this situation. When $\lambda(\mathbf{X}, \boldsymbol{\beta})$ is a GLF, the IRLS computations can be easily done using standard options in GLIM. This is done by specifying the "link function" (which is the inverse of the regression function) and the predictor variables of interest. GLIM can also be used when the regression function is intrinsically nonlinear in the parameters. This requires the partial derivatives of $\lambda(\mathbf{X}, \boldsymbol{\beta})$ with respect to the β_j. As an example, consider the nonlinear regression function for the split-dose experiment—see (1.2) and (1.4),

$$\lambda(d, t) = \kappa\{\gamma d + [1 - 2f(1 - f)(1 - e^{-t/\tau})]d^2\}.$$

To obtain ML estimates of the parameters using GLIM, we wrote a GLIM macro named *fitnl*. A listing of the GLIM driver program, the macro *fitnl*, and detailed computational results for the split-dose experiment in Section 3.2 can be obtained from the authors.

Identical results can also be obtained, for example, by using a FORTRAN program PREG (Frome, 1981), the system *S* (Becker and Chambers, 1984), or the statistical program SAS (Goodnight and Sall, 1982; see also Frome and MaClain, 1984). Each of these approaches requires the partial derivatives, initial estimates of the parameters, and some convergence criteria. Additional examples of Poisson regression with nonlinear models are given in Frome and Beauchamp (1968), Frome (1983), and Frome and Checkoway (1985). Further note that the computational approach described here can be extended to situations where the response variable y is in the regular exponential family for a general nonlinear regression function (Charnes, Frome, and Yu, 1976). If the regression function is a GLF (see Nelder and Wedderburn, 1972) then the analysis can be done using standard options in GLIM. An excellent account of GLF for the exponential family is given by McCullagh and Nelder (1983), and application of these regression methods to discrete data has been reviewed by Frome (1985).