

Statistical Methods for Biological Dosimetry

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ABSTRACT

Statistical methods most commonly employed at HPA for purposes of biological dosimetry by analysis of chromosomal aberrations in blood lymphocytes are reviewed and discussed together with worked examples. They serve to illustrate practical aspects of the techniques. A novel method of computation of dose and the corresponding 95% limits, taking into account both the Poisson derived uncertainties in induced aberration yield as well as uncertainties associated with the calibration curve, is presented. A very good agreement of the latter approach with a more simplified one, which merely takes into consideration the Poisson uncertainties in yield, further validates the approximations used over many years for dose assessment.

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1 INTRODUCTION

The measurement of chromosome aberration frequencies in human lymphocytes is a valuable tool for assessing absorbed doses of ionising radiation to individuals (IAEA, 2001). As such, biological dosimetry has an important role to play in the investigations of radiation accidents as it can provide useful information on the likely future stochastic and deterministic health consequences. Alternatively, the knowledge that after a radiation incident no raised level of chromosomal damage is present can give reassurance to patients, their families and physicians.

Human lymphocytes are long-lived cells that can be easily obtained from a blood sample. Since most are not cycling, they represent predominantly a population of G₀ phase cells, i.e. those held before DNA replication. These cells can be stimulated *in vitro* to undergo mitotic division by the introduction of phytohaemagglutinin (PHA) (Nowell, 1960) and can be stopped at their first metaphase with the use of the arresting agent Colcemid after about 45h of culture at 37°C. The slides are then prepared and stained with Giemsa or FISH (fluorescence *in situ* hybridization) probes and scored accordingly. A check for contamination with second-division metaphases is recommended and is carried out by incorporating bromodeoxyuridine (BrdU) in conjunction with fluorescence plus Giemsa (FPG) staining. The full details of these techniques can be found in the IAEA manual (2001).

In the case of traditional Giemsa staining, the scored aberrations comprise mainly dicentrics (exchanges between centromeric pieces of chromosomes accompanied by the acentric pieces of these chromosomes), centric rings (exchanges between two breaks on separate arms of the same chromosome), and acentric fragments. For biological dosimetry, dicentrics are the preferred aberration due to their low background incidence, approximately 0.5-1 dicentrics per 1000 cells, and the reliability of detection. Centric rings are approximately 5% as frequent as dicentrics (Lloyd and Purrott, 1981). The main disadvantage of the dicentric assay is that the damage is unstable and damaged cells are removed from the pool of peripheral blood lymphocytes at the rate with which cells are replaced. It is presently believed that for persons with normal haematological parameters the disappearance rate is exponential with a half-life of approximately 3 years (Lloyd *et al*, 1980), although significant individual variation is observed. In this respect, the introduction of the FISH technique (Pinkel *et al*, 1986) permits the more persistent aberrations, such as stable translocations, to be scored providing a reliable retrospective and long-term exposure dosimetry system (Lindholm and Edwards, 2004; Darroudi, 2000). The progress in cytogenetic methods of biological dosimetry using FISH has recently been reviewed by Edwards *et al* (2005) and Whitehouse *et al* (2005).

An additional technique occasionally employed in biodosimetry is the cytokinesis blocked micronucleus assay (IAEA, 2001). This identifies fragments, or sometimes whole chromosomes, that do not segregate properly at mitosis to daughter nuclei. They are excluded into the cytoplasm as discrete chromatin positive bodies seen alongside the two daughter nuclei. The most important advantage of this technique stems from the speed with which micronuclei can be scored which, it has been suggested, is particularly convenient in the case of initial triage of large numbers of persons involved in a major

radiation incident (Köteles, 1996). However, the value of the technique is limited mainly by the larger and more variable background frequency of micronuclei as compared with dicentrics.

The aim of this document is to review and discuss the statistical methods employed at HPA for the analysis of chromosome aberrations.

2 STATISTICAL APPROACH

2.1 Dose-response calibration curves

In the majority of cases, accidental overexposures are to x- or γ -radiation sources. Therefore, the determination of *in vitro* dose-response calibration curves for these types of radiation are the priority for laboratories involved in biological dosimetry and ought to be supported by reliable and accurate physical dosimetry. Important considerations, discussed in detail in IAEA (2001), to bear in mind are: (i) the uniform irradiation of the blood sample, which can be ensured by adjusting the distance between the source and the sample to be sufficiently long; (ii) the production of charged particle equilibrium, achieved by surrounding the sample with materials of similar composition to blood; (iii) the dose rate for the *in vitro* simulation of an acute exposure which should be chosen such that the required dose is delivered in less than 15 minutes, and (iv) the geometry and materials of and surrounding the ionisation chamber should be selected such that the corrections for distance, absorption, scattering and the mismatch at the blood/holder interface are minimized.

From a statistical point of view, in order to carry out a satisfactory calibration curve fitting procedure, a sufficient number of degrees of freedom is necessary. This translates to around 10 doses in the range 0.1 – 5.0 Gy for low linear energy transfer (LET) radiation such as x-rays or γ -rays. Doses higher than 5 Gy show progressive deviation from a curve of simple form eventually producing saturation of the aberration yield (see discussion at the end of this section; Lloyd and Edwards, 1983). It is still a matter of some debate as to how to treat the background level of aberrations in the fitting procedure. The problem arises due to the very low incidence of background dicentrics that may give a zero yield in a scored data point. The inclusion of the point of zero in the curve fitting may lead to a fitted negative control level, which is not biologically meaningful. The data point in question could be omitted from the fitting procedure and the curve forced to pass through the origin. However, this too is unrealistic because there is a large body of control data showing that, while low, the background is positive. Therefore, at HPA, the solution that has been chosen is to fit the dose-response with a small but positive yield with a large uncertainty assigned to the zero dose data point.

