

Do low dose-rate bystander effects influence domestic radon risks?

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(Received 9 February 2001; accepted 20 December 2001)

Abstract.

Purpose: Radon risks derive from exposure of bronchio-epithelial cells to high-linear energy transfer (LET) α -particles. α -particle exposure can result in bystander effects, where irradiated cells emit signals resulting in damage to nearby unirradiated bystander cells. This can result in non-linear dose–response relations, and inverse dose-rate effects. Domestic radon risk estimates are currently extrapolated from miner data, which are at both higher doses and higher dose-rates, so bystander effects on unhit cells could play a large role in the extrapolation of risks from mines to homes. Therefore, we extend an earlier quantitative mechanistic model of bystander effects to include protracted exposure, with the aim of quantifying the significance of the bystander effect for very prolonged exposures.

Materials and methods: A model of high-LET bystander effects, originally developed to analyse oncogenic transformation *in vitro*, is extended to low dose-rates. The model considers radiation response as a superposition of bystander and linear direct effects. It attributes bystander effects to a small subpopulation of hypersensitive cells, with the bystander contribution dominating the direct contribution at very low acute doses but saturating as the dose increases. Inverse dose-rate effects are attributed to the replenishment of the hypersensitive subpopulation during prolonged irradiation.

Results: The model was fitted to dose- and dose-rate-dependent radon-exposed miner data, suggesting that one directly hit target bronchio-epithelial cell can send bystander signals to about 50 neighbouring target cells. The model suggests that a naïve linear extrapolation of radon miner data to low doses, without accounting for dose-rate, would result in an underestimation of domestic radon risks by about a factor of 4, a value comparable with the empirical estimate applied in the recent BEIR–VI report on radon risk estimation.

Conclusions: Bystander effects represent a plausible quantitative and mechanistic explanation of inverse dose-rate effects by high-LET radiation, resulting in non-linear dose–response relations and a complex interplay between the effects of dose and exposure time. The model presented provides a potential mechanistic underpinning for the empirical exposure–time correction factors applied in the recent BEIR–VI for domestic radon risk estimation.

1. Introduction

1.1. Bystander effect and radon

By far the largest component of the background radiation dose equivalent is from domestic radon

exposure (NCRP 1987). For a variety of reasons, however, direct epidemiological assessment of the risks from domestic radon exposure is difficult, resulting in risk estimates with wide confidence intervals (Lubin *et al.* 1995b). Consequently, domestic radon risk estimates are currently based on extrapolation of data from miner studies, largely at considerably higher radon exposures and exposure rates. At present, a linear extrapolation of the risks from high to low radon exposures is generally considered to have the strongest biophysical rationale (NRC 1999).

At an average home radon concentration, few potential target cells in the bronchial epithelium of a home resident will be struck or traversed by an α -particle in, say, 1 year (NRC 1999)—and this observation remains true even at high domestic radon levels (figure 1).

This inhomogeneous energy deposition by α -particles is of potential relevance to the radon problem because there is convincing evidence, at least *in vitro*, that irradiated cells can send out signals that can result in damage to nearby unirradiated ‘bystander’ cells. The evidence is particularly strong for high-LET radiation, with a broad variety of endpoints (summarized, for example, by Sawant *et al.* 2001b) including chromosomal damage and oncogenic transformation. Some recent results suggest that bystander effects can be induced by high-LET radiation even when the bystander cells have been previously exposed to low doses of low-LET radiation (Sawant *et al.* 2001a).

1.2. Modelling the bystander effect

Brenner *et al.* (2001) suggested a model for acute exposure to high-LET particles that incorporated both bystander effects and the more classical ‘direct effects’. The basic picture proposed of the bystander effect was that:

- it is a binary ‘all or nothing’ phenomenon in which a signal is sent out by cells whose nuclei are directly hit, but at high-LET more hits to one cell nucleus do not lead to an increased bystander response in its neighbours;
- the cell population contains, at any given time, a

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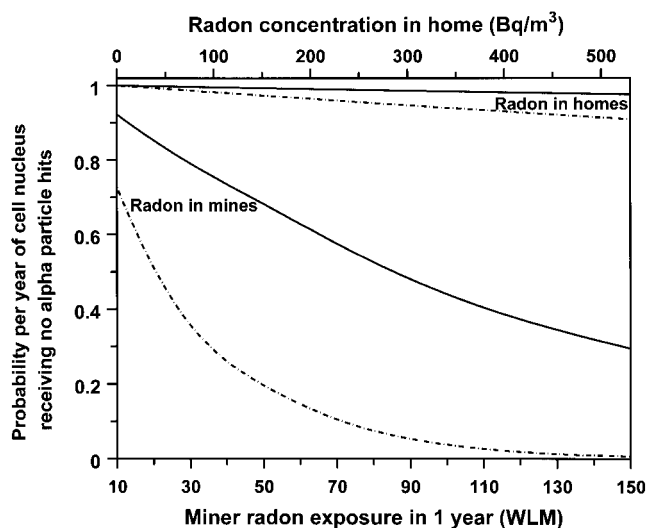


Figure 1. Probability of a cell nucleus in the bronchial epithelium not being traversed by an α -particle over 1 year (based on data in NRC 1999). Solid curves: basal cell nuclei; broken curves: secretory cell nuclei. Lower curves (refer to lower horizontal axis) are for exposed miners; for reference, the mean radon exposure rate for the Colorado Plateau uranium miners was about 150 WLM year⁻¹, for other miner cohorts, about 30 WLM year⁻¹. Upper curves (refer to upper horizontal axis) are for domestic radon exposure; for reference, the mean radon concentration in US homes is around 46 Bq m⁻³ (1.3 pCi l⁻¹) (NRC 1999), comparable with approximately 0.23 WLM year⁻¹.

small subpopulation of cells that are hypersensitive in their response to the bystander signal; and

- a cell from this hypersensitive subpopulation is also very sensitive to direct particle traversals of the nucleus, such a traversal generally resulting in cell death.

This approach produced results consistent with data then available from *in vitro* experiments designed to probe the bystander effect (e.g. Miller *et al.* 1999, Sawant *et al.* 2001b) for oncogenic transformation, and also with data published since then (e.g. Belyakov *et al.* 2001). Broadly speaking, it predicts dose-response curves for acute high-LET irradiation that rise rapidly to a plateau at low doses (due to the bystander effect) and then further increase at higher doses (due to 'direct' effects). Thus, at low doses, the curve is downwardly curving (figure 2a, solid curve), a pattern apparent for lung cancer incidence in animals acutely exposed to radon (Cross 1992, Gilbert *et al.* 1996, Monchaux *et al.* 1999). ('Downwardly curving' for the yield, Y , as a function of dose, D , is taken to mean that for any sufficiently small doses, D_1 and D_2 , $Y[(D_1 + D_2)/2] > [Y(D_1) + Y(D_2)]/2$.) This downward

curvature for acute exposure is of potential significance for dose-rate effects, as will now be discussed.

1.3. Significance of exposure protraction to radon risk

The bystander model discussed above was for a single acute dose. By contrast, domestic exposure to radon is protracted over a lifetime, and even miner exposure is typically protracted over several years (average 5.7 years) (NRC 1999). It is important, therefore, to extend models of bystander response from acute to protracted exposure when analysing the extrapolation of radon risks from higher to lower exposures.

