INSTITUTE OF PHYSICS PUBLISHING PHYSICS IN MEDICINE AND BIOLOGY

Phys. Med. Biol. **47** (2002) 3197–3209 PII: S0031-9155(02)37834-5

Equivalence of the linear–quadratic and two-lesion kinetic models

M Guerrero[1], Robert D Stewart[2], Jian Z Wang[1] and X Allen Li[1]

[1] Department of Radiation Oncology, University of Maryland, Baltimore, MD 21201, USA
[2] Radiation and Biophysics Research, Pacific Northwest National Laboratory, Richland, WA 99352, USA

E-mail: mguer001@umaryland.edu

Received 10 June 2002
Published 21 August 2002
Online at stacks.iop.org/PMB/47/3197

Abstract

Double strand breaks (DSBs) are widely accepted as the main type of DNA damage responsible for cell killing in the range of doses and dose rates relevant to radiation therapy. Although the standard linear–quadratic (LQ) model with one first-order repair term often suffices to explain the results of some radiobiological experiments, converging lines of evidence suggest that DSBs are rejoined at two or more distinct rates. A two-lesion kinetic (TLK) model has been proposed to provide a direct link between biochemical processing of the DSBs and cell killing. A defining feature of the TLK model is that the family of all possible DSBs is subdivided into simple and complex DSBs, and each kind may have its own unique repair characteristics. Break-ends associated with both kinds of DSB are allowed to interact in pairwise fashion to form irreversible lethal and non-lethal chromosome aberrations.

This paper examines the theoretical and practical linkages between the TLK and LQ models. The TLK formalism is used to derive an LQ formula with two first-order repair terms (dose protraction factors) and to relate the intrinsic radiosensitivity parameters used in one model to the parameters used in the other. Two extensive radiobiological datasets, one for CHO 10B2 cells and one for C3H 10T1/2 cells, are analysed using the TLK and LQ models. The LQ with two repair terms and the TLK are equally capable of explaining the CHO 10B2 and C3H 10T1/2 cell survival data. For the doses and dose rates most relevant to radiation therapy, tests of model equivalence indicate that an LQ formula with two first-order repair terms is an excellent approximation to the TLK model. We find the LQ and TLK models useful complementary tools for the analysis and prediction of radiobiological effects.

1. Introduction

The linear–quadratic (LQ) formalism is the most prevalent model used to predict the radiation killing of cells in clinical applications. The mechanistic basis for the standard LQ model has been discussed extensively in the literature (reviewed in Brenner *et al* 1998, Sachs *et al* 1997). A particularly attractive feature of the LQ formalism is that trends in the cell surviving fraction as a function of dose and dose rate can be explained using a minimum number of adjustable parameters. Other more mechanistic models use systems of ordinary differential equations to link the formation and repair of double strand breaks (DSBs) to cell killing. The lethal–potentially lethal (LPL) model (Curtis 1986) and the repair–misrepair (RMR) model (Tobias 1985) are two outstanding examples of such kinetic models. In the LPL and RMR models, DSB rejoining is modelled using first-order (linear) and second-order repair mechanisms. Second-order repair is also termed binary misrepair or pairwise damage interaction. Alternatives and extensions to the LPL and RMR models have been reviewed by Sachs *et al* (1997).

Others (Curtis 1986, Sachs *et al* 1997, Brenner *et al* 1998) have shown that the LPL and RMR models can be used to derive the standard LQ formula (one first-order repair term) in the limit of small doses and dose rates. The standard LQ is also an approximate solution for kinetic models that postulate saturable repair mechanisms instead of non-saturable first- and second-order repair mechanisms (Brenner *et al* 1998). However, as a model to link the biochemical processing of the DSB to cell killing, the LPL is not completely satisfactory. That is, the LPL model cannot directly link the formation and repair of DSBs to cell killing without invoking additional ad hoc hypotheses about the nature of the initial damage responsible for cell killing, e.g., only a small subset of the initial DSBs is potentially lethal (e.g., see Stewart (2001)). Similar arguments apply to the RMR and standard LQ model because of their close theoretical linkages to the LPL model.

The two-lesion kinetic (TLK) model has been proposed to make a better link between biochemical processing of the DSB and cell killing (Stewart 2001). A defining feature of the TLK model is that the family of all possible DSBs is subdivided into simple and complex DSBs, and each kind of DSB may have its own unique repair characteristics. Break-ends associated with both kinds of DSB are allowed to interact in pairwise fashion to form irreversible lethal and non-lethal chromosome aberrations. Although the TLK model may suffice to link biochemical processing of the DSB to cell killing (Stewart 2001), the number of parameters used in this model can be prohibitive for some applications, if the parameters are treated as purely ad hoc. Through the use of biologically meaningful equality and inequality constraints, the number of free parameters used in the TLK can be effectively reduced to a level comparable to the LQ or other models.

An alternative to applying equality and inequality constraints to TLK parameters is to derive an LQ formula that approximates the TLK in the limit of small doses and dose rates, as has been done for the LPL and RMR kinetic models[3]. In effect, the LQ formalism reduces the number of adjustable parameters to a minimum by aggregating some of the TLK parameters together. The LQ formula derived from the TLK model uses five parameters to characterize intrinsic radiosensitivity, compared to three for the standard LQ model (α, β and λ) and 16 for the most general form of the TLK. The two first-order repair-rate constants of the TLK model result in two protraction factors compared to one for the standard LQ model. LQ models with two first-order repair processes have been successfully applied to the analysis of survival data in mouse lung (Van Rongen *et al* 1993, Millar and Canney 1993), pig skin (Millar *et al* 1996), mouse kidney (Millar *et al* 1994) and spinal cord (Ang *et al* 1992).

[3] Sachs *et al* (1997) used a two-lesion model similar to the TLK to derive an LQ formula with two first-order repair terms.

The main purpose of this paper is to test the equivalence of the LQ formula as an approximation to the TLK model. The paper also examines some of the theoretical and practical linkages between the TLK and LQ models. The relationship between the radiosensitivity parameters used in the LQ and TLK models can be used to better understand the linkages between LQ parameters and biochemical processing of DSBs and to help integrate information on DSB formation and repair into estimates of LQ radiosensitivity parameters (e.g., to derive biologically based constraints on α and β). Alternatively, the relationship between the LQ and TLK model parameters can be used to identify constraints on the TLK parameters that reflect the wealth of empirical LQ radiosensitivity information available in the literature.

