

6-2010

THE DOSE WINDOW FOR RADIATION-INDUCED PROTECTIVE ADAPTIVE RESPONSES

Ronald E J Mitchel

Atomic Energy of Canada Limited, Chalk River Laboratories, Chalk River, ON Canada

Follow this and additional works at: https://scholarworks.umass.edu/dose_response

Recommended Citation

Mitchel, Ronald E J (2010) "THE DOSE WINDOW FOR RADIATION-INDUCED PROTECTIVE ADAPTIVE RESPONSES," *Dose-Response: An International Journal*: Vol. 8 : Iss. 2 , Article 7.
Available at: https://scholarworks.umass.edu/dose_response/vol8/iss2/7

This Article is brought to you for free and open access by ScholarWorks@UMass Amherst. It has been accepted for inclusion in Dose-Response: An International Journal by an authorized editor of ScholarWorks@UMass Amherst. For more information, please contact scholarworks@library.umass.edu.

THE DOSE WINDOW FOR RADIATION-INDUCED PROTECTIVE ADAPTIVE RESPONSES

Ronald E. J. Mitchel □ Radiation Protection Research and Instrumentation Branch, Atomic Energy of Canada Limited, Chalk River Laboratories, Chalk River, ON Canada

□ Adaptive responses to low doses of low LET radiation occur in all organisms thus far examined, from single cell lower eukaryotes to mammals. These responses reduce the deleterious consequences of DNA damaging events, including radiation-induced or spontaneous cancer and non-cancer diseases in mice. The adaptive response in mammalian cells and mammals operates within a certain window that can be defined by upper and lower dose thresholds, typically between about 1 and 100 mGy for a single low dose rate exposure. However, these thresholds for protection are not a fixed function of total dose, but also vary with dose rate, additional radiation or non-radiation stressors, tissue type and p53 functional status. Exposures above the upper threshold are generally detrimental, while exposures below the lower threshold may or may not increase either cancer or non-cancer disease risk.

Keywords: radiation, adaptive response, dose-threshold, risk, low-dose

INTRODUCTION

Radiation effects and radiation risks are assumed to be proportional to dose at all exposure doses, without a threshold, for both the whole organism and for each tissue of a complex organism (ICRP 2006). This assumption for stochastic, as opposed to deterministic effects, is known as the Linear No-Threshold (LNT) hypothesis, and is used by national regulatory agencies as the basis for radiation protection principles and practices that apply to both humans and the environment. However, a wealth of data from experiments using single and multi-cellular organisms, including mammals, indicates that this hypothesis is biologically incorrect.

Inherent in the LNT hypothesis is the assumption that the nature of the biological response to radiation damage is constant, irrespective of dose. However, exposure of cells or organisms to low dose/low dose rates of low LET ionizing radiation induces an adaptive response, such that the detrimental effects of subsequent or previous damage or events, including spontaneous events, are reduced. This induction of resistance is part of a general cellular response to stress that appeared very early in evolution and has subsequently been observed in all organisms thus far

Address correspondence to Ronald E.J. Mitchel, Radiation Protection Research and Instrumentation Branch, Atomic Energy of Canada Limited, Chalk River Laboratories, Chalk River, ON Canada, K0J 1J0; E-mail: mitchelr@aecl.ca

Radiation-Induced Protective Adaptive Responses

examined. A central feature of that induced resistance in prokaryotes is the increased ability to correctly repair DNA double-strand breaks, and this capability appears to have been tightly conserved during evolution, appearing in single-cell eukaryotes, simple eukaryotes, insects, plants, amphibians, and mammals, including human cells in tissue culture and *ex-vivo*. Since this adaptive response to radiation is part of a general response to stress, other stressors, such as heat and chemicals, can also induce adaption to radiation and influence the outcome of the same or other types of stresses, including radiation exposures (Mitchel, 2006).

Multicellular organisms have additionally evolved other protective mechanisms inducible by an exposure to a low dose of low LET ionizing radiation, including the induction of free radical scavengers to reduce initial damage as well as increased immune surveillance and increased apoptotic cell death to remove unrepaired, misrepaired or cancerous cells. Feinendegen *et al.* (2007) have described the roles of these various protective mechanisms that are stimulated by low doses, and how they reduce the risk of cancer development.

Also integral to the LNT hypothesis is the assumption that effects and cancer risks arise in cells as a result of radiation-generated ionization tracks in those cells. However, at the low doses that are of greatest concern for public or occupational exposure, not all cells are hit by radiation (i.e. do not receive an ionization track) and therefore the assumption predicts that there is no consequence of the exposure in those cells. However, extensive recent evidence indicates that this fundamental assumption is not correct, and that hit cells communicate with non-hit cells and generate so-called bystander effects, sometimes (Morgan and Sowa 2007) considered to increase detrimental effects. However, these damaging events are often considered to be part of a signalling system that leads to the removal of damaged cells, resulting in an adaptive or beneficial response (Wang *et al.* 2004, Mothersill and Seymour 2006, Bauer 2007, Portess *et al.* 2007). Therefore at low doses, bystander effects which can appear to generally increase risk, can result in adaptive responses which decrease risk. Both processes are highly non-linear. There are some indications that, for some cell lines in culture, the ability to induce an adaptive response for increased survival (after a second, high dose) may be absent entirely, and that such cell lines only show detrimental bystander effects (Ryan *et al.* 2009).