The yield of chromosome aberrations (Y) is related to dose (D) by the following equation:

$$Y = c + \alpha D + \beta D^2, \quad (1)$$

where c , α and β are the fitted coefficients. The control value c for dicentric is approximately 0.0005 - 0.001. Some values of α and β measured at HPA are summarised in table 1, however in practice, a synthesis of various experimental sets of data, judged to be the best to date, is used to produce the coefficients routinely used for the purposes of biological dosimetry.

Chromosome aberrations are a good example of a biological effect caused by ionising radiation where it is possible to discriminate differences between radiation qualities that are generally termed low LET. These are qualities for which in radiological protection a deliberate decision has been taken to ascribe a weighting factor (w_R) of 1. Thus, no attempt is made to distinguish between their biological effectiveness (ICRP, 1990). Nevertheless, with dicentric, differences can be seen and are reflected in the linear yield coefficient α and are, thus, most evident at low doses. In practice, laboratories tend to calibrate with orthovoltage x-rays (200-250 kVp) and with a convenient γ -ray source, usually cobalt-60. However, when confronted with the need to estimate a dose in a case that involves exposure to other low LET sources, the practice in HPA is to assume an α coefficient by extrapolating or interpolating between established calibration data. For example, when dealing with lower energy diagnostic x-rays, such as 30 kVp, an α coefficient of ~ 0.05 is assumed. For cobalt-60 γ -ray sources ($\bar{E}=1.2$ MeV) an α value of 0.02 is used but for lower energy sources, also commonly used in industrial radiology such as caesium-137 ($\bar{E}=660$ keV) or iridium-192 ($\bar{E}=317$ keV), α values of 0.025 and 0.03 are used, respectively.

TABLE 1 A summary of values of α and β and their standard errors (SE) for different types of radiation.

Reference	Radiation source	$\alpha \pm \text{SE} (\text{Gy}^{-1})$	$\beta \pm \text{SE} (\text{Gy}^{-1})$
Lloyd <i>et al</i> (1986)	250kVp x-rays	0.046 \pm 0.005	0.065 \pm 0.003
Lloyd <i>et al</i> (1986)	⁶⁰ Co γ -rays	0.014 \pm 0.004	0.076 \pm 0.003
Prosser <i>et al</i> (1983)	³ H β -particles	0.054 \pm 0.005	0.067 \pm 0.004
Lloyd <i>et al</i> (1984)	14.9MeV neutrons	0.195 \pm 0.018	0.119 \pm 0.020
Lloyd <i>et al</i> (1976)	MRC cyclotron generated neutrons ($\bar{E}=7.6$ MeV)	0.482 \pm 0.040	0.064 \pm 0.022
Lloyd <i>et al</i> (1978)	²⁵² Cf fission neutrons ($\bar{E}=2.13$ MeV)	0.600 \pm 0.019	-
Lloyd <i>et al</i> (1976)	AWRE fission neutrons ($\bar{E}=0.9$ MeV)	0.733 \pm 0.024	-
Lloyd <i>et al</i> (1976)	BEPO fission neutrons ($\bar{E}=0.7$ MeV)	0.832 \pm 0.032	-
Lloyd <i>et al</i> (1988)	24keV neutrons	0.821 \pm 0.031	-
Edwards <i>et al</i> (1980)	²⁴² Cm alpha particles	0.286 \pm 0.015	-
Purrott <i>et al</i> (1980)	²³⁹ Pu alpha particles	0.375 \pm 0.024	-
Edwards <i>et al</i> (1986)	8.7 MeV protons (L = 5.1keV/ μ m)	0.044 \pm 0.075	0.058 \pm 0.057
Edwards <i>et al</i> (1986)	23.5 MeV ³ He ions (L = 22keV/ μ m)	0.394 \pm 0.018	-

Values of α and β from table 1 have been used to produce an illustrative comparison of calibration curves for some chosen types of radiation shown in figure 1. It is worth noting that whilst the dose-response curves depend very strongly on the energy in the case of neutrons, they are rather similar in the case of photon radiation but, as discussed earlier, with γ -rays being less effective at producing aberrations at low doses than x-rays. The reason for this stems from differences in the actual physical interaction of ionising radiation of different qualities with tissue. In the case of high doses of low LET, or sparsely ionisation radiation, the ionisations are randomly distributed amongst cells and there are a large number of tracks present. Hence, assuming an equal probability that any DNA damage can interact with a nearby similar break and be converted into an aberration, aberrations should be randomly distributed amongst cells. Indeed, it has been shown that aberrations for low LET radiation conform closely to the Poisson distribution. However, it is not the case for high LET radiation (e.g. neutrons, α -particles), where more cells with multiple and no aberrations are observed than expected from the Poisson distribution. This is because ionisations are non-randomly distributed across the cell, as high LET energy is deposited in discrete tracks. From this it follows that whilst aberrations produced by a single track have a frequency that is proportional to a linear function of dose (β in equation 1 equals zero; e.g. for lower energy neutrons), those induced by two independent tracks have a frequency proportional to the square of dose. The ratio of α/β of the coefficients in equation 1 is equivalent to the dose at which linear and quadratic components contribute equally to the formation of aberrations.