Section 2 summarizes the key equations relating the TLK model to the LQ model(s). Biologically plausible constraints on the TLK parameters and the numerical methods used to solve the TLK model are presented in section 3. In section 4.1, two extensive radiobiological datasets, one for CHO 10B2 cells (Stackhouse and Bedford 1993) and one for C3H 10T1/2 cells (Wells and Bedford 1983), are analysed using the TLK. Section 4.2 uses the TLK parameters obtained from this analysis to examine the accuracy of the LQ formula as an approximate solution to the full TLK model. As expected, the LQ is a good approximation to the TLK model for low doses and low dose rates. In section 4.3, the LQ formula is treated as an independent model, and an alternate set of LQ parameters is obtained by directly fitting the model to the survival data. When treated as a stand-alone model, the LQ fits the survival data as well as the TLK model, regardless of dose, dose rate and intrinsic radiosensitivity.

2. Relationship between the LQ and TLK models

An LQ formula can be derived from kinetic models such as the LPL, RMR and TLK using perturbation theory or other methods (Curtis 1986, Sachs *et al* 1997 and Brenner *et al* 1998). The following two subsections briefly outline the TLK model and the key equations relating the TLK to the LQ. Because the LQ formula derived from the TLK model uses two first-order repair terms to describe the DSB rejoining rate, we will henceforth call this formula the LQ^2 model.

2.1. TLK model

The most general form of the TLK model (Stewart 2001), which uses 16 biologically significant parameters to relate biochemical processing of the DSB to mutagenesis and cell killing, can be reduced to the LPL (Curtis 1986) or RMR (Tobias 1985) models by applying various equality constraints to the TLK parameters. Here, we briefly summarize a variant of the TLK that retains most of the central features of the full model while reducing the number of parameters from 16 to 10. An analysis of DSB rejoining kinetics and CHO-cell survival for a large range of single-dose and split-dose exposure conditions suggests that this model may suffice to directly link biochemical processing of DSBs to cell killing (Stewart 2001)[4].

The following pair of nonlinear differential equations models the time-dependent rate at which DSBs are created and then repaired, misrepaired or fixed:

$$\frac{\mathrm{d}\bar{L}_1(t)}{\mathrm{d}t} = 2\dot{D}(t)Y\Sigma_1 - \lambda_1\bar{L}_1(t) - \eta\bar{L}_1(t)[\bar{L}_1(t) + \bar{L}_2(t)] \tag{1}$$

$$\frac{\mathrm{d}\bar{L}_2(t)}{\mathrm{d}t} = 2\dot{D}(t)Y\Sigma_2 - \lambda_2\bar{L}_2(t) - \eta\bar{L}_2(t)[\bar{L}_1(t) + \bar{L}_2(t)] \tag{2}$$

[4] The formalism of the TLK model does not explicitly account for cell-cycle and other proliferation-related phenomena that are relevant to the analysis of some radiobiological experiments.

where $\dot{D}(t)$ is the instantaneous absorbed dose rate at time t (Gy h^{-1}), $\bar{L}_1(t)$ is the expected number of simple (type 1) DSBs per cell at time t and $\bar{L}_2(t)$ is the expected number of complex (type 2) DSBs per cell at time t. The total number of DSBs expected per cell at time t is $\bar{L}_1(t) + \bar{L}_2(t)$. As a working hypothesis, simple DSBs are assumed to be a section of the DNA 10 to 20 base pairs (bps) in length that contains a break in both strands of the DNA. A complex DSB is a simple DSB that contains additional elementary damage sites (base damage, strand breaks, base deletion, etc) within the same section of DNA (Stewart 2001).

The initial DSB yield (Gy^{-1} $cell^{-1}$) is characterized by the number of bps per cell Y (the factor of 2 converts bp to number of nucleotides) and the formation probabilities Σ_1 and Σ_2. The parameters λ_1, λ_2 and η characterize the rate at which break-ends are rejoined by enzymatic processes. The biophysical interpretations of these parameters are discussed in more detail elsewhere (Stewart 2001). For the special case of $\eta = 0$ (no binary misrepair) the expected number of DSBs per cell decreases exponentially with time after irradiation. For the first-order unsaturated rejoining kinetics, the rate constants λ_1 and λ_2 can be related to the expected amount of time required for a cell to remove half of the initial DSBs created by an acute dose of radiation. That is, the so-called repair half-time for the ith kind of DSB, denoted τ_i, equals $\ln(2)/\lambda_i$.

The time-dependent rate at which DSBs are converted into lethal genetic alterations (point mutations or chromosome aberrations) is modelled by

$$\frac{\mathrm{d}\bar{L}_f(t)}{\mathrm{d}t} = (1-a_1)\varphi_1\lambda_1\bar{L}_1(t) + (1-a_2)\varphi_2\lambda_2\bar{L}_2(t) + \gamma\eta[\bar{L}_1(t) + \bar{L}_2(t)]^2 \tag{3}$$

where a_1 and a_2 represent the fidelity of the linear misrepair mechanism (e.g., $a_i = 1$ indicates correct repair). The probabilities φ_i and γ partition misrepaired damages into lethal and non-lethal genetic alterations ($\varphi_i = 1$ means that linear misrepair of a DSB always produces a fatal lesion). The equation describing the accumulation of non-lethal genetic alterations is

$$\frac{\mathrm{d}\bar{L}_m(t)}{\mathrm{d}t} = [(1-a_1)(1-\varphi_1)\lambda_1\bar{L}_1(t) + (1-a_2)(1-\varphi_2)\lambda_2\bar{L}_2(t)] + (1-\gamma)\eta[\bar{L}_1(t) + \bar{L}_2(t)]^2. \tag{4}$$

2.2. LQ² approximation for the TLK model

To derive the LQ^2 solution, the TLK system of differential equations is integrated using a perturbation theory approach, under the assumption that the binary misrepair probability η is small. The expected number of fatal lesions per cell as time goes to infinity is given by

$$\bar{L}_f(\infty) = \alpha D + (\beta_1 G_1 + \beta_2 G_2)D^2 \tag{5}$$

where

$$G_i = \frac{2}{D^2}\int_{-\infty}^{\infty} \mathrm{d}t\, \dot{D}(t) \int_{-\infty}^{t} \mathrm{d}t'\, \dot{D}(t)\, \mathrm{e}^{-\lambda_i(t-t')} \qquad i = 1, 2. \tag{6}$$

The dose protraction factors, G_i, can be derived and expressed in several ways (see Sachs *et al* (1997) and references therein). The coefficients α and β_i are related to the TLK model parameters as

$$\alpha = (1-a_1)\varphi_1 2Y\Sigma_1 + (1-a_2)\varphi_2 2Y\Sigma_2 \tag{7}$$

$$\beta_1 = \delta + [\gamma - (1-a_1)\varphi_1]\frac{(2Y\Sigma_1)^2}{2\chi_1} \tag{8}$$

$$\beta_2 = \delta + [\gamma - (1 - a_2)\varphi_2]\frac{(2Y\Sigma_2)^2}{2\chi_2} \tag{9}$$

$$\delta \equiv [2\gamma - (1 - a_1)\varphi_1 - (1 - a_2)\varphi_2]\frac{(2Y\Sigma_1)(2Y\Sigma_2)}{2(\chi_1 + \chi_2)} \tag{10}$$

with $\chi_i \equiv \lambda_i/\eta$. Expressions for the expected number of non-lethal mutations $\bar{L}_m(\infty)$ can be derived by substituting $\varphi_i \to (1 - \varphi_i)$ and $\gamma \to (1 - \gamma)$.