Given the inherently non-linear characteristics of bystander and adaptive type biological responses to low doses, it seems highly unlikely that the radiation protection assumption that risk is a linear function of dose could be correct at low doses. Since low doses have been shown to induce adaptive responses that reduce the *in vivo* effects and risks of existing spontaneous cancer (Mitchel *et al.* 2003) as well as radiation-induced cancer (Mitchel *et al.* 1999), it would appear that, at least within a certain

R. E. J. Mitchel

range of low doses, radiation-induced protective adaptive responses outweigh detrimental effects, including any detrimental bystander effects. Outside that dose range, detrimental effects are assumed to predominate. Scott (2008) has developed biologically based models that predict both upper and lower dose thresholds of harm.

This review will consider the evidence examining the low LET radiation dose range, and factors influencing it, within which protective adaptive responses outweigh detrimental effects, and therefore reduce rather than increase risk, in contradiction to the LNT hypothesis.

CELL BASED MEASURES OF RISK

Upper dose thresholds

Since it is well established that high acute doses of radiation can produce detrimental cellular effects, and equally well established that low dose and dose rate exposures can produce protective adaptive responses against those events, it follows that there must be a crossover point where protection turns to detriment as dose increases. Most of the studies examining this issue in mammalian cells have used endpoints like chromosomal aberrations or micronucleus formation, measures somewhat removed from the risk endpoints normally considered in radiation protection and risk estimates. For example, Shadley and Wiencke (1989) measured chromatid deletions in human lymphocytes and showed that 10 mGy but not 500 mGy of X-rays given at high dose rate protected against the effects of a 1.5 Gy challenge dose, indicating an upper threshold for protective adaptive responses between those doses. However, they also showed that 500 mGy could induce an adaptive response against the 1.5 Gy challenge dose, if the dose rate was lowered to less than 10 mGy/min, but could not at dose rates ≥ 100 mGy/min. Similar observations were reported by Broome *et al.* (2002) using normal human fibroblasts.

Protection by low dose can also be detected against the naturally occurring, spontaneous risk of deleterious events; i.e. events not induced by a challenge dose. A report by de Toledo *et al.* (2006) showed that an acute dose of 100 mGy increased micronucleus formation in normal human cells but the same dose delivered over 48 hours reduced the frequency to, or below, spontaneous levels. Those data, and the data of Shadley and Wiencke (1989) and Broome *et al.* (2002) showed that the upper threshold was dependent on both dose and dose rate, and suggested that the critical parameter might be the number of lesions that exist in the cell at a given point or points in time, implying that the rate of lesion repair would also influence the upper dose threshold.

The adaptive response to radiation is part of a general cellular response to stress, and other stressors can modify radiation risk and vice

Radiation-Induced Protective Adaptive Responses

versa (Boreham *et al.* 1997; Mitchel, 2006). Likewise, the total stress on the organism also influences the upper dose threshold for protective effects. For example, exposure of fish cells in culture to a low dose of radiation induced a protective adaptive response against a subsequent exposure to a high dose. However, if the cells were also exposed to increasing levels of chlorine, the total stress on the cells eventually reached a level where the same low radiation dose was no longer able to induce protection against the high radiation dose (Mitchel 2007).

In cells, neoplastic transformation is an endpoint much closer to the cancer risk endpoint normally used in radiation protection risk estimates. Using mouse C3H10T1/2 cells, Azzam *et al.* (1996) showed a reduction in spontaneous neoplastic transformation at doses from 1 to 100 mGy given at 2.4 mGy/min (without a subsequent challenge dose), although higher doses were not examined. There was no significant difference in the magnitude of the reduction over this dose range, indicating that the lowest dose produced the maximum protective response, and there was no further increase (or decrease) in that protective response with increasing dose up to 100 mGy. This lack of change in the magnitude of the protective response for neoplastic transformation over this dose range paralleled a similar lack of change with increasing dose in the magnitude of protection against micronucleus formation in normal human fibroblasts exposed to the same dose range (Broome *et al.* 2002). In a similar experiment, using that same measure of risk in a human hybrid cell line, Redpath *et al.* (2001) showed that doses up to 100 mGy alone (at 3.3 mGy/min) suppressed transformation as it did in the mouse cells. However, at 300 mGy and above (at 41.3 mGy/min), transformation was elevated to a rate consistent with a linear extrapolation through the spontaneous rate of the unexposed cells. Both reports therefore agree that the upper dose threshold for protective adaptive responses against neoplastic transformation is above 100 mGy, for both human and mouse cells.