In practice, prior to fitting a polynomial defined by equation 1 to the data points given at each dose, a test on the distribution of dicentric is carried out in order to determine whether the yields at each data point conform to the Poisson distribution. Following the method of Savage (1970), the so-called u-test of the goodness of fit is employed (CtamPoiss computer program available from HPA). This relies on the value of the dispersion index, which provides a measure of how well the sample's variance corresponds to that predicted by the Poisson distribution. Therefore, any deviation of

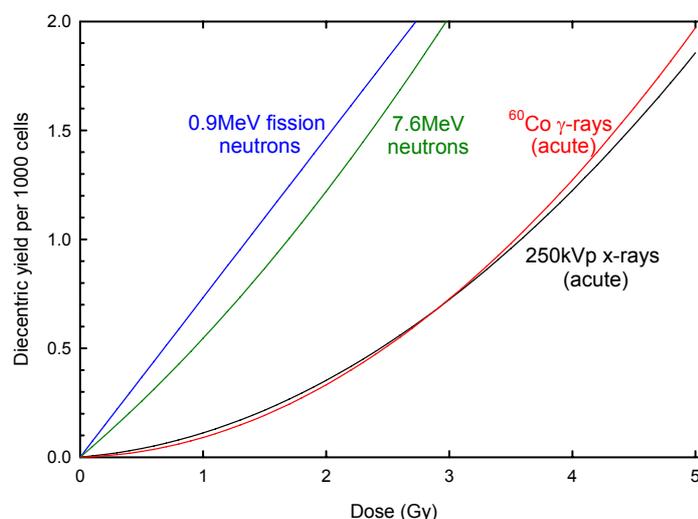


FIGURE 1 Dose-response curves for four selected radiation qualities.

the variance/mean ratio from unity (i.e. under or overdispersion compared with the Poisson distribution) can subsequently be taken into account in the fitting procedure by adjusting the weights of the data points appropriately (by multiplying the Poisson-derived variance of the data points by the ratio of variance/mean). Although some laboratories tend to use the individual ratios of variance/mean in their curve fitting, at HPA it is common practice to check for any trends in the values of variance/mean ratios with increasing dose. This is preferable because the ratios themselves have large statistical uncertainties. If no trends are observed then a mean ratio is calculated and applied to all the data points.

The importance of weighting in curve fitting cannot be overstressed, as more accurate points have to be considered more seriously than the inaccurate ones and, indeed, many commercially available curve fitting programs simply ascribe equal weights to each point. This has led to erroneous dicentric response curves being published. The most common approach is to minimize the sum of squared differences (SSD) between the observed and fitted values, with weights of the data points given by the inverse of their variance. It was suggested by Papworth (1975) to base the estimates of variance on the value of the fitted yield rather than the observed one. This can be done iteratively until a self-consistent solution is found (MIPol computer program available from HPA). This method of iteratively reweighted least squares is equivalent to that of the maximum-likelihood (Papworth, 1975). The goodness of the fit can be tested assuming that SSD follows a chi-square distribution with degrees of freedom (DF) equal to the number of data points less the number of fitted parameters (normally three; c , α , β). A close correspondence between SSD and DF implies a good fit. Whilst values of SSD significantly greater than DF suggest an uncertainty due to the lack of fit, SSD considerably less than DF hint to the data points being too close, within their errors, to the fitted curve. Generally, the uncertainties in the parameters should be based on the Poisson estimate of the variance, however, in the case of lack of fit, where $SSD > DF$, the uncertainties based on Poisson statistics should be increased by $(SSD/DF)^{1/2}$.

The consequence of the inclusion of data points obtained at 5 Gy and above in the fitting procedure can be demonstrated using data from an early classic experiment of Norman and Sasaki (1966), who irradiated human lymphocytes with x-rays to doses up to 30 Gy. As mentioned before, approximately from 5 Gy onwards, a saturation of dose-response curves is observed and, therefore, special care must be taken in the selection of the data points used in the fit. As shown in table 2, the inclusion of higher dose data points in the fit to the quadratic model (equation 1) using the maximum likelihood

TABLE 2 The effect of sequentially removing the higher dose levels on the results of fitting the data of Norman and Sasaki (1966).

Highest dose (Gy)	$\alpha \pm SE \times 10^{-3}$	$\beta \pm SE \times 10^{-2}$	χ^2	DF
30	180.0 ± 37.0	0.95 ± 0.24	205	10
25	149.0 ± 34.0	1.35 ± 0.28	142	9
20	120.0 ± 31.0	1.79 ± 0.32	99	8
12	49.0 ± 21.0	3.20 ± 0.35	30	7
8	16.0 ± 15.0	4.18 ± 0.36	11	6
6	3.4 ± 13.1	4.67 ± 0.37	5.9	5
5	-5.9 ± 4.4	5.16 ± 0.44	2.5	4

method leads to a decrease in the values of the β coefficient and a simultaneous increase in the α coefficient. This is due to the fact that α and β are correlated and, hence, lower values of one must be compensated by enlarged values of the other. From table 2, it can be deduced that the best fit in this case is obtained when doses up to 6 Gy are included in the fit.