The standard, three-parameter LQ model can be mimicked using the TLK formalism by imposing the equality constraints: $a_i = a, \varphi_i = \varphi, \lambda_i = \lambda, 2\Sigma_i = \Sigma$. For these constraints, $G_1 = G_2 \to G$ and $\beta = (\beta_1 + \beta_2)$ so that equation (5) reduces to $\bar{L}_f(\infty) = D[\alpha + \beta G D]$ with

$$\alpha = 2Y\Sigma(1 - a)\varphi \tag{11}$$

$$\beta = \eta(2Y\Sigma)^2[\gamma - (1 - a)\varphi]/2\lambda. \tag{12}$$

Equations (7)–(12) relate the radiobiological parameters of the LQ, LQ^2 and TLK models. In equation (7), each type of DSB contributes to the LQ^2 radiosensitivity parameter α with a term directly proportional to the yield of DSBs per cell per Gy and to the fraction of DSBs that becomes fatal due to incorrect repair, $(1 - a_i)\varphi_i$. According to equations (8)–(10), the parameters β_i are related to the square of the individual yields and their cross product, indicating the interaction among DSBs. There is also a correlation between β_i and the ratios η/λ_i and $\eta/(\lambda_1 + \lambda_2)$. These relationships are important regarding the question of whether the repair mechanisms postulated by the TLK are a good description of cellular recovery kinetics. For example, a positive correlation between the values of α and β has been reported (Peacock *et al* 1992) and a correlation between β and the repair time is also known to exist (Brenner 1992). Equations (7)–(10) show that the TLK predicts a positive correlation between α and β since they both depend on the yields of DSBs and equations (8)–(10) demonstrate a proportionality of β to the repair time.

The validity of the LQ^2 formula as an approximation to the TLK model ultimately hinges on the relative numbers of DSBs removed by first- and second-order repair. Others have shown that the standard LQ model is a low dose, low dose-rate approximation for kinetic models such as the LPL and RMR models (Tobias 1985, Curtis 1986, Sachs *et al* 1997, Brenner *et al* 1998). Similarly, the LQ^2 is a low dose, low dose-rate approximation of the TLK. The standard LQ is a good approximation to the LPL and RMR models for doses less than about α/β and dose rates less than about $\lambda\alpha/\beta$ (Sachs *et al* 1997, Brenner *et al* 1998). The accuracy of the LQ^2 model as an approximation to the full TLK also improves as α/β increases.

3. Methods

3.1. Numerical solution of the TLK model

A virtual cell (VC) radiobiology computer program[5] has been developed to solve the TLK model for an arbitrary dose-rate function $\dot{D}(t)$. In this software, Visual Numeric's (http://www.vni.com) IMSL® DIVPAG routine is used to integrate the TLK system of differential equations forward in time using Gear's backward differentiation algorithm (Gear 1971, Shampine and Gear 1979). Prior to irradiation, cells are assumed undamaged so that $\bar{L}_1(0) = \bar{L}_2(0) = \bar{L}_f(0) = 0$. The expected fraction of the cells capable of producing viable progeny at time t is given by $S(t) = \exp\{-\bar{L}_f(t)\}$ (Sachs *et al* 1997, Stewart 2001). The VC

[5] R D Stewart, virtual cell (VC) radiobiology software. PNNL-13579, July 2001 (update 1.10J released March 2002). Available online at http://www.pnl.gov/berc/kbem/vc/.

software includes an automated (least-squares) procedure to identify an optimal set of TLK parameters from cell survival data. Details of this procedure are described elsewhere (Stewart 2001).

3.2. Biological constraints on TLK model parameters

Effective strategies to calibrate the TLK model require the judicious application of equality and inequality constraints. For mammalian cells irradiated *in vitro*, analysis of cell survival data using the LQ formalism often yields values of α (Gy^{-1}) in the range [0, 1] and values of β (Gy^{-2}) in the range $[10^{-3}, 0.2]$ (Deschavanne *et al* 1990, Peacock *et al* 1992). Reasonable biophysical considerations (Stewart 2001) suggest the following constraints on TLK model inputs:

$$\begin{aligned}
&\gamma = 0.25\\
&0 \leqslant a_i \leqslant 0.995\\
&0 \leqslant \varphi_i \leqslant 2.5 \times 10^{-2}\\
&0 \leqslant (1 - a_i)\varphi_i \leqslant 2.5 \times 10^{-2}\\
&0.02 \leqslant \tau_1 \leqslant \tau_2 \leqslant 25\,\mathrm{h}\\
&0\,\mathrm{h}^{-1} \leqslant \eta \leqslant 10^{-2}\,\mathrm{h}^{-1}\\
&Y\Sigma_1 = 3Y\Sigma_2\\
&20\,\mathrm{Gy}^{-1}\,\mathrm{cell}^{-1} \leqslant 2Y(\Sigma_1 + \Sigma_2) \leqslant 80\,\mathrm{Gy}^{-1}\,\mathrm{cell}^{-1}.
\end{aligned} \tag{13}$$

This set of constraints provides a relatively high degree of flexibility for the analysis of cell survival data, imposes some reasonable restrictions on the allowed range of values, and effectively decreases the number of 'adjustable' TLK parameters from ten to six or less. Of course these constraints should be adjusted to reflect any extra, cell-specific information that may be available.