Before considering a specific model of bystander response to protracted exposures, it is useful to note that with some limitations (see below), one can estimate the effect of protraction in a model-independent way. To take the simple example of splitting the dose into two fractions (figure 2), one can picture the overall response to be the result of repeating the dose-response relation for each fraction (Rossi *et al.* 1982, Brenner and Sachs 2000). Then, if the acute dose-response relation has an upward curvature (as in, for example, the 'classic' linear-quadratic relationship), protraction would be expected to decrease the response (figure 2b); a

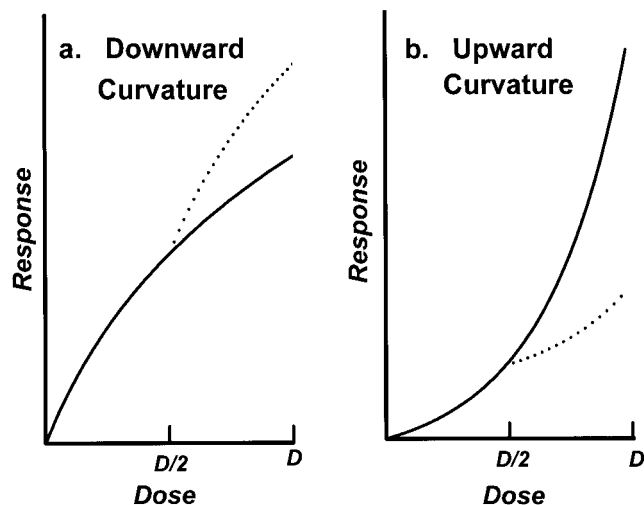


Figure 2. Potential influence of the shape of the acute dose-response curve on the effect of protracting the dose, here by dividing the dose into two fractions. The solid curves respectively show downwardly and upwardly curving acute dose-response relations. The dotted curves, for the second of two fractions, result from rigidly displacing the initial part of the solid curves; they illustrate how upwardly curving acute dose-response relations can yield 'direct' dose-rate effects (decreasing risk with increasing protraction), whilst downwardly curving acute dose-response relations can yield 'inverse' dose-rate effects (increasing risk with increasing protraction).

decrease of response with increasing dose protraction is often called a *direct* dose-rate effect. On the other hand, downward curvature in the acute dose-response relation, which appears to be the scenario relevant to bystander responses, would imply that protraction increases the response (figure 2a), giving an *inverse* dose-rate effect. Finally, a system whose dose-response relationship for acute irradiation is linear would be expected to show little protraction effect.

The applicability of this repeat rule, that the effect of protraction approximates repeated applications of the same initial part of the dose-response curve (figure 2), depends on how a cell population changes during a protracted exposure. An acute exposure preferentially removes more radiosensitive cells from a heterogeneous population, and the repeat rule will then hold if, during the irradiation, there is continual restoration towards the pre-irradiation distribution of radiosensitivity. For the bystander model briefly outlined above, this rule would be expected to hold (Brenner *et al.* 2001). After an initial acute exposure, the small proportion of hypersensitive cells (sensitive both to bystander signals and direct killing) would be decreased to still lower values due to direct cell killing or transformation, but over time this proportion should return to its pre-irradiation level due to normal redistribution effects (Hahnfeldt and Hlatky 1998). If the distribution of cellular sensitivities is restored on a time-scale shorter than or comparable with the protraction time, then protraction would increase response relative to an acute exposure (figure 2a)—an inverse dose-rate effect.

1.4. Observed inverse dose-rate effects for protracted high-LET exposure

In fact, both laboratory and epidemiological evidence strongly suggest that increasing protraction of exposure to α -particles increases the oncogenic risk. Of particular interest is that inverse dose-rate effects have been demonstrated in miners exposed to radon-progeny α -particles over differing periods. In comparisons between different epidemiological studies involving different average radon-progeny exposure rates, Howe *et al.* (1987) and Darby and Doll (1990) inferred inverse dose-rate effects. Within individual miner cohorts, inverse dose-rate effects were reported by Hornung and Meinhardt (1987), Hornung *et al.* (1998), Stram *et al.* (1999), Gilliland *et al.* (2000), Sevc *et al.* (1988), Tomášek *et al.* (1993), Lubin *et al.* (1990), and Xuan *et al.* (1993). In a joint analysis of 11 cohorts of miners exposed to radon, with data stratified both by exposure and exposure time, Lubin *et al.* (1995a) and BEIR-VI (NRC 1999) clearly

demonstrated the existence of a statistically significant inverse dose-rate effect.

In parallel to these epidemiological studies, there have been many laboratory reports of inverse dose-rate effects for oncogenesis or oncogenic transformation induced by high-LET radiations (Hill *et al.* 1982, 1985, Yang *et al.* 1987, Miller *et al.* 1988, 1990, Bettega *et al.* 1992, Gilbert *et al.* 1996, Monchaux *et al.* 1999).

As discussed above, average domestic radon exposures are much lower than average miner exposures, to the extent that multiple α -particle traversals in target bronchial cell nuclei from domestic exposure will be much less common than for miners. The case where multiple traversals can occur in one target differs in an important way from the case where multiple traversals are very rare: specifically, several authors (Barendsen 1985, Curtis 1989, Brenner 1993) have argued that at low doses, a lack of multiple traversals may preclude *any* dose-rate effects, inverse or direct. Subsequent studies, both epidemiological (Lubin *et al.* 1995a, Hornung *et al.* 1998, NRC 1999) and in animals (Gilbert *et al.* 1996, Monchaux *et al.* 1999), have indeed confirmed that inverse dose-rate effects do decrease as the dose decreases, essentially disappearing at fluences of around one α -particle per target cell nucleus. We shall argue that when bystander effects are taken into account, a similar situation should hold, provided the concept of 'one target' is appropriately enlarged to include all the bystander cells that can be signalled from a hit cell.

1.5. Modelling the influence of bystander effects on domestic radon risk

We are concerned with the extrapolation of radon risks from miners (comparatively high dose, significant numbers of multiple α -particle traversals per nucleus, relative short exposure time) to the domestic situation (low dose, almost no multiple nuclear α -particle traversals, long exposure time). The evidence discussed here suggests that dose-rate effects must be taken into account in such an extrapolation. In fact, the most recent analysis of domestic radon risk, in the BEIR-VI report (NRC 1999), does make an empirical correction for dose-rate effects based on a phenomenological fit to miner data involving a range of exposure and exposure rates. For example, the BEIR-VI phenomenological model incorporates an increase in risk by a factor of about 4 for a radon exposure of >35 years compared with a typical miner exposure of, say, 6 years.

The goal in our current work is to provide a possible quantitative mechanistic underpinning for these empirical dose-rate-based correction factors —

these being a central component of the extrapolation of radon risk from mines to homes. Specifically, we extend the bystander model (Brenner *et al.* 2001) for high-LET acute exposure to high-LET exposure at lower dose-rates. The model is comparatively parsimonious, involving only four essential adjustable parameters. As discussed below, it is probably not appropriate to apply directly model parameters estimated in the analysis of *in vitro* data to the *in vivo* situation. Therefore, we provide some validation of this extended model by comparing it with the data provided by Lubin *et al.* (1995a) for lung cancer mortality rates in miners, with data stratified both by exposure and by exposure time. Finally, we use this extended model to draw conclusions about the basis for the complex interplay of dose and dose-rate (Brenner *et al.* 1994) in extrapolating radon risks from mines to homes, providing a mechanistic rationale for the phenomenologically based dose-rate corrections adopted in BEIR-VI (NRC 1999).