2.2 Dose assessment in whole body exposure

Deriving the dose from a given yield of aberrations does not present any difficulty as it follows directly from equation 1 that:

$$D = \left(-\alpha + \sqrt{\alpha^2 + 4\beta(Y - c)} \right) / 2\beta. \quad (2)$$

However, there are a number of approaches that permit calculation of uncertainties. These are customarily expressed as confidence limits, with 95% being the limit chosen most often. The 95% confidence limits define an interval that embraces the true dose on 95% of occasions. The difficulty in determining the confidence limits arises from the fact that there are two components to the uncertainty: one originates from the Poisson nature of aberrations seen in blood samples from irradiated persons, and the other, is associated with uncertainties in the calibration curve. This problem was addressed by Savage and Papworth (2000) resulting in two fairly complex methods of estimating the confidence limits. In the first approach, the confidence limits can be obtained from differentiation of equation 2 and expressing the variance in dose ($\text{var}D$) in terms of variances and co-variances of the fitted parameters α , β and c . Then, 95% confidence limits are given by $D \pm 1.96\text{SE}(D)$, where $\text{SE}(D) = \sqrt{\text{var}D}$. The second approach is similar but relies on uncertainties in yield rather than dose. Full details of the two methods can be found in IAEA (2001).

In this document, it is intended to emphasize the two methods of the confidence limit estimation that are routinely employed at HPA. It has recently been shown that the simplified approach, described in detail below, produces results that are in a very good agreement with a more sophisticated explicit calculation. The simplified approach, illustrated in figure 2, is a modification of the method proposed by Merkle (1983) and involves the following steps:

- Determining the yields corresponding to the lower and upper 95% confidence limit on the observed yield (Y_L and Y_U), assuming the Poisson distribution.
- Determining the dose at which Y_L crosses the dose-response curve. This corresponds to the lower confidence limit (D_L).
- Determining the dose at which Y_U crosses the dose-response curve. This corresponds to the upper confidence limit (D_U).

Example: 25 dicentrics are observed in 500 cells from a person overexposed to γ -rays. The parameters of the γ -ray calibration curve, shown in figure 2, are $c = 0.001$, $\alpha = 0.014$ and $\beta = 0.076$.

- From standard statistical tables or using a computer spreadsheet, the 95% lower and upper confidence limits on 25 observations are 16.18 and 36.91, respectively. Hence, $Y_L = 16.18/500 = 0.032$ and $Y_U = 36.91/500 = 0.074$ dicentrics per cell.
- Y_L intersects the calibration curve at $D_L = 0.56$ Gy.
- Y_U intersects the calibration curve at $D_U = 0.89$ Gy.

It is important to recognize that the simplified approach does not take account of uncertainties associated with the dose-response curve. An initial approach to take into consideration both the uncertainties in the calibration curve as well as the Poisson nature of the yield was made by Merkle (1983) by reading off the value of dose for which Y_L (Y_U) intersects the upper (lower) 95% confidence limit of the dose-response calibration curve. This approach overestimated the effect of uncertainties in the calibration curve.

The second approach discussed here, developed specifically to test the departure of the results of the simplified method from an explicit calculation accounting for all uncertainties, relies on a systematic convolution of the possible values of yield with the possible values of the calibration curve. This is a Bayesian type of approach as uncertainties in the observed yield and in the calibration curve are expressed as continuous probability distributions. More specifically, as shown schematically in figure 3, the values of possible yields are described by the Poisson likelihood function for the particular number of observed dicentrics (red curve in the figure, in this case 25 dicentrics seen in 500 cells). This Poisson likelihood function is then convoluted with a distribution of possible values of dose given, for each value of yield (Y), by dose likelihood functions (blue curves in figure 3). These are assumed to be normal and are determined using the knowledge of the standard errors on each datum point of the calibration curve (obtained in turn from the curve fitting procedure, described earlier in section 2.1). The standard deviation of the normal distribution can be approximated by $\sigma = (D_{UL} - D_{LL})/2$, where D_{LL} and D_{UL} are the lower and upper limits of dose

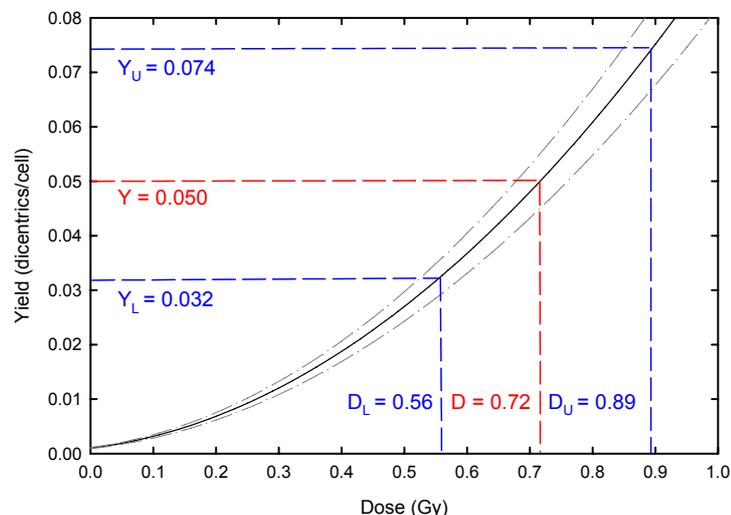


FIGURE 2 A dose-response calibration curve used to estimate uncertainties for the simplified approach. The dot-dash lines represent 95% confidence limits of the calibration curve.