4. Results and discussion

The main purpose of this paper is to test the equivalence of the LQ^2 formula as an approximation to the full TLK model. Presentation of the results and equivalence tests is organized as follows. First in section 4.1, representative TLK parameters for CHO 10B2 and C3H 10T1/2 cells are identified. The CHO 10B2 and C3H 10T1/2 datasets are used because these two cell lines have very different intrinsic radiosensitivities and because survival data are available for a wide range of exposure conditions. As discussed in section 2.2, the accuracy of the low dose and dose-rate approximation used to derive the LQ^2 formula from the TLK depends on intrinsic radiosensitivity. CHO 10B2 cells have an intrinsic radiosensitivity representative of late-responding tissues (small α/β), and C3H 10T1/2 cells are more representative of early-responding tissues (large α/β). The TLK parameters obtained are converted to equivalent LQ^2 parameters using equations (7)–(10). Section 4.2 tests the equivalence of the LQ^2 and TLK models by comparing surviving fractions as a function of dose and dose rate. Section 4.3 compares the TLK-derived LQ^2 parameters to the LQ^2 parameters obtained from a global fit to the CHO 10B2 and C3H 10T1/2 cell datasets. For comparison to the LQ^2 parameters, the standard LQ model is also fit to the CHO 10B2 and C3H 10T1/2 survival datasets.

4.1. Representative TLK parameters for CHO and C3H 10T1/2 cells

Analysis of the cell survival data as a function of dose, dose rate, and dose fractionation indicates that the following set of TLK parameters are optimal for CHO 10B2 cells (Stewart 2001): $2Y(\Sigma_1 + \Sigma_2) = 25.09$ DSB Gy^{-1} cell^{-1}, $2Y\Sigma_2 = 5.09$ DSB Gy^{-1} cell^{-1} (20% of the DSBs are 'complex'), $\tau_1 = 1.03$ h, $\tau_2 = 15.8$ h, $a_1 = 0$, $a_2 = 0$, $\varphi_1 = 1.52 \times 10^{-3}$, $\varphi_2 = 0$, $\gamma = 0.25$ and $\eta = 1.18 \times 10^{-4}$ h^{-1}. Although this set of parameters provides an optimal fit to an extensive set of cell survival data, other parameter sets can be identified that provide an equally good fit to the survival data (discussed in Stewart (2001)). Even if the specific values of the individual TLK parameters are not unique, the collection of parameter values may still be considered a robust characterization of intrinsic radiosensitivity. That is, the family of TLK parameter sets that gives the best fit to the survival data yield about the same LQ model parameter values.

Wells and Bedford (1983) published C3H 10T1/2 cell survival data for a large range of doses delivered at dose rates of 0.06 Gy h^{-1}, 0.17 Gy h^{-1}, 0.29 Gy h^{-1}, 0.49 Gy h^{-1}, 2.4 Gy h^{-1} and 55 Gy h^{-1}. To identify a suitable TLK model calibration, the parameters $2Y\Sigma_1$, τ_1, τ_2, a_1 and η were treated as adjustable parameters, and the remaining parameters were set *a priori* to biologically plausible values (i.e., $2Y\Sigma_2 = 15$ Gy^{-1} cell^{-1}, $a_2 = 0$, $\varphi_i = 2.5 \times 10^{-3}$ and $\gamma = 0.25$). Wells and Bedford (1983) allowed 12 to 24 h for repair before the cells were trypsinized and resuspended in a fresh medium. This period of time was sufficient to minimize the impact on the survival assay of time-to-plating.

We found that several combinations of $2Y\Sigma_1$, τ_1, τ_2, a_1 and η gave equally good fits to the C3H 10T1/2 surviving fraction data. This indicates that the dataset does not contain sufficient information to identify a truly unique best-fit model calibration. The identification of a suitable set of DSB rejoining-rate parameters (τ_1, τ_2 and η) was especially problematic. The best fits to the surviving fraction data were obtained with values of τ_1 in the range from ~0.5 h to 1.5 h, τ_2 from ~3.5 to 15 h and η in the range from 10^{-6} to 5×10^{-5} h^{-1}. A representative set of TLK parameters that gives a good fit to the surviving fraction data is: $2Y(\Sigma_1 + \Sigma_2) = 60.8$ DSB Gy^{-1} cell^{-1}, $2Y\Sigma_2 = 15$ DSB Gy^{-1} cell^{-1} (25% of the DSBs are complex), $a_1 = 0.297$, $a_2 = 0$, $\varphi_i = 2.5 \times 10^{-3}$, $\tau_1 = 0.489$ h, $\tau_2 = 3.91$ h, $\gamma = 0.25$ and $\eta = 2.57 \times 10^{-5}$ h^{-1}.

4.2. Test 1: equivalence of the LQ^2 and TLK models using identical parameters

For CHO 10B2 cells, the best-fit TLK parameters identified in section 4.1 correspond, according to equations (7)–(10), to the following LQ^2 parameters: $\alpha = 3.04 \times 10^{-2}$ Gy^{-1}, $\beta_1 = 1.29 \times 10^{-2}$ Gy^{-2}, $\beta_2 = 1.28 \times 10^{-2}$ Gy^{-2}, $\tau_1 = 1.03$ h and $\tau_2 = 15.8$ h. The ratio $\alpha/(\beta_1 + \beta_2) = 1.18$ Gy. For C3H 10T1/2 cells, the LQ^2 parameters are: $\alpha = 0.118$ Gy^{-1}, $\beta_1 = 7.47 \times 10^{-3}$ Gy^{-2}, $\beta_2 = 6.79 \times 10^{-3}$ Gy^{-2}, $\tau_1 = 0.489$ h and $\tau_2 = 3.91$ h. The ratio $\alpha/(\beta_1 + \beta_2) = 8.27$ Gy. It is interesting to note that for both cell lines the values for β_1 and β_2 are within 10% of each other. For other representative combinations of TLK parameters, β_1 and β_2 may differ by as much as a factor of 2 (not shown).