Lower dose thresholds

Like the molecular and cellular end point data showing upper dose thresholds for protective effects, there is also data describing the existence of lower dose thresholds for a radiation-induced adaptive response.

Wolff *et al.* (1988) showed that 10 mGy of X-rays could protect human lymphocytes against chromosome breaks induced by various chemical mutagens and cross-linking agents, indicating that the lower dose limit for adaption was below this dose.

Shadley and Wiencke (1989) observed that 10 mGy induced an adaptive response against chromatid deletions produced by a 1.5 Gy challenge exposure of human lymphocytes in culture, when the adapting dose was given at 200 mGy/min but not at 5 mGy/min. That data suggested that a lower dose threshold for adaption existed, and that like the upper dose

R. E. J. Mitchel

threshold was also dependent on the number of lesions existing in the cell over a given period of time. As observed for the upper dose threshold, the data predict that the lower dose threshold would be sensitive to the cellular rate of lesion removal.

In another study using normal human fibroblasts in culture, a single dose of 1 mGy, but not 0.1 mGy, produced an adaptive response that reduced the frequency of micronuclei after a subsequent large dose (Broome *et al.* 2002). In that regard, a report by Rothkamm and Lobrich (2003), using γ -H2AX foci formation as a measure of radiation-induced DNA double strand breaks in non-dividing cultures of primary human fibroblasts, showed that while DNA repair proceeded after single doses of 5 mGy or more, foci persisted for 24h or more after doses of 1.2 mGy. However, if these cells were allowed to divide, they showed substantial cell death by apoptosis. That result suggested that while the mechanism of protection may depend on the dose, the lower dose threshold for protective effects was below 1.2 mGy in these cells.

The well reported phenomenon of low dose hyper-radiosensitivity followed by high dose radioresistance could be considered evidence for a lower dose threshold (at high dose rate) for cell killing of human cells in tissue culture (Joiner *et al.* 2001), although adaptive responses do not normally occur at these doses (0.3-1.0 Gy) after high dose rate exposure. However, in some cell lines, the absolute ability to induce an adaptive response was linked to the ability of the cell to show a hyper-radiosensitive response (Ryan *et al.* 2009)

A lower dose threshold has also been observed at the cellular level for chromosomal inversions measured in cells from mice exposed *in vivo* (Hooker *et al.* 2004; Zeng *et al.* 2006), where a dose of 1 mGy produced a protective response and reduced spontaneous inversions, but a dose of 0.01 mGy did not, and actually increased spontaneous inversion frequency. This latter observation may reflect the disposition of persistent lesions reported by Rothkamm and Lobrich (2003) in non-dividing cells.

Using a human hybrid cell line Elmore *et al.* (2008) have reported that 100 mGy at dose rates of 1-4 mGy/day was able to induce an adaptive response that protected against spontaneous neoplastic transformation. However, at dose rates below about 1 mGy/day that suppression was lost, again suggesting that the lower dose threshold for adaption depends on the presence of a minimum number of lesions per unit time.

Since the presence of a certain number of lesions per unit time reflects not only the dose rate but also the DNA repair capacity of a cell, this result implies that the lower dose threshold will also reflect the inherent repair capacity associated with different tissue cell types, contributing to tissue specific differences. Additionally, genetic based DNA repair differences between individuals would be expected to further modify the lower dose threshold. Further, since the upper dose threshold for

Radiation-Induced Protective Adaptive Responses

adaption is also known to be dose rate sensitive (Broome *et al.* 2002), similar considerations and variability would likely apply there as well.

ANIMAL BASED MEASURES OF RISK

Upper dose thresholds

Exposure of either *Trp53* normal or cancer prone *Trp53* heterozygous mice to doses from 1-4 Gy, given at high dose rate, resulted in a reduction in median lifespan that was a linear function of the dose, indicating that at high dose rate, the upper dose threshold for protective effects was below 1 Gy (Carlisle *et al.* 2009, in press). That result was consistent with the observations for malignant transformation of human cells in culture (Redpath *et al.* 2001).

Exposure of mice to a dose of 100 mGy at low dose rate induced an adaptive response that protected the mice by increasing latency for myeloid leukemia induced by a 1 Gy exposure, indicating that any upper dose threshold for adaption was above 100 mGy (at low dose rate) in these *Trp53* normal mice (Mitchel *et al.* 1999).

Both 10 and 100 mGy, given at low dose rate, reduced the risk of spontaneous lymphomas in cancer-prone *Trp53* heterozygous mice, indicating an upper dose threshold above 100 mGy for that tissue (Mitchel *et al.* 2003). However, in the same experiment, the 10 mGy but not the 100 mGy exposure protected against osteosarcomas. That result indicated that different tissues in the same animal display different upper dose thresholds, and overall risk or protection for the whole animal will reflect the contributions of all responding tissues. The existence of different upper dose thresholds for different tissues implies that the overall protection of the whole animal at low doses will transition gradually, rather than abruptly, to overall increased risk as the dose rises.