2. Materials and methods

2.1. General approach

The number of normal cells ‘activated’ by radiation damage will be modelled, where an activated cell is one that has an increased probability that one or more of that cell’s progeny will progress to cancer. We view such activation events as parts of the chain of events in the carcinogenesis process, which can also occur endogenously, providing a rationale for modelling relative, rather than an absolute risk. ‘Activation’ here does not necessarily correspond to the same step as in standard multistep models (e.g. Moolgavkar and Knudson 1981, Yakovlev *et al.* 1997, Luebeck *et al.* 1999); indeed, it could refer to an early or later event in the carcinogenesis process.

2.2. $\mathcal{B}\mathcal{D}$ (Bystander and Direct) model for acute high-LET exposure *in vitro*

The earlier model for bystander effects induced by acute high-LET exposure (Brenner *et al.* 2001) was suggested by results (Miller *et al.* 1999, Sawant *et al.* 2001b) of experiments using a single-cell, single-particle microbeam irradiator (Randers-Pehrson *et al.* 2001) designed to probe the systematics of the bystander phenomenon. The endpoint of the experiments was *in vitro* oncogenic transformation by α -particles. The following model assumptions were discussed and motivated in detail by Brenner *et al.* (2001).

- (1) A distinction is drawn between cells that are directly hit, i.e. have an α -particle traverse

their nucleus, and bystander cells which are not directly hit. The yield, Y , is due to a superposition of bystander and direct effects:

$$Y = \mathcal{B} + \mathcal{D} \quad (1)$$

- (2) A cell that is directly hit sends out a bystander signal to its k nearest neighbour cells, where k is treated as an adjustable parameter. The model does not attempt to analyse the nature of this bystander signal.
- (3) At any instant, a small subpopulation of cells is hypersensitive to the bystander signal. Specifically, suppose a cell is hit directly by an α -particle. Then any hypersensitive cells among the k nearest neighbours will be activated, but no other bystander cells are activated.
- (4) For cells that are not hypersensitive, activation can only be by direct hits, and the yield for a Poisson-distributed number of particles with average N per cell nucleus is given by a standard linear formula, independent of dose-rate:

$$\mathcal{D} = \mu N \quad (2)$$

where μ is another adjustable parameter.

- (5) If a hypersensitive cell is hit directly by an α -particle, it will be killed rather than activated.

These assumptions gave a reasonable fit to *in vitro* data (Brenner *et al.* 2001); it was pointed out, there, that the assumptions when applied to a protracted exposure would be expected to lead to an inverse dose-rate effect because, over the course of the protracted irradiation, the number of hypersensitive cells would be expected to be replenished by endogenous processes. We describe here an extension to the model quantifying these dose-rate effects.

2.3. Extension of the $\mathcal{B}\mathcal{D}$ model to protracted exposure

As discussed, we consider only the radiation-dependent stages of the carcinogenesis process. It will be assumed that the picture just outlined, including equations (1) and (2), carries over to the *in vivo*, low exposure-rate situations of present interest. As above, it is assumed that the target cell population includes a small subpopulation of hypersensitive cells. The extended model focuses on how the number of these hypersensitive cells, $\mathcal{H}(t)$, changes as a function of time, t , during irradiation.

It is assumed that in the absence of irradiation, the number, \mathcal{H} , of hypersensitive cells is kept at a dynamic equilibrium, \mathcal{E} , by a balance between cell production and cell loss. Production (e.g. from suitable progenitor cells and/or by cells changing phenotype from normal to hypersensitive) is taken to be at

a constant rate, r ; endogenous losses (e.g. by cell death or by phenotypic change from hypersensitive to normal) are taken to occur at rate $\rho\mathcal{H}$, where ρ is a rate constant. It follows that $r = \rho\epsilon$.

During irradiation, two additional effects tend to deplete the hypersensitive subpopulation: killing and activation. According to the picture carried over from the *in vitro* model: first, killing of hypersensitive cells occurs at the rate $\mathcal{H}(t) \times A\phi(t)$, where A is the cross-sectional area of a target cell nucleus and ϕ is the α -particle flux density (proportional to dose-rate); second, activation of hypersensitive cells occurs at the rate $k\mathcal{H}(t) \times A\phi(t)$, since on average each hypersensitive cell is among the k nearest neighbours of k different cells. The assumption here that cells hypersensitive to the bystander signal for oncogenic activation are also hypersensitive to killing by direct hits is not essential to the results; dropping the assumption would give quite similar results as far as the present data are concerned. The assumption was included for consistency with the earlier *in vitro* model where it plays a more important role.

Assembling these observations gives the two main equations of our model for protracted exposure. The rate of change with time of the hypersensitive cell number is:

$$d\mathcal{H}/dt = \rho(\epsilon - \mathcal{H}) - (k+1)\mathcal{H}A\phi(t) \quad (3)$$

The rate at which bystander cells are activated is:

$$d\mathcal{B}/dt = k\mathcal{H}A\phi(t) \quad (4)$$

The differential equation (3), with initial condition $\mathcal{H}(0) = \epsilon$, can be integrated to obtain $\mathcal{H}(t)$ (see appendix). For a constant dose-rate, the flux density, ϕ , is a constant. In this case, inserting the resulting form of $\mathcal{H}(t)$ into equation (4) and integrating gives the number, \mathcal{B} , of activated bystander cells at time T (see appendix):

$$\mathcal{B} = [k/(k+1)]\epsilon \left\{ h/(1+y) + [y^2/(1+y)^2] \times [1 - \exp(-h - h/y)] \right\} \quad (5)$$

where

$$h = (k+1)A\phi T, \quad y = h/\rho T \quad (6)$$

The variable h is dimensionless and proportional to total dose or total cumulative exposure. It has a useful intuitive interpretation, as follows. Since a hypersensitive cell can be influenced by hits on its own nucleus or the nuclei of k neighbours, consider a supracellular ‘collective target’ consisting of $k+1$ cell nuclei and, therefore, having total cross-sectional area $(k+1)A$. The total number of α -particles that hit this supracellular collective target at a constant particle flux density ϕ (i.e. at a constant dose-rate)

during the radiation period T is thus $h = (k+1)A\phi T$. The variable y is the corresponding collective-target hit-rate, h/T , made dimensionless by rescaling with the hypersensitive-cell replacement rate constant, ρ .

2.4. Applying the model for protracted exposure

Combining equations (1), (2), (5) and (6) gives a model with four adjustable parameters: μ , ϵ , k and ρ . The model was fitted, using standard maximum-likelihood methods (Press *et al.* 1986), to the combined data reported by Lubin *et al.* (1995a) for lung cancer mortality rates in 11 different cohorts of radon-exposed underground miners. The data are stratified by exposure and duration of exposure, and are adjusted for attained age, cohort and other concomitant factors.

It is assumed that excess relative risk is proportional to the yield of activated cells, i.e. $ERR = c(\mathcal{B} + \mathcal{D})$. The proportionality factor, c , does not act as a further adjustable parameter because it is merely a multiplicative factor for μ and ϵ , i.e. only the combinations $c\mu$ and $c\epsilon$ are relevant to the data.