Figure 1 shows the per cent difference in the LQ^2 and TLK predicted surviving fractions as a function of dose and dose rate. For the entire range of doses and dose rates considered, the LQ^2 formalism tends to predict higher levels of cell killing than the TLK model. The LQ^2 model predicts higher levels of cell killing because the rate of DSB rejoining is slower than that predicted using the TLK model. That is, the same value for τ_1 and τ_2 are used to model first-order rejoining kinetics, but the TLK model also removes some DSBs through second-order repair processes. At 2 Gy, the maximum per cent difference (i.e., at $\dot{D} = 10^5$ Gy h^{-1}) between the LQ^2 and TLK solutions is 0.01% for C3H 10T1/2 cells and 0.16%

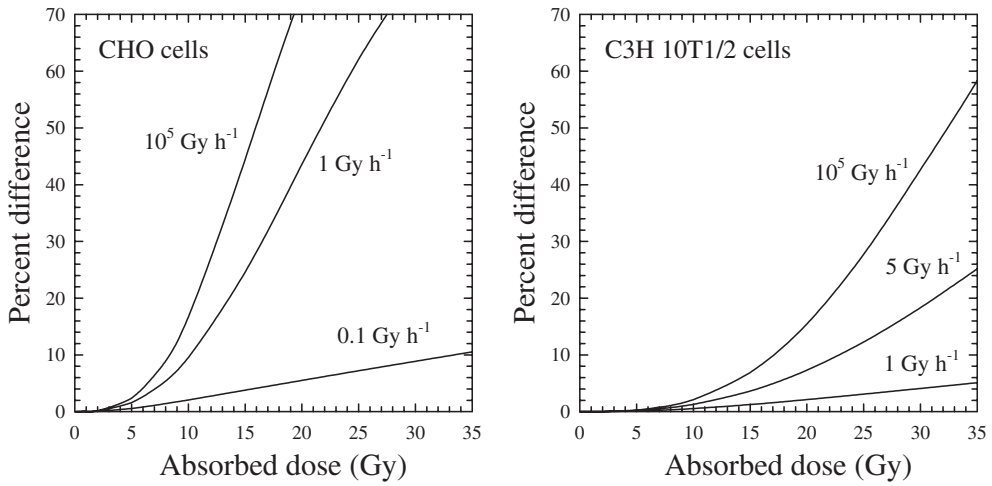

Figure 1. Per cent difference, $(S_{\mathrm{TLK}} - S_{\mathrm{LQ}})/S_{\mathrm{TLK}}$, between the surviving fractions predicted by the LQ^2 and full TLK solutions. Results are for a single dose of radiation delivered at the indicated dose rate. See main text (section 4.1) for TLK parameters. Left panel: CHO cell result. LQ^2 parameters are: $\alpha = 3.04 \times 10^{-2}$ Gy^{-1}, $\beta_1 = 1.29 \times 10^{-2}$ Gy^{-2}, $\beta_2 = 1.28 \times 10^{-2}$ Gy^{-2}, $\tau_1 =$ 1.03 h and $\tau_2 = 15.8$ h. Right panel: C3H 10T1/2 cells. LQ^2 parameters are: $\alpha = 0.118$ Gy^{-1}, $\beta_1 = 7.47 \times 10^{-3}$ Gy^{-2}, $\beta_2 = 6.79 \times 10^{-3}$ Gy^{-2}, $\tau_1 = 0.489$ h and $\tau_2 = 3.91$ h.

for CHO 10B2 cells. For a 5 Gy dose of radiation, the maximum per cent difference increases to 0.27% and 2.37% for C3H 10T1/2 and CHO 10B2 cells, respectively. At 10 Gy, the maximum per cent difference between the TLK and LQ^2 solutions is 2.11% and 16.8% for C3H 10T1/2 and CHO 10B2 cells, respectively. For higher doses, the per cent differences between the LQ^2 and TLK predicted surviving fractions continue to increase.

The per cent difference between the LQ^2 and TLK results are consistently smaller for the C3H 10T1/2 cells than for the CHO 10B2 cells, as was expected on the basis of their radiosensitivity. The LQ^2 formalism is a better approximation to the full TLK model for cells with a large α/β ratio (e.g., C3H 10T1/2 cells) than for cells with a smaller α/β ratio (e.g., CHO cells). For CHO 10B2 cells, the per cent difference between the LQ^2 and TLK solutions increases rapidly in the dose-rate range from 0.1 to 1 Gy h^{-1} (figure 1, left panel). For C3H 10T1/2 cells, similar changes occur in per cent differences for dose rates in the range from 1 to 5 Gy h^{-1} (figure 1, right panel).

For most of the doses and dose rates of interest in radiation therapy (i.e., doses less than about 2 to 5 Gy), the results shown in figure 1 clearly demonstrate that the LQ^2 and TLK predict surviving fractions which are within a few per cent of each other (i.e., the LQ^2 formula is an excellent approximation to the full TLK model). The good agreement between the LQ^2 and TLK surviving fractions for lower doses and dose rates indicates that first-order repair processes are responsible for rejoining most of the DSBs. For higher doses, second-order repair processes remove a larger fraction of the DSBs, and the differences in the LQ^2 and TLK predicted surviving fractions become more apparent (figure 1).

4.3. Test 2: comparison of direct-fit and TLK-calculated LQ parameters

The LQ^2 parameters derived from the best-fit TLK parameters may or may not provide the optimal values to predict cell survival. As an independent way to identify a set of best-fit

Table 1. Summary of LQ and LQ^2 model parameters for CHO 10B2 and C3H 10T1/2 cells. Estimates of the parameters are derived from global (least-squares) fits to survival data for all doses and dose rates. For numerical purposes only, the parameter estimates are reported to three significant digits.

	CHO 10B2 parameters			C3H 10T1/2 parameters		
	LQ^2 model		LQ model	LQ^2 model		LQ model
Parameter	TLK predicted	Direct analysis	Direct analysis	TLK predicted	Direct analysis	Direct analysis
α (Gy^{-1})	0.0304	0.0418	0.0474	0.118	0.112	0.124
β_1 (Gy^{-2})	0.0129	0.0109	0.0103	0.007 47	0.0136	0.006 75
β_2 (Gy^{-2})	0.0128	0.0113	0.0103	0.006 79	0.001 80	0.006 75
$\beta = (\beta_1 + \beta_2)$ (Gy^{-2})	0.0257	0.0222	0.0206	0.0143	0.0154	0.0135
α/β (Gy)	1.18	1.88	1.90	8.27	7.27	9.12
τ_1 (h)	1.03	0.990	6.20	0.489	0.970	1.62
τ_2 (h)	15.8	13.9	6.20	3.91	15.8	1.62

LQ^2 parameters and as a second method of testing the equivalence of the LQ^2 and TLK models, we used the NLINFIT function from MATLAB® 6.0 to perform a global fit of the LQ^2 model to the CHO 10B2 and C3H 10T1/2 datasets. The NLINFIT function is a nonlinear (Gauss–Newton), least-squares algorithm. For comparison, the standard three-parameter LQ model was also fit to these two datasets. Table 1 summarizes the results of the least-squares analysis. The predicted LQ^2 parameters calculated from the best-fit TLK model inputs are also listed.