Since the adaptive response to radiation is part of an evolutionarily conserved general response to stress (Mitchel 2006), the upper dose threshold for protective effects in animals is also influenced by the total stress on the organism, just as it is in cells in culture. For example, in mice that also received a high acute dose of 4 Gy, 10 mGy but not 100 mGy was protective for lymphomas (Mitchel *et al.* 2004), while in the absence of the additional 4 Gy stress, protection against the same cancer type was still evident at 100 mGy (Mitchel *et al.* 2003).

Lower dose thresholds

In animals, the lifespan of immune compromised mice was extended by continuous lifetime radiation exposure. However, exposure at 0.35 mGy/h was less effective than exposure at 1.2 mGy/h, and continuous exposure that stopped after 5 weeks at either dose rate was less effective than the lifetime exposure (Ina and Sakai 2004, 2005). Those results

R. E. J. Mitchel

implied that at some even lower dose and/or dose rate there could be a threshold below which the protective effects in mice could disappear. That possibility has subsequently been tested for both the risk of cancer and non-cancer disease in mice.

Cancer

Only one investigation has specifically tested for the existence of a lower dose threshold for the induction of protective adaptive responses against cancer *in vivo* (Mitchel *et al.* 2008). That report tested the influence of very low dose/low dose rate, chronic fractionated exposures (0.33 mGy/day, 0.7 mGy/h, 5d/week for 30, 60 or 90 weeks) on cancer risk in C57BL/6 mice that were either normal for *Trp53* or were cancer prone due to heterozygosity for *Trp53*.

Exposures for less than 60 weeks were below the level necessary to induce overall protective adaptive responses against cancer in *Trp53* normal mice. In fact, exposure for 30 weeks increased risk (higher frequency, decreased latency) for some cancer types (lymphomas) but not others (sarcomas), indicating that like the upper dose threshold, the lower dose threshold is tissue type dependent and that risk will gradually transition from no or increased risk to protection, as was observed (Mitchel *et al.* 2008). The increased cancer risk from exposures below the lower threshold *in vivo* was similar to the increased risk of chromosomal inversions seen at doses below the protective threshold in cells of mice exposed *in vivo* (Hooker *et al.* 2004; Zeng *et al.* 2006).

Continuing the exposures for 60 weeks and hence increasing the total dose, significantly decreased the risk of sarcomas (lower frequency and increased latency) compared to the unexposed control mice, and exposure for 90 weeks eliminated the increased risk of lymphomas. These results indicated that in order to induce a protective adaptive response against cancer in *Trp53* normal mice, the dose had to exceed a lower dose threshold, and doses below that threshold could produce either detrimental or no effects, depending on the specific tissue type. These observations suggest, therefore, that below the lower dose threshold for induction of protective adaptive responses *in vivo*, detrimental bystander effects could outweigh protective effects in some tissue types, while above that threshold the converse was true.

Like *Trp53* normal mice, *Trp53* heterozygous mice exposed to single low doses also display a protective adaptive response against cancer (Mitchel *et al.* 2003). However, in contrast to the *Trp53* normal mice, *Trp53* heterozygous mice showed no influence, by any of the chronic 30, 60 or 90 week exposures, on overall life span in mice with or without cancer or on the frequency or latency of cancers of any tumor type (including B- or T-cell lymphomas) (Mitchel *et al.* 2008). These results indicate that the elevated risk seen in the *Trp53* normal mice exposed to the lower

Radiation-Induced Protective Adaptive Responses

(30 week) chronic fractionated doses was completely dependent on full *Trp53* gene function. They also indicate that reduced *Trp53* functionality elevated the lower dose threshold for both the known protective effects and any possible detrimental effects.

The chronic fractionated exposures given to the *Trp53* normal and heterozygous mice totalled about 48, 97 and 146 mGy after 30, 60 or 90 weeks respectively (Mitchel *et al.* 2008). Previous data for the *Trp53* heterozygous mice showed that single exposures of either 10 or 100 mGy protected against spontaneous lymphoma formation in these mice (Mitchel *et al.* 2003). In contrast, the lack of protection seen in the same *Trp53* heterozygous mice after fractionated doses up to 146 mGy clearly indicates that “dose” thresholds for protective effects are not actually a fixed function of dose, but rather depend upon the existence of a certain level of damage per unit of time, i.e. are dependent on dose rate. This *in vivo* evidence for the dose rate dependency of protective adaptive response thresholds is therefore entirely consistent with the cell based evidence (Shadley and Wiencke 1989; Broome *et al.* 2002; Elmore *et al.* 2008).

Mathematical modelling of the adaptive response and dose thresholds suggests that dose thresholds will be sensitive to the LET of the exposure, since the dose per cell of a single radiation track will vary with this parameter (Leonard 2008).