Applying the model requires estimating the total number of α -particle hits, $N = A\phi T$, on a target cell nucleus for a given cumulative exposure (WLM). Based on the analysis reported in BEIR-VI (NRC 1999), it was assumed that a cumulative exposure of 100 WLM results in $N \approx 1$ α -particle traversal per target cell nucleus; this value is for target bronchial basal cell nuclei, with a cross-sectional area of about $25 \mu\text{m}^2$. The figure of 0.01 hits/WLM implies that 60 years of exposure in a house with a radon concentration of 100 Bq m^{-3} results in $N \approx 0.3$ particles traversals per basal cell nucleus (there will be turnover of the cells during this time). A total of 1 WLM roughly corresponds to a dose of about 5 mGy $100 \text{ keV } \mu\text{m}^{-1}$ α -particles in these target cells, but conversions of WLM to Gy (or to Sv) will not be needed in our calculations.

3. Results

We have fitted the extended $\mathcal{B}d\mathcal{D}$ model described above to the combined data of Lubin *et al.* (1995b) for lung cancer mortality rates in 11 cohorts of radon-exposed underground miners, stratified both by cumulative exposure and by duration of exposure, and adjusted for attained age, cohort and other concomitant factors. The results are shown in figure 3. The estimated parameters were $k = 49.8$ (the number of bystander cells signalled by one hit cell nucleus), $\rho = 24.3 \text{ year}^{-1}$ (the rate constant for replacement of hypersensitive cells), $c\epsilon = 0.092$ (the contribution of bystander effects to the ERR if all hypersensitive cells initially present were activated by

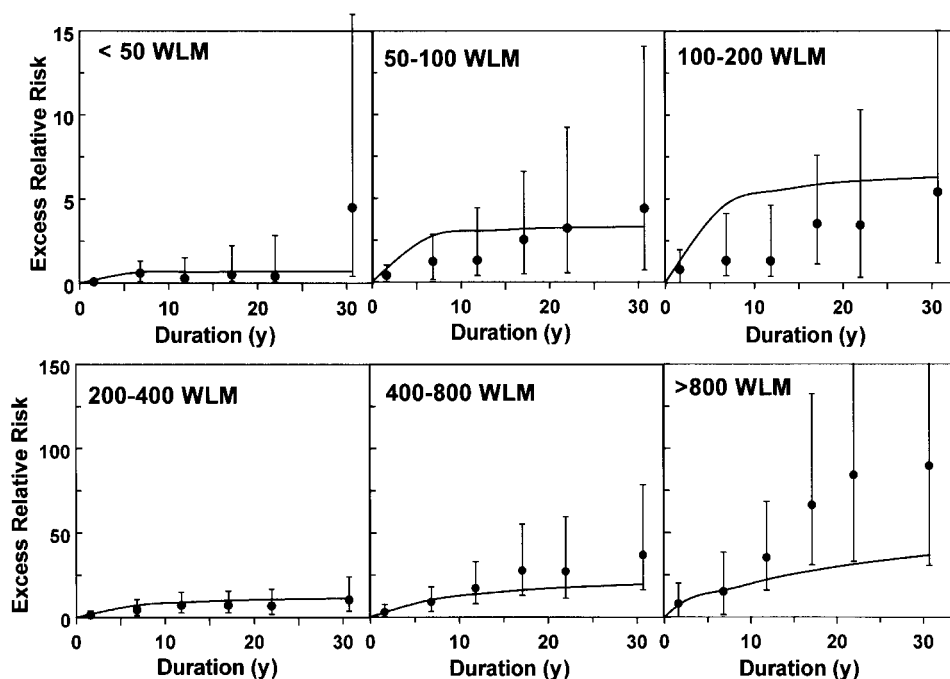


Figure 3. Fit of the extended BA model described here to the data of Lubin *et al.* (1995a) for lung cancer mortality rates in 11 cohorts of radon-exposed underground miners, stratified both by cumulative exposure (in WLM) and by duration of exposure, and adjusted for attained age, cohort and other concomitant factors. Note the different vertical scales in the upper and lower panels. The inverse dose-rate effect (increasing risk with increasing time) is clearly significant at high WLM, but less so at low WLM.

bystander signals), and $c\mu=0.25$ (the contribution of direct effects to the ERR if the average hit number for a target cell nucleus is 1). Here \mathcal{E} is the equilibrium number of hypersensitive cells in the absence of radiation, and μ is the number of non-hypersensitive cells activated by direct hits when each target cell nucleus is hit by an average of one α -particle—but, as discussed above, only the combinations $c\mathcal{E}$ and $c\mu$ can be estimated from the data.

The estimated parameters were used to extrapolate the miner data to lower doses, and for a 60-year exposure. The results are shown in figure 4a: for the comparatively short miner exposures (solid curve; for illustrative purposes, we used 6 years, the average time of miner exposure in the data), the dose-response relation is linear at very high doses (where the direct effect dominates). It can be seen, however, that at intermediate doses, where the bystander response starts to become important, the 6-year exposure (solid) curve become non-linear. At these intermediate doses the risks from a 60-year exposure (dotted line) are larger than those for a 6-year exposure (solid line)—the inverse dose-rate effect. At still lower doses, dose-rate effects become small, so the 6- and 60-year exposures produce the same risk.

Figure 4a also shows a linear extrapolation of the miner data (Lubin *et al.* 1994) in which the effects of

dose-rate are ignored. It can be seen that ignoring dose-rate effect and simply using a linear extrapolation from the miner to the domestic situation would result (using our estimated parameters) in an underestimation of the low-dose radon risk by about a factor of 4.5. This underestimation is comparable with the corresponding empirically estimated factor in BEIR-VI (NRC 1999) of about 3.7.

For comparison, plotted in figure 4a are results from domestic radon case-control studies as meta-analysed by Lubin and Boice (1997). The spread and uncertainties of the results are such that they are consistent with both the current mechanistically based low dose-rate/low-dose extrapolation, the BEIR-VI phenomenological low dose-rate/low-dose extrapolation, and also the ‘naïve’ low-dose extrapolation from miner data that ignores the effect of dose-rate. Thus, it is unlikely that further comparison with the results of domestic radon case-control studies will be informative in this regard. It is important, however, to note that these data typically represent above-average cumulative radon exposures and that, assuming low-dose linearity, most radon-related deaths will be at still lower cumulative exposures.

Figure 4b shows the proportion of the overall risk that, using our estimated parameters, can be attributed to bystander effects rather than to direct effects.