For CHO 10B2 cells, the different methods of LQ parameter estimation yielded estimates of α that varied by 56%, i.e., from 3.04×10^{-2} Gy^{-1} (TLK predicted) to 4.74×10^{-2} Gy^{-1} (LQ direct analysis). The ratio $\alpha/(\beta_1 + \beta_2)$ obtained from the LQ direct analysis was higher by a factor of 1.61 than the TLK predicted value of 1.18 Gy. The estimates of τ_1 and τ_2 obtained from the TLK parameters and direct LQ^2 are within 5 to 10% of each other. Both LQ^2 and TLK analyses indicate that CHO 10B2 cells repair some DSBs quickly ($\tau_1 \sim 1$ h) and some very slowly ($\tau_2 \sim$ 13–15 h). In contrast, the LQ analysis suggests an average first-order repair half-time of 6.2 h. This repair half-time is very similar to the 5.68 h repair half-time determined from a global LPL-model fit to this same dataset (Stewart 2001).

Figure 2 compares the measured surviving fractions to the LQ and LQ^2 surviving fractions (direct-fit radiosensitivity parameters). The estimates of the surviving fraction obtained with the LQ^2 model are almost indistinguishable from those obtained using the full TLK model (not shown). Despite the relatively large differences in the model inputs (table 1), the surviving fractions predicted by the LQ and LQ^2 models are strikingly similar for the single-dose exposure conditions (figure 2, left panel). However, the LQ^2 model does better at predicting the surviving fractions for the split-dose exposures than the LQ model (figure 2, right panel). Two first-order repair terms evidently provide a better explanation for the survival data than a single first-order term. A direct examination of the measured CHO cell survival and DSB rejoining-rate data using the TLK provides even more compelling evidence for (at least) two distinct rates of DSB repair (Stewart 2001).

For C3H 10T1/2 cells, the estimates for α varied by 11% (i.e., from 0.112 Gy^{-1} to 0.124 Gy^{-1}). The ratio $\alpha/(\beta_1 + \beta_2)$ ranged from 7.27 Gy (LQ^2 direct analysis) to 9.12 Gy (LQ model), a 25% difference. With the TLK model, we found that several different sets of DSB rejoining-rate parameters gave equally good fits to the C3H 10T1/2 survival data. Several

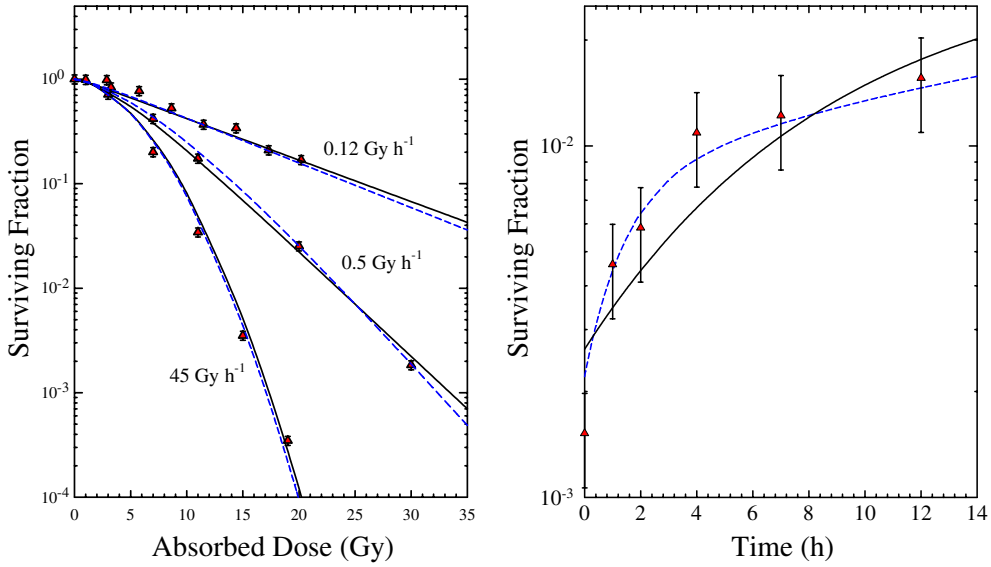

Figure 2. Survival of plateau phase CHO 10B2 cells irradiated by ^{137}Cs gamma-rays. Solid lines: LQ model ($\alpha = 4.74 \times 10^{-2}$ Gy^{-1}, $\beta = 1.25 \times 10^{-2}$ Gy^{-2} and $\tau = 6.2$ h). Dashed lines: LQ^2 model ($\alpha = 4.18 \times 10^{-2}$ Gy^{-1}, $\beta_1 = 1.09 \times 10^{-2}$ Gy^{-2}, $\beta_2 = 1.12 \times 10^{-2}$ Gy^{-2}, $\tau_1 = 0.99$ h and $\tau_2 = 13.9$ h). Left panel: cell survival after a single dose of radiation; measured data (Stackhouse and Bedford 1993) are shown with an estimated standard error of 10% (J S Bedford, personal communication). Right panel: cell survival following split-dose irradiation (8 Gy + 8 Gy); measured data (Stackhouse and Bedford 1993) are shown with an estimated standard error of 30% (J S Bedford, personal communication).

different attempts at model calibration suggested that τ_1 must lie in the range from ∼0.5 h to 1.5 h and τ_2 in the range from ∼3.5 to 15 h. For comparison, the LQ^2 analysis gave a 95% confidence interval of [0.54 h, 4.94 h] for τ_1 and [5.72 h, >25 h] for τ_2. The confidence intervals on τ_1 and τ_2 obtained from the LQ^2 analysis overlaps the estimated range of values obtained from the TLK analysis. The results shown in figure 3 illustrate that, even with dramatically different first-order rate constants, the LQ^2 and TLK models predict essentially the same levels of cell killing for a wide range of single-dose exposure conditions. The LQ model also does an adequate job at predicting the surviving fraction data using the parameters listed in table 1 (not shown).

The analyses of the CHO 10B2 and C3H 10T1/2 cell survival datasets illustrate some of the practical difficulties associated with the extraction of accurate biological parameters from even large experimental datasets. The uncertainties associated with the repair half-times obtained from the LQ^2 and TLK analyses are quite large for C3H 10T1/2 cells ($\tau_1 \sim 0.5$ h–5 h and $\tau_2 \sim 3.5$–25 h). For CHO 10B2 cells, the first-order repair rates determined from the LQ^2 and TLK analyses are nearly the same ($\tau_1 \sim 1$ h and $\tau_2 \sim 13$–15 h). This observation suggests that reliable estimates for τ_1 and τ_2 cannot always be determined from single-dose survival data. This difficulty arises because survival data for low and high dose rates contain very little information about the rates of DSB rejoining. When the exposure time is short compared to τ_i (high dose rates), a negligible amount of repair occurs during the exposure and the dose protraction factor is close to unity. When the exposure time is large compared to τ_i (low dose rates), the dose protraction factor is close to zero. To probe first-order repair rates in an effective manner, the experimental design should include exposure conditions that give intermediate values of the dose protraction factor (i.e., $G_i \sim 0.25$–0.75).