Non-cancer disease

Chronic ulcerative dermatitis is a severe, spontaneous, autoimmune-related skin disease that appears in aging C57BL/6 mice (Andrews *et al.* 1994). *Trp53* heterozygous mice had a significantly lower frequency of severe disease, but it appeared significantly earlier than in *Trp53* normal mice, indicating a link between the appearance of this disease and the level of *Trp53* function (Mitchel *et al.* 2007). The link between chronic ulcerative dermatitis and oxidative stress (Lawson *et al.* 2005) suggests that in animals with fully or partially functional *Trp53*, radiation may act on ulcerative dermatitis in a manner similar to its action in radiation carcinogenesis, where a low dose induces an adaptive response that protects against the carcinogenic effects of an oxidative stress from a subsequent radiation exposure (Mitchel *et al.* 1999, 2004).

Chronic exposure of *Trp53* normal mice to fractionated doses, beginning at 6 weeks of age and continuing for either 30, 60 or 90 weeks (total doses 48, 97 or 146 mGy) showed that the 90-week exposure (but not the 30 or 60 week exposures) induced an adaptive response in the older mice (>657 days of age), that significantly slowed the appearance and reduced the severity of the disease and also significantly increased the life span of animals that ultimately required euthanization as a result of severe skin disease (Mitchel *et al.* 2007). Those data suggested that in *Trp53* normal

R. E. J. Mitchel

mice a lower dose threshold must be passed before a protective adaptive response is initiated *in vivo* against this non-cancer disease.

However, reduced *Trp53* functionality modified the dose thresholds and extent of the adaptive response *in vivo*. The same chronic fractionated exposure of *Trp53* heterozygous mice that stopped prior to (at 30 weeks of age) or midway through ulcerative dermatitis disease development (at 60 weeks of age) increased (as compared to having no effect in the *Trp53* normal mice) both the frequency and severity of the disease in older animals. Those results indicate that for these doses, detrimental effects outweighed any constitutive or induced protective mechanisms when *Trp53* function was reduced. However, if the chronic, fractionated exposure was continued past 60 weeks, for essentially the whole life span of the *Trp53* heterozygous animals, the frequency or severity of the disease were not different from those observed in the unexposed control heterozygotes. That result showed that even with reduced *Trp53* function, a larger total dose was able to induce adaptive responses that at least balanced the otherwise detrimental effects of the exposure. Again, the results suggest that protective adaptive responses are activated only when a lower damage threshold is surpassed, and that for this non-cancer disease, the threshold was similar in both *Trp53* normal and heterozygous mice.

SIGNIFICANCE OF GENETICS

Radiation dose limits established by national bodies are assumed to provide adequate protection for all persons, and typically do not consider persons who may be at increased risk for genetic reasons. It is assumed that a conservative approach to setting dose limits adequately compensates for any quantitative differences. Inherent in this assumption is the idea that the biological risk of radiation in genetically cancer prone individuals is qualitatively similar to that of genetically normal individuals. These assumptions have not been tested in humans. However, if cancer prone humans were abnormally sensitive to low doses, such as those typical of public and most occupational exposures, then those persons would be at higher risk from such exposures.

Only one gene, *Trp53*, has been extensively investigated for its influence on the *in vitro* and *in vivo* effects of low dose adaptive responses. The *Trp53* gene is inducible by radiation, and functions in regulatory pathways for apoptosis, DNA repair and cell cycle delay, all processes considered critical for cellular responses to radiation damage (Mitchel 2005). Using mouse cells and measuring dicentric frequency, Sasaki *et al.* (2002) showed that the adaptive response to low doses of radiation was dependent on the presence of functional p53. Cells that were *Trp53* normal or *Trp53* heterozygous and were exposed to a low dose showed an adaptive response to a challenge dose of radiation, but cells with no functional *Trp53* did not.

Radiation-Induced Protective Adaptive Responses

The influence of the adaptive response on neoplastic transformation in rodent and human cells has shown that exposures *in vitro* to low doses reduced the frequency of spontaneous neoplastic transformation. This could indicate that the repair system induced during the adaptive response was an error-free system with the characteristics of homologous recombination (Mitchel *et al.*, 2004; Mitchel *et al.* 1997; Dolling *et al.* 1997). A report showing that the *Trp53* protein channels radiation induced DNA double-strand breaks into this pathway, as well as turning off signals for the error-prone NHEJ recombinational pathway, supports this hypothesis (Sasaki *et al.* 2002). Alternatively or additionally, low dose exposure of non-transformed cells is known to induce intercellular signalling that specifically enhances induction of apoptosis of transformed cells, as well as inducing autocrine self-destruction of the transformed cells (Bauer 2007; Portess *et al.* 2007).