computed from the standard error in Y (see figure 3). In the case of γ -rays, the standard errors on the yields at individual data points incorporated in the calculation varied from very large at very low doses to approximately 2-3% on doses from 0.8 Gy onwards. The summation of all the dose likelihood functions, weighted according to the likelihood given in red, produces the final distribution of dose (green curve), from which a mean, a most likely or a median value, as well as 95% limits, can be calculated. The development of the computer program required checks of convergence of the dose results depending on the increments in yield and dose used for the calculation. Moreover, in order to simulate a biologically realistic situation, it has been assumed that all negative dose values correspond to zero dose. In table 3, the estimated doses obtained employing the simplified method are compared to those from the explicit calculation. Strictly, the 95% confidence limits in the simplified method and the 95% limits in the calculation are not equivalent, nevertheless, a very good agreement between the two set of data for the commonly encountered numbers of aberrations entirely justifies the use of the simplified method for the purposes of biological dosimetry. However, for very high and very low yields of aberrations notable departures from the results of the simplified approach may be expected. Whilst at low doses the difference is due to negative doses not being permitted in the Bayesian calculation, at high doses it is the errors in the curve that become increasingly significant. For further discussion on the minimum detectable dose by biodosimetry see Lloyd *et al* (2006). The investigation has also confirmed the general feeling that the uncertainties on a *good* calibration curve contribute little to the total convoluted uncertainty of the estimated dose in contrast to the uncertainties associated with the Poisson nature of the

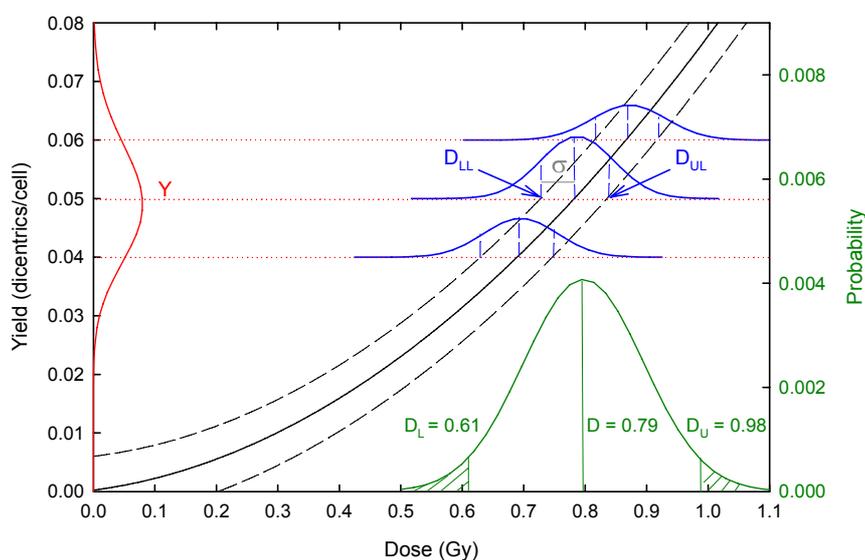


FIGURE 3 Schematic illustration of the explicit dose calculation which takes into account both the Poisson nature of the yield distribution and the uncertainties in the calibration curves: black curve - γ -ray calibration curve $Y = 0.0005 (\pm 0.0004) + 0.0128 (\pm 0.0031)D + 0.064 (\pm 0.0021)D^2$; black dashed curves - standard error on the calibration curve (exaggerated in the figure for clarity); red curve - the yield likelihood function for 25 dicentric cells scored in 500 cells; blue curves - dose likelihood functions; green curve - dose distribution. For more details see text.

yield distribution. Indeed in the case of γ -rays for the yields considered in table 3, it has been verified that σ would have to be increased by a factor larger than three in order to observe considerable differences in the 95% limits of dose.

The above mentioned procedures for the dicentric assay such as principles of curve fitting, distribution testing, derivation of uncertainties apply equally to the FISH assay as well as the micronucleus assay. In the latter case, it is worth noting that micronuclei are inherently overdispersed. For FISH, statistical analysis using the frequency of aberrations detected with chromosome painting probes depends on, amongst others, the number of cells analysed, the cocktail of chromosomes used and the exposure time. The full genome translocation frequency is given by $F_G = F_p / 2.05f_p(1 - f_p)$, where F_p is the aberration frequency detected by FISH and f_p is the fraction of genome hybridized (Lucas, 1992) taking into account the gender of the subject. Values of f_p can be obtained from Morton (1991). It is important to note that the control level of aberrations in the case of FISH analysis is much higher than in the case of the dicentric assay and is variable, with age being the main confounding factor. This is primarily due to the fact that FISH is a retrospective technique and, thus, reflects a cumulative lifetime dose to the bone marrow. In figure 4, the results of the most comprehensive study to date of the dependence of the background levels of stable translocations on age is shown, where the age group of 80 years or more produced approximately 15 translocations per 1000 cells. To summarize, the calibration curves used in the case of FISH for the dose assessment differ from those for the dicentric assay in that the c coefficient in equation 2 is age-dependent and the quadratic term is equal to zero for all qualities of radiation as the exposure is protracted, i.e. the probability of a translocation being produced by two or more different tracks is negligible.