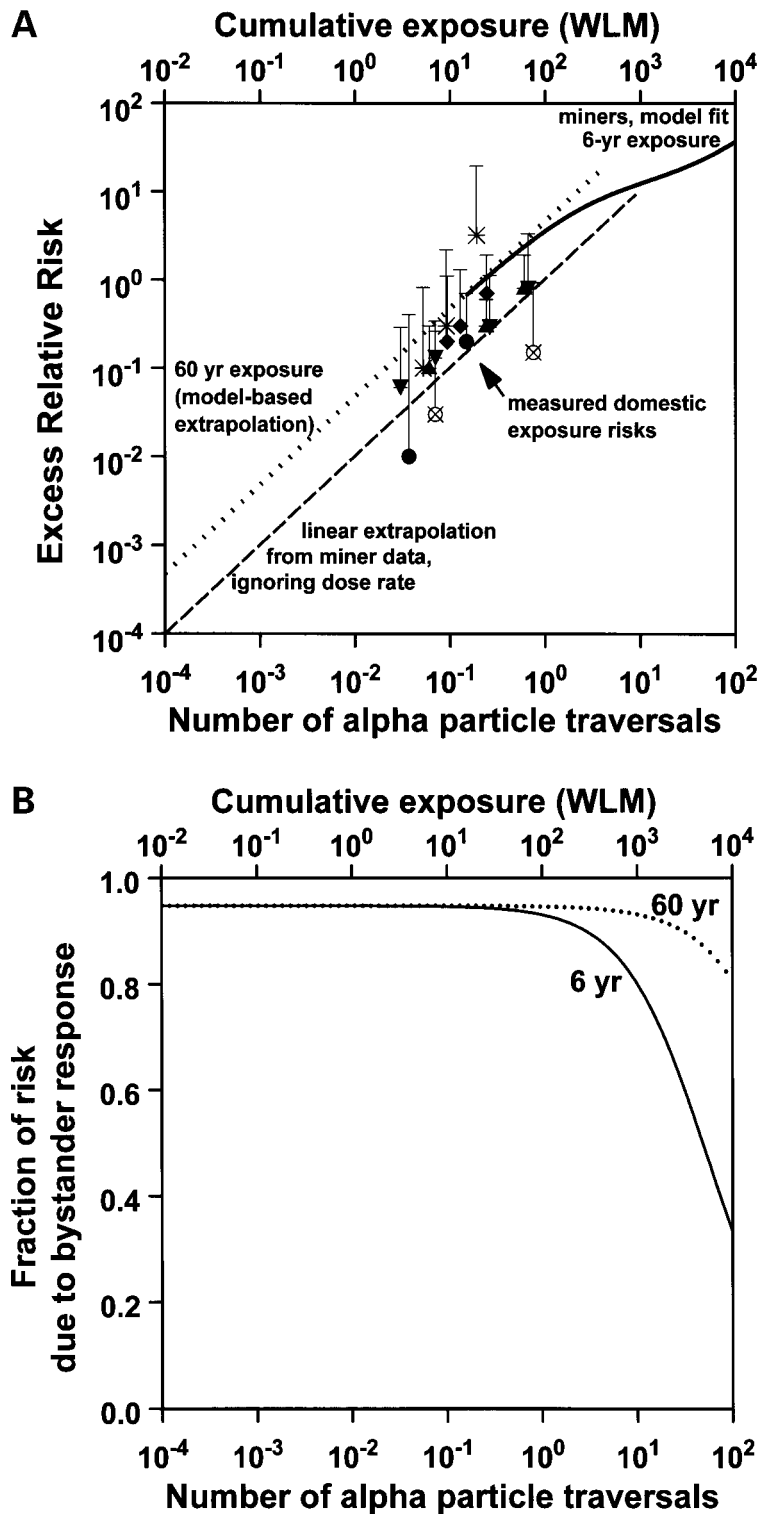


Figure 4. a. Model fits and extrapolations to low doses and dose-rates: In this log-log plot, any linear (no threshold) response appears as a straight line at 45°, with the response per unit dose specified by the height in the log-log plot (*not* by the slope). Solid curve: excess relative risk computed with the parameters fixed by miner data (see figure 3) and assuming a fixed exposure of 6 years, the average for the miner data (NRC 1999). Dotted line: corresponding prediction for a 60-year exposure; note the inverse dose-rate effect relative to the 6-year exposure, at high but not at low doses. Dashed line: linear (no threshold) extrapolation of miner data to low doses (Lubin *et al.* 1994), in which doses-rate effects are not accounted for. Note this ‘naïve’ extrapolation underestimates the low-dose risk estimate in which dose-rate has been accounted for, by about a factor of 4. For comparison, data are shown from domestic radon case-control studies (typically representing cumulative radon exposures above the overall domestic average), as meta-analysed by Lubin and Boice (1997); for visual clarity, only the top halves of the error bars are shown. b. Proportion of the total risk estimated to be due to bystander effects, using the same (best-fit) model parameters as in figure 4a.

At low doses, bystander-induced damage dominates the risk. With increasing dose, the proportion of the risk due to bystander effects decreases, though more slowly for long compared with short exposure times, as long exposure allows for replenishment of cells that are hypersensitive to bystander signals. At very low doses, the fraction of the overall risk that is attributable to bystander effects becomes independent of dose and dose-rate; it is simple to show that this fraction is $1 - \mu/k\epsilon$, which, using our parameter estimates, is about 0.95.

The specific parameter estimates derived for the model parameters seem reasonable: $k = \sim 50$ cells for the number of bystander cells signalled by a hit cell is consistent with the large values found *in vitro* (e.g. Belyakov *et al.* 2001). The replacement rate constant of $\rho = \sim 2$ per month is comparable with the estimated 1-month cell cycle time for the target cells (Adamson 1985).

The numerical values of $c\epsilon$ and $c\mu$ are harder to interpret because c , which cannot be estimated using the current approach, encapsulates the entire process of tumour development with its many unknown features. As we now argue, however, the ratio ϵ/μ is a measure of the relative importance of the bystander to the direct effect at low doses, and as such can be directly compared with the corresponding value derived from analysing *in vitro* data. It is assumed that $k \gg 1$, as appears to be the case both *in vivo* and *in vitro*, so there will exist low doses such that $N \ll 1$ and $Mk \gg 1$. At such a dose, the number of cells activated by the bystander effect is essentially ϵ , and the number of cells activated by the direct effect is essentially μN . Consequently, at this dose, the ratio of the bystander contribution to the direct contribution to the overall risk is $\epsilon/\mu N$. Thus, the ratio ϵ/μ is, in this sense, a measure of the relative importance of the bystander effect at low doses and can be compared directly with the corresponding quantity estimated from an analysis of *in vitro* data (using the notation described in Brenner *et al.* 2001, this is the ratio σ/ν). In fact, from the miner data, our estimates above give $\epsilon/\mu = \sim 0.4$ hits per nucleus, whereas analysing *in vitro* data (Brenner *et al.* 2001) we estimated $\sigma/\nu = \sim 4$ hits per nucleus, about 10 times larger. Such a difference, corresponding to a relatively smaller role for the bystander effect *in vivo* than *in vitro* (provided the signalled neighbour number k is not too small), would not be surprising in view of the many differences between the two situations.

4. Discussion

Radon risks derive from exposure of bronchio-epithelial cells to high-LET α -particles. α -particle

exposure can result in bystander effects, where irradiated cells emit signals resulting in damage to nearby unirradiated bystander cells. This can result in non-linear dose–response relations, and inverse dose-rate effects. Domestic radon risk estimates are currently extrapolated from miner data, which are at both higher doses and higher dose-rates, so bystander effects on non-hit cells could play a large role the extrapolation of risks from mines to homes. Therefore, we have extended an earlier quantitative mechanistic model of bystander effects to include protracted exposure with the aim of quantifying the significance of the bystander effect for very prolonged exposures.