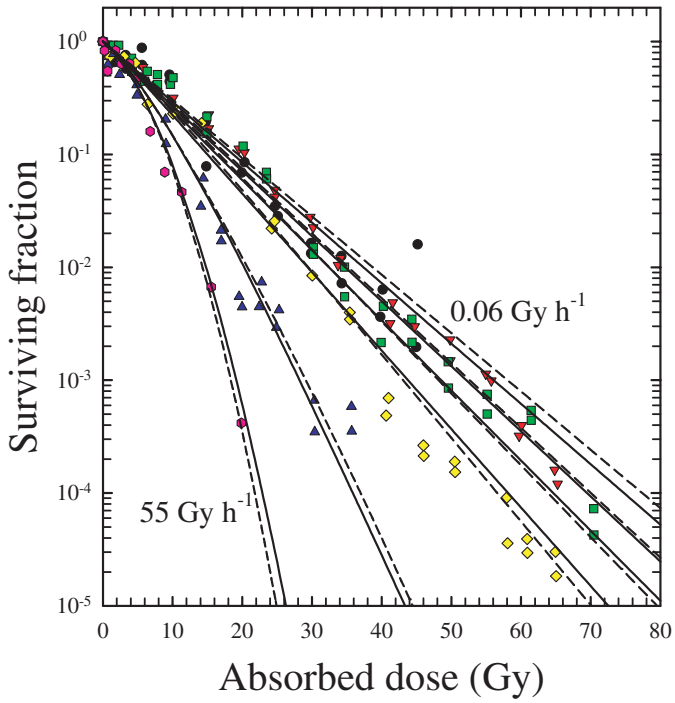

Figure 3. A comparison of measured, LQ^2 and TLK surviving fractions as a function of dose and dose rate (0.06 Gy h^{-1}, 0.17 Gy h^{-1}, 0.29 Gy h^{-1}, 0.49 Gy h^{-1}, 2.4 Gy h^{-1} and 55 Gy h^{-1}). Filled symbols: measured data (Wells and Bedford 1983). Solid lines: TLK model ($2Y(\Sigma_1 + \Sigma_2) = 60.8\,\mathrm{Gy}^{-1}\,\mathrm{cell}^{-1}$, $a_1 = 0.297$, $\tau_1 = 0.489$ h, $\tau_2 = 3.91$ h and $\eta = 2.57 \times 10^{-5}\,\mathrm{h}^{-1}$). Other parameters set *a priori* to biologically plausible values; see main text. Dashed lines: LQ^2 model ($\alpha = 0.112\,\mathrm{Gy}^{-1}$, $\beta_1 = 1.36 \times 10^{-2}\,\mathrm{Gy}^{-2}$, $\beta_2 = 1.8 \times 10^{-2}\,\mathrm{Gy}^{-2}$, $\tau_1 = 0.973$ h and $\tau_2 = 15.79$ h).

5. Summary and conclusions

This paper examines the theoretical and practical linkages between the LQ and TLK models. The TLK formalism is used to derive an equivalent LQ^2 formula and to relate the intrinsic radiosensitivity parameters used in one model to the parameters used in the other. Two extensive radiobiological datasets, one for CHO 10B2 cells (Stackhouse and Bedford 1993) and one for C3H 10T1/2 cells (Wells and Bedford 1983) are analysed using the TLK and LQ models. As a first test of model equivalence, predictions of the surviving fraction as a function of dose and dose rate are compared using LQ parameters derived from the best-fit TLK parameters. Equations (7) through (10) are used to covert the TLK parameters into an equivalent set of LQ^2 parameters. As expected, the LQ^2 formula is a good approximation to the full TLK for low doses and dose rates.

As a second test of model equivalence, parameters for the LQ^2 model are obtained by performing a global fit to the CHO 10B2 and C3H 10T1/2 datasets. Both the LQ^2 and TLK models are equally capable of explaining the CHO 10B2 and C3H 10T1/2 datasets. The LQ^2 parameters calculated from the best-fit TLK parameters differ noticeably from the direct-fit parameters (table 1). The direct-fit LQ^2 parameters provide a better fit of the survival data than the TLK-calculated ones. However, for the doses and dose rates most relevant to radiation therapy, the LQ^2 model with the TLK-calculated parameters and the full TLK model give surviving fractions that are within a few per cent of each other (figure 1). We find that the LQ^2

formula is generally an excellent approximation to the full TLK model, and the direct-fit and TLK-derived LQ^2 parameters should both be acceptable for doses below about 5 or 10 Gy.

The extraction of rate constants associated with DSB repair was found to be problematic, especially if only single-dose survival data are available. Both LQ^2 and TLK analyses indicate that CHO cells repair some DSBs quickly ($\tau_1 \sim 1$ h) and some very slowly ($\tau_2 \sim 13$–15 h). In contrast, the LQ analysis suggests an average first-order repair half-time of 6.2 h. This repair half-time is very similar to the 5.68 h repair half-time determined from a global LPL-model fit to this same dataset (Stewart 2001). The comparison of split-dose survival data in figure 2 (right panel) and a previous TLK analysis (Stewart 2001) of both the CHO-cell survival *and* DSB rejoining data strongly suggest that DSBs are repaired at two or more distinct rates. The TLK and LQ^2 analysis of the C3H 10T1/2 cell survival data also tends to support two rates of DSB repair, although the evidence is much less compelling because of the lack of split-dose (and DSB rejoining-rate) data to test the models against.

Recently, Fowler (1999) proposed a second-order repair model as an alternative to multi-exponential repair. In principle, such model can be recovered from the TLK by setting $\lambda_1 = \lambda_2 = 0$. However, with the present formulation of the TLK, the assumption $\lambda_1 = \lambda_2 = 0$ gives only a term linear in the dose for the expected number of fatal lesions, independent of the exposure conditions. Some additional hypothesis is needed to obtain an LQ formula for the expected number of fatal lesions when linear repair terms are absent. Nevertheless, Dale *et al* (1999) developed an incomplete repair model based on Fowler's second-order repair hypothesis. Using the Dale *et al* (1999) model, we performed a global fit to the CHO dataset and found an excellent description for both the single fraction and split-dose data (not shown). The issue of whether the rate of DSB rejoining is predominately first- or second-order cannot be decided from an analysis of cell survival data alone.