TABLE 3 A comparison between the dose and the 95% limits obtained using the simplified approach and the explicit calculation for various numbers of dicentrics scored in 500 cells in the case of γ -ray and fission neutron irradiation. The dose-response curves used are shown in brackets.

Dicentrics	Simplified method			Explicit calculation		
	95% LCL	Mean dose (Gy)	95%UCL	95% LL	Mean dose (Gy)	95%UL
γ -rays: $Y = 0.0005(\pm 0.0004) + 0.0128(\pm 0.0031)D + 0.064(\pm 0.0021)D^2$						
0	0	0	0.242	0	0.070	0.249
5	0.129	0.297	0.505	0.164	0.326	0.506
20	0.519	0.692	0.883	0.538	0.704	0.882
50	0.978	1.151	1.336	0.990	1.154	1.334
Neutrons: $Y = 0.0005(\pm 0.0005) + 0.832(\pm 0.009)D$						
0	0	0	0.008	0	0.002	0.008
5	0.003	0.011	0.028	0.005	0.013	0.025
20	0.029	0.048	0.074	0.028	0.046	0.068
50	0.089	0.120	0.158	0.083	0.112	0.148

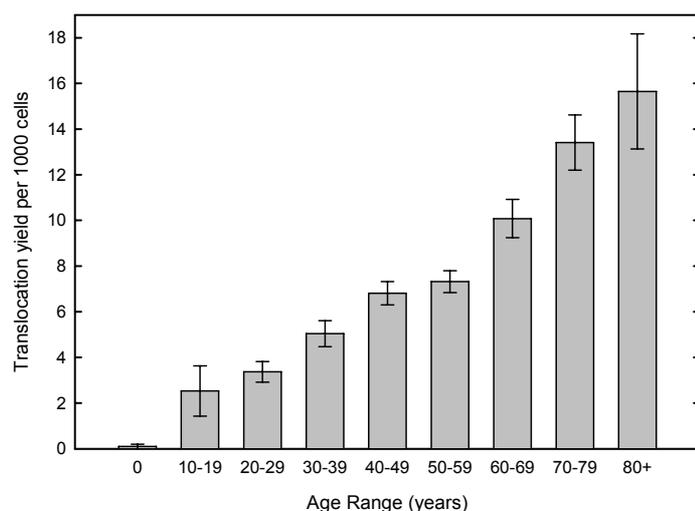


FIGURE 4 Dependence of the background level of stable translocations on age measured with FISH (Whitehouse *et al*, 2005).

3 EXAMPLES OF DOSE ESTIMATION

3.1 Criticality

In a criticality scenario the body is irradiated by both neutrons and γ -rays. Since the ratio of doses due to neutrons and γ -rays is often known from physical measurement, the separate doses due to the two types of radiation can be obtained iteratively as follows:

- (1) Initially, all aberrations are assumed to be due to neutrons, and from the measured yield of dicentrics, a dose is estimated.
- (2) The ratio of neutron to γ -ray is used to estimate the dose due to γ -rays.
- (3) From the estimate of γ -ray dose a yield of dicentrics due to γ -rays is obtained.
- (4) The neutron yield is obtained by subtracting the yield of dicentrics in (3) from the measured yield.
- (5) A new estimate of neutron dose is made and stages (2) to (5) are repeated until a self-consistent solution is found.

Example: Following a criticality accident 100 cells are scored and 100 dicentrics are observed. The neutron to γ -ray ratio available from physical measurements is 2:3. Cytogenetic dose estimates are to be made with 0.7 MeV fission neutron and ^{60}Co γ -ray calibration curves:

$$\text{Neutron: } Y = 0.0005 + 0.83D$$

$$\gamma\text{-rays: } Y = 0.0005 + 0.014D + 0.076D^2.$$

- (1) 100 dicentrics/100 cells = 1.0 dicentric per cell is equivalent to 1.20 Gy neutron dose.
- (2) $1.20 \times 3/2 = 1.81$ Gy γ -rays.
- (3) 1.81 Gy γ -rays is equivalent to 0.275 dicentrics per cell.
- (4) $1.0 - 0.275 = 0.725$, which is the dicentric yield attributable to neutrons.
- (5) 0.725 dicentrics per cell is equivalent to 0.87 Gy neutron dose.

In table 4, the sequence of steps (1) to (5) is laid out. After a few iterations a solution is found with doses of approximately 1.0 and 1.5 Gy due to neutrons and γ -rays, respectively.

An alternative approach developed for criticality incidents involves an elaborate Bayesian type of calculation (Brame and Groer, 2003), where uncertainties in all parameters can be rigorously taken into account by expressing them in terms of probability distributions. This technique, although time-consuming, is particularly useful in situations where the γ /neutron ratio is uncertain (Voisin *et al*, 2004). However, the two methods give results in good agreement.

TABLE 4 Sequence of steps for estimation of doses for mixed neutron and γ -ray irradiation.