The model considers radiation response as a superposition of bystander and linear direct (\mathcal{Bd}) effects. It attributes bystander effects to a small subpopulation of hypersensitive cells, with the bystander contribution dominating the direct contribution at very low acute doses but saturating as the dose increases. Inverse dose-rate effects are attributed to replenishment of the hypersensitive subpopulation during prolonged irradiation. The essential features of the argument are summarized in figure 5, which uses a number of over-simplifications but illustrates most of the key implications of the model.

The main conclusions of this analysis are as follows.

- At high doses, the model predicts saturation effects and inverse dose-rate effects in the bystander response. At sufficiently low doses, in agreement with general microdosimetric arguments, the predicted response is linear in dose and independent of dose-rate.
- Parameter estimates based on applying the model to dose- and dose-rate-dependent miner data suggest that a single directly hit target bronchial basal cell can send bystander signals to about 50 neighbouring cells.
- The model parameter values obtained from this analysis of epidemiological data, in as much as they can be compared with parameter values obtained from *in vitro* analyses, are significantly different. Thus, model parameters estimated from analysis of *in vitro* studies cannot necessarily be applied to the *in vivo* situation, as attempted, for example, by Little and Wakeford (2001).
- The high-dose saturation and inverse dose-rate effects in the bystander response suggest that a linear extrapolation from miner data, which does not properly take into account dose-rate effects would underestimate the domestic radon risk by about a factor of 4—a value comparable with the empirical estimate applied in BEIR–VI (NRC 1999) on radon risk estimation.

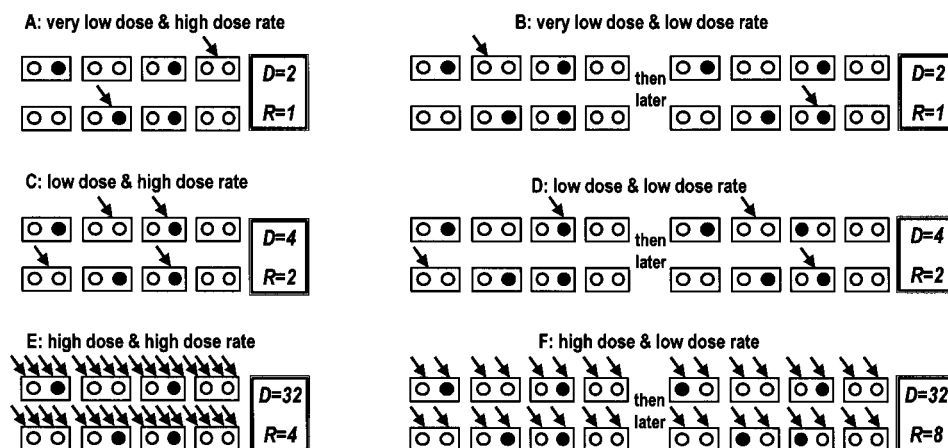


Figure 5. Cartoon illustrating the main results regarding the interplay of risk between dose and dose-rate: the small boxes represent collective, supracellular targets, defined by the property that a hit on any target cell nucleus in the collective target results in a bystander signal to all cells in that collective target. Estimates suggest about 50 target cells per collective target, but for visual clarity, each collective target is shown as containing just two cells. In a few cases, a collective target may contain a hypersensitive cell, shown here as solid. The average number of α -particle hits is labelled 'D' to emphasize its proportionality to dose, and the bystander response is labelled 'R'. (A hypersensitive cell in a hit collective target will, according to the model, be killed if its own nucleus is hit, and activated if any other nucleus in the collective target is hit; since the estimated odds are about 50:1, that activation, rather than killing, occurs, R is taken simply to represent the yield of activated cells.) (A) Dose that is 'very low' in the sense that most collective targets are not hit, and the chance for two α -particle hits in one collective target is negligible. (B) To illustrate the effect of dose-rate on very low dose risks, the same very low dose was split into two separate fractions. The pattern of hypersensitive cells can change between fractions, but it is seen that a very low total dose will produce the same average response, R . Thus, at very low doses, inverse dose-rate effects are negligible. (C, D) Dose is twice as large, but it remains low in the sense that the chance of two hits per collective target is negligible. In agreement with general microdosimetric arguments, the response is also doubled, i.e. it is linearly proportional to dose, and dose-rate effects remain negligible. (E) At a high acute dose, the chance of more than one α -particle per collective target is no longer negligible and this panel represents the case where an average of four hits occurs per collective target (the actual distribution of hits, approximately Poisson, is represented here for simplicity by exactly four α -particles in each collective target). For acute doses, four α -particles in one collective target are no more effective, in terms of the bystander response, than one α -particle; the bystander response, therefore, increases less rapidly than linearly with dose because of 'saturation'—some of the α -particles are 'wasted'. (F) If the high dose is split into two fractions separated by a time interval (long enough for hypersensitive cells to be replaced), the response is doubled, i.e. there is an inverse dose-rate effect at high doses. Overall, comparing (E) with (B) shows that a linear extrapolation of risk from a high acute dose to low dose and low dose-rate may underestimate this risk, in this schematic case by a factor of 4, due to saturation and to inverse dose-rate effects in the bystander response.

It is important to stress that we have in no sense 'proven' the relevance of bystander phenomena to low-dose radon risks. Rather, we have described a mechanistic model that is parsimonious in its number of parameters (four parameters, making the model potentially highly testable), and which is consistent with a large body of epidemiological and laboratory data.

In conclusion, bystander effects represent a plausible quantitative and mechanistic explanation of inverse dose-rate effects by high-LET radiation, resulting in dose-response relations that are non linear and which feature a complex interplay between the effects of dose and exposure time. The model presented here provides a potential mechanistic underpinning for the empirical exposure-time

correction factors applied in BEIR-VI (NRC 1999) on domestic radon risk estimation.

Acknowledgements

The authors are grateful to Mark Little who suggested extending the BAD model to include dose-rate effects and who applied it to uranium miner data, and to Jay Lubin for providing data, results and many useful comments. They also thank Michael Chan for helpful comments. D.J.B. acknowledges the support of the Miller Institute of the University of California at Berkeley. The Columbia University Radiological Research Accelerator Facility (RARAF) is an NIH-Supported Resource Center. The work was supported by Grants RR-11623 and CA-49062

from the National Institutes of Health, and by Grants DE-FG02-01ER63226, DE-FG03-00ER62909 and DE-FG02-98ER62686 from the US Department of Energy.

Appendix

Details are given on the solutions of the differential equations, and intuitive interpretations of the central equations of the model (5 and 6).

The general solution of equation (3) is essentially that for a simple compartmental model with just one compartment:

$$\mathcal{H}(T) = \mathcal{H}(0) +$$

$$\int_0^T \exp\{-\rho(T-t) + (k+1)A[\Phi(t) - \Phi(T)]\} dt$$

where Φ is the α -particle fluence, i.e. the time integral of the particle flux density, ϕ . For a constant α -particle flux density, $\Phi(t) = \phi t$, this expression becomes:

$$\mathcal{H}(T) = [\mathcal{E}/(1+y)] \{1 + y \exp(-h - h/y)\} \quad (7)$$

where

$$\mathcal{E} = \mathcal{H}(0)$$

Here, h and y are given by equation (6). h is the total number of particles that hit a supracellular collective target and it is proportional to dose; y is the corresponding dimensionless particle number rate, proportional to dose-rate.