Animals with either partial or complete defects in *Trp53* are cancer prone (Harvey *et al.* 1993). Conversely, mice that have been modified to have a constitutively activated *Trp53* are nearly free of cancer risk (Tyner *et al.* 2002). Similar to mice, humans with partial *Trp53* defects are at increased cancer risk. A single amino acid change in the p53 protein (proline for arginine at codon 72) impaired apoptosis (Dumont *et al.*, 2003) and human cancer mortality was increased 2.5 fold (van Heemst *et al.* 2005).

Adaptive responses occur in cancer-prone *Trp53* heterozygous mice, and those responses reduce risk in qualitatively the same way as in *Trp53* normal mice, supporting the radiation protection assumption that radiation risk is qualitatively the same in normal and cancer prone humans. However, as might be expected from this genes' apparently central role in the adaptive response process, the *Trp53* heterozygous mice showed quantitative changes in the response compared to the normal mice. Heterozygosity for *Trp53* reduced the magnitude of the protective effect against higher doses, reduced the upper dose threshold (Mitchel *et al.* 2004) and raised the lower dose threshold (Mitchel *et al.* 2008) for protective effects against radiation-induced and spontaneous cancer. At doses below the lower dose protective threshold, reduced *Trp53* function eliminated the elevated cancer risk seen in *Trp53* normal mice (Mitchel *et al.* 2008), indicating that the cancer risk at these low doses was entirely dependent on full *Trp53* function, and not due to the dose itself. For a non-cancer disease, the reverse was observed (Mitchel *et al.* 2007), whereby reduced *Trp53* function was associated with elevated risk below the lower dose threshold, while the dose threshold itself was similar in mice with normal or reduced *Trp53* function.

DOSE THRESHOLDS AND HUMAN EPIDEMIOLOGY

The main source of information on radiation-induced human cancer risks has been the epidemiological evaluation of data on the Japanese

R. E. J. Mitchel

atomic bomb survivors, using a linear non-threshold model to fit the data. It has been recognized that these estimates do not show an increased cancer risk in adult humans below about 100 mSv for the acute exposure (Tubiana *et al.* 2006) and risk estimates at these lower doses are based on a linear extrapolation from high doses. However, Hoel and Li (1998) reported that “for both the incidence data and the mortality data, the addition of a threshold term significantly improves the fit to the linear or linear-quadratic dose response”. Doses below 100 mGy are those normally identified with an adaptive response, and 100 mGy is close to the reported upper dose threshold for protective effects in both human cells and mice *in vivo*.

For radiation protection purposes, a linear no-threshold extrapolation has been used to estimate cancer risk at the lower doses relevant to the general population and radiation workers (ICRP 2006). The resulting assumption of harm for humans exposed to low doses is controversial and the difficulties with that assumption of risk, from both a physical and biological perspective, have recently been pointed out (Feinendegen and Neumann, 2005). Interestingly, recent reports of American and French national groups of experts have reached opposite conclusions. The American National Academy of Sciences report (National Research Council 2005) recommends the use of the LNT relationship for assessing the risks of small or very small doses. Conversely, the Joint Report of the French National Academy of Sciences and Medicine (Joint Report 2005) states that the use of LNT for assessing the risks of doses below 20 mSv is unjustified and should be discouraged. The relative merits of those reports have recently been compared by Tubiana *et al.* (2006), who note that much animal data is not considered by the American report. However, that comparison has not resolved the controversy (see Letters 2008). This controversy is not new, and some reports spanning a decade argue for positive health effects in the Japanese A-bomb survivors and other exposed groups (Luckey, 1999, 2008). Other reports (using LNT assumptions) that warn of the increased cancer risks with the increase in CT scans, (Hall and Brenner 2008) have met strong opposing views (Scott *et al.*, 2008). Brenner and Sachs (2006) argued that we do not understand the mechanisms involved at low doses and therefore it is premature to revise the assumption of linearity. In that context, however, it should be noted that the current assumption of a LNT response was made without knowledge of mechanisms. However, the debate has now broadened, and the general assumption of linearity for most toxic agents has been challenged. It has been argued that beneficial effects arise from exposure to low levels of a wide variety of agents, not only ionizing radiation, that are generally considered to be detrimental to human health at high levels (Calabrese, 2005a,b; Calabrese and Baldwin, 2003a,b).

Radiation-Induced Protective Adaptive Responses

Recent attempts to extend the risk estimates for humans to doses below 100 mSv have had mixed results. One study (Cardis *et al.* 2005) of six cohorts of nuclear workers with an average cohort cumulative dose of about 20 mSv, showed no increase in leukemia or solid cancer risk for five of the six cohorts, either individually or collectively. Inclusion of the sixth cohort, which individually showed a large increase in solid cancer risk, produced an overall significant increase in solid cancer risk. However, this result would seem to point to problems with either the dose records or assumptions associated with the sixth cohort rather than a real risk. In contrast, another recent study of Canadian Nuclear Power industry workers showed a significant reduction in risk in the 1–49 mSv category compared to the lowest category (<1 mSv) with a relative risk of 0.699 (95% CI: 0.548, 0.892). Above 100 mSv the risk “appeared to increase” (Zablotska *et al.*, 2004). This latter finding is consistent with the upper dose thresholds identified in both the *in vivo* animal data and the data for mouse or human cells in culture noted above. Similarly, a review of the literature for the risk of lung cancer in smokers reported a typical reduction in risk of about 40% in persons exposed to <100 mSv of low LET radiation (Sanders 2008).