Step 1 & 5 Neutron dose (Gy)	Step 2 γ -ray dose (Gy)	Step 3 γ -ray yield (dics/cell)	Step 4 Neutron yield (dics/cell)
1.20	1.81	0.257	0.725
0.87	1.31	0.149	0.851
1.02	1.54	0.202	0.798
0.96	1.44	0.178	0.822
0.99	1.48	0.188	0.812

3.2 Low dose over-exposure

A case of a worker who returned a thermoluminescence dosimeter badge, which recorded a dose of 66 mSv is considered. This was reported to be due to γ -rays of energy around 100keV and it was not possible to discount it as a false reading due to various circumstances. Subsequently, the dose was allocated to the worker's dose record with a consequence of the worker being removed from radiological work. This case was referred for a cytogenetic test, which resulted in an observation of 1 dicentric in 1000 metaphases. From the calibration curve $Y = 0.001 + 0.04D + 0.06D^2$ a dose estimate of zero with 95% confidence limits ranging from 0 to 100 mGy was obtained and, thus, a dose of 66 mSv could not be discounted. However, there is a different way in which results can be presented. If only two possibilities are taken into consideration: zero dose or 66 mGy, then the odds in favour of zero dose come out as 4.5:1. This is obtained from the relative probabilities of observing 1 dicentric from the two doses. If the dose was zero, then the expected number of dicentric to be observed is 1 in 1000 (the presumed background). For a dose of 66 mGy, 3.9 dicentrics are expected in 1000 cells

by reference to the calibration curve. Therefore, the chances of observing 1 dicentric when 1 and 3.9 are expected, derived from a Poisson distribution, are $e^{-1} = 0.368$ and $3.9 \times e^{-3.9} = 0.08$, respectively. The ratio of the two values is approximately 4.5.

In this particular case, the latter approach of presenting the results might be more appropriate because on the balance of probabilities this was a dose received by the badge and not the person. Whilst long-term health effects at such low doses are unlikely, there are legal and social consequences. In table 5, 95% confidence limits for various low yields of dicentrics in 1000 cells are shown together with odds ratios representing the probability of zero or 100 mGy dose.

TABLE 5 95% upper and lower confidence limits on various low yields of dicentrics in 1000 cells and the odds ratios showing the likelihood of zero or 100 mGy dose ($Y=0.001+0.04D+0.06D^2$).

Observations (dicentrics)	Dose (Gy)			Odds ratio P(0Gy):P(0.1Gy)
	LCL	Mean	UCL	
0	-	0	0.06	180:1
1	0	0	0.10	25:1
2	0	0.02	0.13	4:1
3	0	0.05	0.16	1:1
4	0	0.07	0.18	1:9
5	0.02	0.10	0.21	1:50
6	0.03	0.11	0.23	1:310
7	0.04	0.13	0.25	1:1900

3.3 Partial body exposure

In the case of a partial body exposure a more realistic estimate of dose may be given than simply stating the mean to the whole body. For partial body exposures, where a high dose is received inhomogeneously over the body, the distribution of dicentrics amongst all scored cells is overdispersed and this is used to estimate the extent of partial body irradiation. The observed distribution of cells is assumed to comprise: (i) a Poisson distribution, which represents the irradiated fraction of the body, and (ii) the remaining unexposed part. It should be stressed that it is a simplified way of approximating a real situation where a gradient of doses is expected. Undamaged cells include two subpopulations, those from the unexposed fraction and irradiated cells representing the first term of the Poisson series (e^{-Y} , i.e. irradiated but no dicentrics observed). Maximum likelihood estimates of the fraction, f , of cells scored which were irradiated and the corresponding mean yield, Y , may be made using the following equations (Lloyd *et al*, 1991):

$$\frac{Y}{1 - e^{-Y}} = \frac{X}{N - n_0} \quad (3)$$

$$f = \frac{X}{NY}, \quad (4)$$

where N is the number of cells scored, X is the number of dicentrics observed and n_0 is the number of cells free of dicentrics. Equation (3) can only be solved iteratively. Then, the mean dose to the irradiated fraction of the body can be estimated using the standard dose-response curves. In order to derive an actual fraction of body exposed to radiation, f needs to be corrected for the effects of mitotic delay and interphase death (apoptosis), as these are the factors reducing the ability of cells to reach metaphase in a 48 hour culture. If the fraction of irradiated cells which reach metaphase was p , the fraction of the body irradiated, F , is given by:

$$F = \frac{f / p}{1 - f + f / p} \quad (5)$$

At HPA, the computer program CtamPoiss (available on request) is used to solve the contaminated Poisson problems. For more details on the limitations of the above-mentioned approach see IAEA (2001).

An alternative approach to estimate a dose delivered in a partial body exposure was proposed by Sasaki and Miyata (1968) and is known as the Qdr method. In this case, the yield of dicentrics and rings in just the damaged cells is considered, assuming that these cells were present at the time of the irradiation. Then, Qdr is the expected yield of dicentrics and rings (X) amongst the damaged cells (N_d) and is given by:

$$Qdr = X / N_d = Y_1 / (1 - \exp(-Y_1 - Y_2)), \quad (6)$$

where Y_1 and Y_2 are yields of dicentrics plus rings and excess acentrics, respectively. Despite the limitations of the original Qdr method, which include the lack of any information on the size of the fraction irradiated as well as incorrect assumptions that excess acentric aberrations conform to a Poisson distribution and that the background frequency of dicentrics plus rings is zero, the agreement between the two methods described is good.

Additionally, in an attempt to reduce the oversimplification of regarding the exposure geometry as two fractions an unirradiated and a uniformly irradiated component, Sasaki (2003) suggested an approach whereby the chromosome aberration distribution is unfolded into a mixed Poisson distribution and, from that, into a dose-distribution profile.