The factor $\mathcal{E}/(1+y)$ has the following interpretation: if the α -particle flux density, ϕ , is constant (corresponding to a constant dose-rate), and is applied for a long period, the number of hypersensitive cells will settle down to $\mathcal{E}/(1+y)$ (which is $< \mathcal{E}$) as a result of a new dynamic balance between production of hypersensitive cells (due to endogenous processes) and their disappearance (due to endogenous processes as well as to being activated or killed by radiation).

For the bystander yield we get from equations (4) and (7):

$$\mathcal{B} = kA \int_0^T \mathcal{H}(t) dt = [k/(k+1)] \mathcal{E} \{h/(1+y) + [y^2/(1+y)^2] [1 - \exp(-h - h/y)]\} \quad (8)$$

Equation (8) implies that, as expected from our intuitive picture, $\mathcal{B}(h, y)$ is a decreasing function of y when h is fixed, i.e. there is an inverse dose-rate effect. To prove this formally, we differentiate and introduce the auxiliary variable $x = h(1+y)/y$, obtaining $\partial \mathcal{B} / \partial y = C(h, y) f(x)$, where $C(h, y)$ is a

positive function (for $h > 0$, $y > 0$) and $f(x) = 2 - x - [(1+x)/\exp(x)]$. Since $f(x)$ is 0 at $x=0$, and has a negative derivative for all $x > 0$, $f(x)$ is negative for $x > 0$, and $\partial \mathcal{B} / \partial y$ is negative as claimed. The interpretation of this inverse dose-rate effect is that as the dose-rate is lowered, more hypersensitive cells can be replaced during the irradiation period.

For $y \ll 1$ and h fixed, equation (8) gives:

$$\mathcal{B} = [k/(k+1)] \mathcal{E} h \quad (9)$$

which is interpreted as follows. For a very low dose-rate, there is plenty of time to replace any hypersensitive cell that is killed or activated. Consequently, the total number of activated hypersensitive cells is the number, $\mathcal{E} h$, of hypersensitive collective targets that are hit, multiplied by a correction factor, $k/(k+1)$, to account for the fact that hitting a collective target sometimes kills, instead of activating, its hypersensitive cell.

The opposite limit, of an acute irradiation, is not directly relevant to the present data but helps clarify the model. For acute irradiation, $y \rightarrow \infty$ with h fixed and equation (8) gives:

$$\mathcal{B} [k/(k+1)] \mathcal{E} [1 - \exp(-h)] \quad (10)$$

which is interpreted as follows. For acute irradiation, no replacement of hypersensitive cells takes place, so several hits on a collective target are no more effective than one hit. The chance of at least one hit is $1 - \exp(-h)$, since the chance of no hits is $\exp(-h)$ by Poisson statistics; the number of activated hypersensitive cells is, therefore, given by equation (10).

For a very small dose, specifically if $h \ll 1$ and thus a low probability that a collective target is, then $[1 - \exp(-h)] \approx h$, and equation (10) reduces to equation (9), i.e. the inverse dose-rate effect becomes negligible. This is a special case of the general microdosimetric argument that, at sufficiently low doses, the response is linear in dose and independent of dose-rate (Kellerer and Rossi 1972); the significant difference here, however, is that 'low dose' is now interpreted as meaning that a *collective target* is hit at most once, whereas the usual interpretation is that an individual cell nucleus is hit at most once.

References

- ADAMSON, I. Y. R., 1985, Cellular kinetics of the lung. In H. P. Witschi and J. P. Brain (eds), *Toxicology of Inhaled Materials* (Berlin: Springer), pp. 289-317.
- BARENSEN, G. W., 1985, Do fast neutrons at low dose rate enhance cell transformation *in vitro*? A basic problem of microdosimetry and interpretation. *International Journal of Radiation Biology*, **47**, 731-734.
- BELYAKOV, O. V., MALCOLMSON, A. M., FOLKARD, M., PRISE, K. M. and MICHAEL, B. D., 2001, Direct evidence