Two recent studies examined risks from low dose chronic occupational exposures. One (Zielinski *et al.* 2009a) examined Canadian medical workers for cancer risk. While cancer and non-cancer mortality was lower than the general Canadian population, thyroid cancer incidence was significantly elevated both among males and females, with a combined Standardized Incidence Ratio of 1.74. A second study (Zielinski *et al.* 2009b) examined cardiovascular disease mortality risk in occupationally exposed Canadian nuclear workers as well as medical, dental and industrial workers, and reported a strong positive association between dose and risk. Again, the risk was lower than in the general population. Both studies suffer from uncertainties in dosimetry and adjustment for non-radiation risk factors. Risks lower than the general population may reflect a healthy worker effect, but could also reflect protection by low doses, or some combination of the two. Unfortunately, it is not possible to distinguish between these possibilities.

A current epidemiological focus on the effects of low dose is the estimate of risks from people exposed in the Techa River area of the Southern Urals. Aside from the acknowledged problems with the dose estimates for this cohort (Cardis 2007), the people were exposed to a variety of mixed high and low LET radiation, both internally and externally. Since radiation weighting factors that are used for high LET radiation were developed from high dose studies and are generally unknown at low doses, and the animal experiments indicate that there are likely to be tissue specific differences, it will be difficult for classical epidemiological studies of this mixed LET cohort to be helpful for informing about the

R. E. J. Mitchel

low dose effects of low LET exposure. On the other hand, at least some types of high LET particles are able to produce an adaptive response (Iyer and Lehnert 2002) and it has also been shown that adaptive signals generated by γ -irradiated cells can protect against the bystander effects produced by α -irradiated cells (Sawant *et al.* 2001).

It is uncertain if conventional epidemiological studies of human populations will ever be able to clarify the risks versus benefits of low dose exposure. In addition to the issues above, other problems associated with such studies include the comparison of the results obtained for different exposure patterns (for example, acute external irradiation versus protracted internal irradiation). Aside from these inherent problems, some methodological practices tend to obscure any observation of low dose protective effects, presenting additional difficulties. These include dose lagging (discarding some of the dose), averaging risk over wide dose intervals and including low dose exposed individuals in the low dose control. It has been argued that using such practices may result in unwarranted support for an LNT-type dose-response curve and obscure evidence for risk thresholds or reduced risk at low doses and dose rates (Scott, 2008; Scott *et al.* 2008). Nonetheless, the issue of whether the underlying mechanisms and hence the risk from low or protracted dose exposures are different from those of high dose exposures is now receiving increased attention, and recent reviews by Mullenders *et al.* (2009), Little *et al.* (2009), Jaworowski (2009), Cohen (2008) and Tubiana *et al.* (2009) consider those questions.

While the resolution of low dose responses from the human data will undoubtedly be difficult, data from low dose experiments in cells and animals should be used as guidance for epidemiological studies. Figure 1 gives a schematic representation of that dose response. Based on all the evidence obtained thus far, from lower organisms up to mammals *in vivo*, it would appear that a linear non-threshold response to low LET radiation is highly improbable on an evolutionary basis, and use of this dose response model for humans would therefore appear to be unwarranted. The animal data suggest that low doses are most likely to impact tumor latency rather than incidence, and analysis of the human data should take this into account. Additionally, any detrimental effects found will need to be carefully considered for outcomes that are restricted to specific human genetic variations, rather than effects applicable to the general population. Those results suggest that molecular, rather than conventional epidemiological techniques will be required. The animal data also suggests that at low total doses (<100 mGy), different exposure doses and times cannot be simply all considered together. Even at these low doses, the dose range and dose rate for both protective and detrimental effects of human exposures will likely be subject to both upper and lower thresholds that are variable with genetics and tissue type, as well as with dose

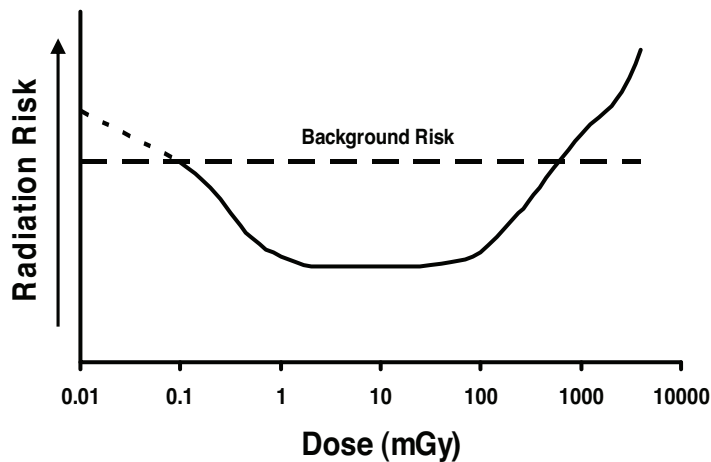
Radiation-Induced Protective Adaptive Responses