Example: An *in vitro* experiment was performed to simulate partial body exposure, whereby 50% of irradiated cells (5 Gy, 250kVp x-ray) were mixed with unirradiated ones from the same donor (Lloyd *et al*, 1991). The number of dicentrics scored was 45 in 247 cells, with the following distribution: 225 cells with zero, 4 with one, 13 with two and 5 with three dicentrics. This produced the ratio of variance to mean of 2, therefore, an overdispersion expected from partial body exposure was revealed. The application of the contaminated Poisson method using the dose-effect curve of $Y=0.0005+0.04D+0.06D^2$ and the value of $D_0=3$ Gy ($p = e^{-D/D_0}$), which represents the relative selection against irradiated cells reaching metaphase, resulted in an estimate of the mean dose of (4.9 ± 0.5) Gy delivered to an estimated $(40 \pm 6)\%$ of the body. Only $(0.11 \pm 0.02)\%$ of the scored cells appeared to have been irradiated, which demonstrates the need to compensate for the reduction due to interphase death and mitotic delay. In order to carry out the Qdr analysis, the number of dicentrics plus rings, 48 in this case, as well as the number of damaged cells, 31, and the following dose-

response calibration curves are required: $Y_{dics+rings} = 0.0005 + 0.043D + 0.063D^2$ and $Y_{acentrics} = 0.0035 + 0.051D + 0.11D^2$. By substitution into equation (6):

$$Qdr = 48 / 31 = 1.55 = \frac{0.0005 + 0.043D + 0.063D^2}{1 - \exp(-0.0035 - 0.051D - 0.11D^2)}. \quad (7)$$

The equation can be solved for D iteratively and gives an estimate of 4.4 Gy. Sasaki and Miyata (1968) did not state how uncertainties on dose could be calculated and it is not at all obvious how this should be done. The problem lies in the fact that both the number of dicentrics and rings as well as the number of damaged cells carry uncertainties, which are correlated. It might be possible to use Monte Carlo simulation techniques to solve the problem but this has not, to our knowledge, been done. This problem becomes even more complex when the errors in the calibration curves are considered. Nevertheless, the contaminated Poisson approach and the Qdr method yield consistent results.

3.4 Protracted and fractionated exposure

Protraction or fractionation of exposure can result in reduced numbers of observed chromosome aberrations. Whilst it is not the case for high LET radiation, because the dose-response curve is linear, in the case of low LET radiation the β coefficient in equation 1 is reduced. This is due to repair mechanisms that have time to operate during the course of the protracted exposure. It has been shown that the decrease in the frequencies of aberrations appears to follow an exponential function with a mean time of approximately 2 hours.

Lea and Catchside (1942) considered a time-dependant factor, known as the G function, to facilitate the modification of the dose squared coefficient as follows:

$$Y = \alpha D + \beta G(x) D^2, \quad (8)$$

where $G(x) = 2(x - 1 + \exp(-x)) / x^2$ and $x = t / t_0$, t is the time over which the radiation occurred and t_0 is the mean lifetime of the breaks (~ 2 hours). Applying these ideas to protracted and fractionated cases requires a certain amount of information about the incident such as the time over which exposure happened and the constancy of the dose rate. The analysis is only worthwhile in instances where large doses and long exposure times (up to few days) are experienced. For a dose being delivered over a very long period of time, $G(x)$ reaches zero since equation 1 reduces to $Y = c + \alpha D$. For interfraction times shorter than about 6 hours, the G function can be written as $\exp(-t_1 / t_0)$, where t_1 is the time between fractions.

Example: In 1998 a serious accident occurred in Turkey when a ^{60}Co teletherapy source was sold as scrap metal. Ten persons were exposed, mainly during one day, with exposure times ranging from 2 to 7 hours. Here, a case of one of the subjects exposed during 6 hours is considered. The pooled results from three laboratories yielded 474 dicentrics scored in 1363 cells. From the acute dose-response curve $Y = 0.001 + 0.02D + 0.06D^2$, the acute dose ($\pm \text{SE}$) of 2.2 ± 0.1 Gy is obtained. The application of the G function with $x = t / t_0 = 6 / 2 = 3$ gives $G(x) = 0.46$, hence, the dose-

response becomes $Y = 0.001 + 0.02D + 0.028D^2$ and the dicentric frequency corresponds to a 6 hour exposure of 3.2 ± 0.1 Gy.

4 SUMMARY

Statistical methods in biological dosimetry are well developed as over the years a wealth of information has been gleaned through the experience of dealing with a number of different radiological incidents as well as *in vitro* experiments designed to explore possible irradiation scenarios. In this report, appropriate statistical techniques have been reviewed and discussed in order to highlight the scope of their application. These are accompanied by worked examples, often extracted from actual incidents, which further elucidate the principles underlying each of the approaches. It is worth emphasising that different radiological scenarios require different sets of information in order to carry out meaningful analyses. It is also pleasing to confirm that approximations used routinely in the calculation of 95% confidence limits on dose are in a very good agreement with a stringent Bayesian-like calculation which takes into account both the Poisson derived uncertainties in yield as well as uncertainties in the dose-response calibration curve.

PC compatible programs available on request from HPA: CtamPoiss and MIPol.

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