- for a bystander effect of ionizing radiation in primary human fibroblasts. *British Journal of Cancer*, **84**, 674–679.
- BETTEGA, D., CALZOLARI, P., CHIORDA, G. N. and TALLONE-LOMBARDI, L., 1992, Transformation of C3H10T1/2 cells with 4.3 MeV alpha particles at low doses, effects of single and fractionated doses. *Radiation Research*, **131**, 66–71.
- BRENNER, D. J., 1994, The significance of dose rate in assessing the hazards of domestic radon exposure. *Health Physics*, **67**, 76–79.
- BRENNER, D. J. and SACHS, R. K., 2000, Protraction effects in radiation studies: basic biophysics. *Radiation Research*, **154**, 736–737.
- BRENNER, D. J., LITTLE, J. B. and SACHS, R. K., 2001, The bystander effect in radiation oncogenesis. II. A quantitative model. *Radiation Research*, **155**, 402–408.
- CROSS, F. T., 1992, A review of experimental animal radon health effects data. In J. D. Chapman, W. C. Dewey and G. F. Whitmore (eds), *Radiation Research: A Twentieth-Century Perspective*, vol. 2 (San Diego: Academic Press), pp. 476–481.
- CURTIS, S. B., 1989, A possible role of the inverse dose-rate effect in the radon exposure problem. In K. F. Baverstock and J. W. Stather (eds), *Low Dose Radiation, Biological Bases of Risk Assessment* (London: Taylor & Francis), pp. 547–553.
- DARBY, S. C. and DOLL, R., 1990, Radiation and exposure rate. *Nature*, **344**, 824.
- GILBERT, E. S., CROSS, F. T. and DAGLE, G. E., 1996, Analysis of lung tumor risks in rats exposed to radon. *Radiation Research*, **145**, 350–360.
- GILLILAND, F. D., HUNT, W. C., ARCHER, V. E. and SACCOMANNO, G., 2000, Radon progeny exposure and lung cancer risk among non-smoking uranium miners. *Health Physics*, **79**, 365–372.
- HAHNFELDT, P. and HLATKY, L., 1998, Cell resensitization during protracted dosing of heterogeneous cell populations. *Radiation Research*, **150**, 681–687.
- HILL, C. K., BUONOGURO, F. J., MYERS, C. P., HAN, A. and ELKIND, M. M., 1982, Fission-spectrum neutrons at reduced dose rate enhance neoplastic transformation. *Nature*, **298**, 67–68.
- HILL, C. K., CARNES, B. A., HAN, A. and ELKIND, M. M., 1985, Neoplastic transformation is enhanced by multiple low doses of fission-spectrum neutrons. *Radiation Research*, **102**, 404–410.
- HORNUNG, R. W., DEDDENS, J. A. and ROSCOE, R. J., 1998, Modifiers of lung cancer risk in uranium miners from the Colorado Plateau. *Health Physics*, **74**, 12–21.
- HORNUNG, R. W. and MEINHARDT, T. J., 1987, Quantitative risk assessment of lung cancer in U.S. uranium miners. *Health Physics*, **52**, 417–430.
- HOWE, G. R., NAIR, R. C., NEWCOMBE, H. B., MILLER, A. B., BURCH, J. D. and ABBAT, J. D., 1987, Lung cancer mortality (1950–1980) in relation to radon daughter exposure in a cohort of workers at the Eldorado Port Radium uranium mine: Possible modification by exposure rate. *Journal of the National Cancer Institute*, **79**, 1255–1260.
- KELLERER, A. M. and ROSSI, H. H., 1972, The theory of dual radiation action. *Current Topics in Radiation Research Quarterly*, **8**, 85–158.
- LITTLE, M. P. and WAKEFORD, R., 2001, The bystander effect in C3H10T1/2 cells and radon-induced lung cancer. *Radiation Research*, **156**, 695–699.
- LUBIN, J. H. and BOICE, J. D., JR, 1997, Lung cancer risk from residential radon: meta-analysis of eight epidemiologic studies. *Journal of the National Cancer Institute*, **89**, 49–57.
- LUBIN, J. H., BOICE, J. D., JR and SAMET, J. M., 1995b, Errors in exposure assessment, statistical power and the interpretation of residential radon studies. *Radiation Research*, **144**, 329–341.
- LUBIN, J. H., BOICE, J. D., JR, EDLING, C., HORNUNG, R. W., HOWE, G., KUNZ, E., KUSIAK, R. A., MORRISON, H. I., RADFORD, E. P., SAMET, J. M., TIRMARCHE, M., WOODWARD, A., XIANG, Y. S. and PIERCE, D. A., 1995a, Radon-exposed underground miners and inverse dose-rate (protraction enhancement) effects. *Health Physics*, **69**, 494–500.
- LUBIN, J. H., BOICE, J. D., JR, HORNUNG, R. W., EDLING, C., HOWE, G., KUNZ, E., KUSIAK, R. A., MORRISON, H. I., RADFORD, E. P., SAMET, J. M., TIRMARCHE, M., WOODWARD, A., XIANG, Y. S. and PIERCE, D. A., 1994, *Radon and Lung Cancer Risk: A Joint Analysis of 11 Underground Miner Studies*. National Institute of Health Publication No. 94-3644 (Bethesda: NIH).
- LUBIN, J. H., QIAO, Y. L., TAYLOR, P. R., YAO, S. X., SCHATZKIN, A., MAO, B. L., RAO, J. Y., XUAN, X. Z. and LI, J. Y., 1990, Quantitative evaluation of the radon and lung cancer association in a case control study of Chinese tin miners. *Cancer Research*, **50**, 174–180.
- LUEBECK, E. G., HEIDENREICH, W. F., HAZELTON, W. D., PARETZKE, H. G. and MOOLGAVKAR, S. H., 1999, Biologically based analysis of the data for the Colorado uranium miners cohort: age, dose and dose-rate effects. *Radiation Research*, **152**, 339–351.
- MILLER, R. C., BRENNER, D. J., GEARD, C. R., KOMATSU, K., MARINO, S. A. and HALL, E. J., 1988, Oncogenic transformation by fractionated doses of neutrons. *Radiation Research*, **114**, 589–598.
- MILLER, R. C., BRENNER, D. J., RANDERS-PEHRSON, G., MARINO, S. A. and HALL, E. J., 1990, The effects of the temporal distribution of dose on oncogenic transformation by neutrons and charged particles of intermediate LET. *Radiation Research*, **124**, S62–68.
- MILLER, R. C., RANDERS-PEHRSON, G., GEARD, C. R., HALL, E. J. and BRENNER, D. J., 1999, The oncogenic transforming potential of the passage of single alpha particles through mammalian cell nuclei. *Proceedings of the National Academy of Sciences, USA*, **96**, 19–22.
- MONCHAUX, G., MORLIER, J. P., ALTMAYER, S., DEBROCHE, M. and MORIN, M., 1999, Influence of exposure rate on lung cancer induction in rats exposed to radon progeny. *Radiation Research*, **152** (suppl. 6), S137–140.
- MOOLGAVKAR, S. H. and KNUDSON, A. G., JR, 1981, Mutation and cancer: a model for human carcinogenesis. *Journal of the National Cancer Institute*, **66**, 1037–1052.
- NATIONAL COUNCIL ON RADIATION PROTECTION AND MEASUREMENTS (NCRP), 1987, *Ionizing Radiation Exposure of the Population of the United States*. NCRP Report 93.
- NRC, 1999, *Health Effects of Exposure to Radon, BEIR-VI, Committee on the Health Risks of Exposure to Radon* (Washington, DC: National Research Council).
- PRESS, W. H., FLANNERY, B. P., TEUKOLSKY, S. A. and VETTERLING, W. T., 1986, *Numerical Recipes: The Art of Scientific Computing* (Cambridge: Cambridge University Press).
- RANDERS-PEHRSON, G., GEARD, C. R., JOHNSON, G., ELLISTON, C. D. and BRENNER, D. J., 2001, Technical characteristics of the Columbia University single-ion microbeam. *Radiation Research*, **156**, 210–214.
- ROSSI, H. H., HALL, E. J. and ZAIDER, M., 1982, The role of neutrons in transformation research. I. Theory. In J. J. Broerse and G. B. Gerber (eds), *Neutron Carcinogenesis*.

- Report EUR 8084 (Luxembourg: Commission of the European Communities), pp. 371–380.
- SAWANT, S., RANDERS-PEHRSON, G., GEARD, C. R., BRENNER, D. J. and HALL, E. J., 2001b, The bystander effect in radiation oncogenesis, I. Transformation in C3H10T½ cells *in vitro* can be activated in the unirradiated neighbors of irradiated cells. *Radiation Research*, **155**, 397–401.
- SAWANT, S., RANDERS-PEHRSON, G., METTING, N. F. and HALL, E. J., 2001a, Adaptive response and the bystander effect in C3H10T½ cells in culture. *Radiation Research*, **156**, 177–180.
- ŠEVC, J., KUNZ, E., TOMÁŠEK, L., PLACEK, V. and HORACEK, J., 1988, Cancer in man after exposure to Rn daughters. *Health Physics*, **54**, 27–46.
- STRAM, D. O., LANGHOLZ, B., HUBERMAN, M. and THOMAS, D. C., 1999, Correcting for exposure measurement error in a reanalysis of lung cancer mortality for the Colorado Plateau Uranium Miners cohort. *Health Physics*, **77**, 265–275.
- TOMÁŠEK, L., DARBY, S. C., FEARN, A. J., SWERDLOW, A. J., PLACEK, V. and KUNZ, E., 1994, Patterns of lung cancer mortality among uranium miners in West Bohemia with varying rates of exposure to radon and its progeny. *Radiation Research*, **137**, 251–261.
- XUAN, X.-Z., LUBIN, J. H., LI, J.-Y., YANG, L.-F., LUO, Q. S., YANG, L., WANG, J.-Z. and BLOT, W. J., 1993, A cohort study in southern China of tin miners exposed to radon and radon decay products. *Health Physics*, **64**, 120–131.
- YAKOVLEV, A., MUELLER, W., PAVLOVA, L. and POLIG, E., 1997, Do cells repair precancerous lesions induced by radiation? *Mathematical Biosciences*, **142**, 107–117.
- YANG, T. C., CRAISE, L. M., MEI, M.-T. and TOBIAS, C. A., 1987, Dose protraction studies with low- and high-LET radiations on neoplastic cell transformation *in vitro*. *Advances in Space Research*, **6**, 137–147.