Biological models are playing an increasingly important role in the design, optimization and evaluation of radiation treatment plans (e.g., see Mohan *et al* 2000, Brahme 2001, Buffa *et al* 2001, Ling *et al* 2000). The theoretical linkages among the LQ and TLK models could be exploited to improve the design and optimization of radiation therapy in a number of ways. By exploiting the relationship between the TLK and LQ model parameters, it may also be possible to devise a mechanism-based strategy to estimate distributions of radiosensitivity parameters (e.g., to reflect inter-patient variability in radiation sensitivity). The TLK formalism could be used to explore how uncertainties associated with specific biological mechanisms affect LQ radiosensitivity parameters. *In vitro* data on DSB formation or repair could be used to identify plausible constraints on LQ parameters, using equations (7) to (12). Conversely, empirical knowledge of the LQ parameters can be used to identify plausible constraints on the TLK parameters (e.g., see section 3.2). Equations (7) through (12) could also be used to develop scaling rules that integrate different kinds of *in vitro* data into a single biomarker of response (i.e., a multi-endpoint predictive assay). We find that the LQ, LQ^2 and TLK models are all useful, complementary tools for the analysis and prediction of radiobiological effects.

Acknowledgments

The authors thank Dr J C McDonald for helpful comments on the manuscript and thank Mr J R Weber for excellent editorial assistance. Research is supported by grant R01HL62213 from the National Institute of Health and by a Low Dose Radiation Research Program (www.lowdose.org) grant to the Pacific Northwest National Laboratory (PNNL) from the US Department of Energy's (DOE) Office of Biological and Environmental Research (BER). Battelle operates PNNL for the US DOE under contract DE-AC06-76RLO 1830.

References

Ang K K, Jiang G L, Guttenberger R, Thames H D, Stephens L C, Smith C D and Feng Y 1992 Impact of spinal cord repair kinetics on the practice of altered fractionation schedules *Radiother. Oncol.* **25** 287–94

Brahme A 2001 Individualizing cancer treatment: biological optimization models in treatment planning and delivery *Int. J. Radiat. Oncol. Biol. Phys.* **49** 327–37

Brenner D J 1992 Correlations between α/β and T1/2: implications for clinical biological modelling *Br. J. Radiol.* **65** 1051–4

Brenner D J, Hlatky L R, Hahnfeldt P J, Huang Y and Sachs R K 1998 The linear–quadratic model and most other common radiobiological models result in similar predictions of time-dose relationships *Radiat. Res.* **150** 83–91

Buffa F M, Davidson S E, Hunter R D, Nahum A E and West C M 2001 Incorporating biologic measurements (SF(2), CFE) into a tumor control probability model increases their prognostic significance: a study in cervical carcinoma treated with radiation therapy *Int. J. Radiat. Oncol. Biol. Phys.* **50** 1113–22

Curtis S B 1986 Lethal and potentially lethal lesions induced by radiation—a unified repair model *Radiat. Res.* **106** 252–70

Curtis S B 1989 *Radiat. Res.* **119** 584 (erratum)

Dale R G, Fowler J F and Jones B 1999 A new incomplete-repair model based on a 'reciprocal-time' pattern of sublethal damage repair *Acta Oncol.* **38** 919–29

Deschavanne P J, Fertil B, Chavaudra N and Malaise E P 1990 The relationship between radiosensitivity and repair of potentially lethal damage in human tumor cell lines with implications for radioresponsiveness *Radiat. Res.* **122** 29–37

Fowler J F 1999 Is repair of DNA strand break damage from ionizing radiation second-order rather than first-order? A simpler explanation of apparently multiexponential repair *Radiat. Res.* **152** 124–36

Gear C W 1971 *Numerical Initial Value Problems in Ordinary Differential Equations* (Englewood Cliffs, NJ: Prentice-Hall) ch 11

Ling C C, Humm J, Larson S, Amols H, Fuks Z, Leibel S and Koutcher J A 2000 Towards multidimensional radiotherapy (MD-CRT): biological imaging and biological conformality *Int. J. Radiat. Oncol. Biol. Phys.* **47** 551–60

Millar W T and Canney P A 1993 Derivation and application of equations describing the effects of fractionated protracted irradiation, based on multiple and incomplete repair processes: Part 2. Analysis of mouse lung data *Int. J. Radiat. Biol.* **64** 293–303

Millar W T, Jen Y M, Hendry J H and Canney P A 1994 Two components of repair in irradiated kidney colony forming cells *Int. J. Radiat. Biol.* **66** 189–96

Millar W T, Van Den Aardweg G J M J, Hopewell J W and Canney P A 1996 Repair kinetics in pig epidermis: an analysis based on two separate repair rates *Int. J. Radiat. Biol.* **69** 123–40

Mohan R, Wu Q, Manning M and Schmidt-Ullrich R 2000 Radiobiological considerations in the design of fractionation strategies for intensity-modulated radiation therapy of head and neck cancers *Int. J. Radiat. Oncol. Biol. Phys.* **46** 619–30

Peacock J H, Eady J J, Edwards S M, McMillan T J and Steel G G 1992 The intrinsic alpha/beta ratio for human tumour cells: is it a constant? *Int. J. Radiat. Biol.* **61** 479–87

Sachs R K, Hahnfeld P and Brenner D J 1997 Review: The link between low-LET dose- response relations and the underlying kinetics of damage production/repair/misrepair *Int. J. Radiat. Biol.* **72** 351–74

Shampine L F and Gear C W 1979 A user's view of solving stiff ordinary differential equations *SIAM Rev.* **21** 1–17

Stackhouse M A and Bedford J S 1993 An ionizing radiation-sensitive mutant of CHO cells: irs-20. II. Dose-rate effects and cellular recovery processes *Radiat. Res.* **136** 250–4

Stewart R D 2001 Two-lesion kinetic model of DSB rejoining and cell killing. *Radiat. Res.* **156** 365–78

Tobias C A 1985 The repair–misrepair model in radiobiology: comparison to other models *Radiat. Res.* **104** (Suppl) 77–95

Van Rongen E, Thames H D Jr and Travis E L 1993 Recovery from radiation damage in mouse lung: interpretation in terms of two repair rates *Radiat. Res.* **133** 225–33

Wells R L and Bedford J S 1983 Dose-rate effects in mammalian cells: IV. Repairable and nonrepairable damage in noncycling C3H 10T 1/2 cells *Radiat. Res.* **94** 105–34