FIGURE 1. Schematic representation of the risk of cancer from a single low dose rate, low LET radiation exposure. The figure is based on cell and animal data. The window for maximum adaptive response protection occurs at doses between about 1 and 100 mGy, where risk is reduced below the background or spontaneous level of cancer risk. As dose increases above this range, risk gradually increases to background value at the upper dose threshold and then moves to values above background risk. The change is gradual, because at the individual level, different tissue types have different thresholds, and at the population level there will be genetic variations between individuals. At doses below the range for the maximum level of protection, the risk again gradually increases, and reaches background value at the lower dose threshold for protection. Below this dose, data are sparse and risk may or may not increase above background risk. The dose values of both the upper and lower dose thresholds are subject to multiple variables, including dose rate, cell type, genetic variation, and additional stressors including radiation, heat and chemicals.

and dose rate. For specific human groups or individuals, epidemiological analyses will also need to consider the influence of other, non-radiation stressors that will impact on the ability of low doses to induce protective responses, and/or modify the dose thresholds.

REFERENCES

- Andrews AG, Dysko RC, Spilman SC, Kunkel RG, Brammer D, and Johnson KJ. 1994. Immune complex vasculitis with secondary ulcerative dermatitis in aged C57BL/6Nnia mice. *Vet Pathol* 31:293-300.
- Azzam EI, de Toledo SM, Raaphorst GP, and Mitchel REJ. 1996. Low-dose ionizing radiation decreases the frequency of neoplastic transformation to a level below the spontaneous rate in C3H10T1/2 cells. *Radiat Res* 146:369-373.
- Bauer G. 2007. Low dose radiation and intercellular induction of apoptosis: potential implications for control of oncogenesis. *Int J Radiat Biol* 83:873-888.
- Boreham DR, Dolling J-A, Maves SR, Miller S, Morrison DP, and Mitchel REJ. 1997. Heat-induced thermal tolerance and radiation resistance to apoptosis in human lymphocytes. *Biochemistry Cell Biology*, 75:7-11.
- Brenner DJ, and Sachs RK. 2006. Estimating radiation-induced cancer risks at very low doses: rationale for using a linear no-threshold approach. *Radiat Environ Biophys* 44:253-256.
- Broome E J, Brown D L, and Mitchel R E J. 2002. Dose responses for adaption to low doses of ^{60}Co - γ and ^3H - β radiation in normal human fibroblasts. *Radiat. Res* 158:181-186.
- Calabrese EJ. 2005a. Paradigm lost, paradigm found: the re-emergence of hormesis as a fundamental dose response model in the toxicological sciences. *Environ Pollut* 138(3):379-411.

R. E. J. Mitchel

- Calabrese EJ. 2005b. Historical blunders: how toxicology got the dose-response relationship half right. *Cell Mol Biol (Noisy-le-grand)*. 51(7):643-654.
- Calabrese EJ, and Baldwin LA. 2003a. Toxicology rethinks its central belief. *Nature*. 421(6924):691-692.
- Calabrese EJ, and Baldwin LA. 2003b. The hormetic dose-response model is more common than the threshold model in toxicology. *Toxicol Sci* 71(2):246-250.
- Cardis E. 2007. Commentary: Low dose-rate exposures to ionizing radiation. *International Journal of Epidemiology* 36:1046-1047.
- Cardis E, Vrijheid M, Blettner M, Gilbert E, Hakama M, Hill C, Howe G, Kaldor J, Muirhead CR, Schubauer-Berigan M, Yoshimura T, Bermann F, Cowper G, Fix J, Hacker C, Heinmiller B, Marshall M, Thierry-Chef I, Utterback D, Ahn YO, Amoros E, Ashmore P, Auvinen A, Bae JM, Solano JB, Biau A, Combalot E, Deboodt P, Diez Sacristan A, Eklof M, Engels H, Engholm G, Gulis G, Habib R, Holan K, Hyvonen H, Kerekes A, Kurtinaitis J, Malker H, Martuzzi M, Mastauskas A, Monnet A, Moser M, Pearce MS, Richardson DB, Rodriguez-Artalejo F, Rogel A, Tardy H, Telle-Lamberton M, Turai I, Usel M, and Veress K. 2005. Risk of cancer after low doses of ionising radiation: retrospective cohort study in 15 countries. *BMJ* 331(7508):77.
- Carlisle SM, Burchart PA, and Mitchel REJ. 2009. Cancer and non-cancer risks in normal and cancer-prone *Tp53* heterozygous mice exposed to high dose radiation. *Radiat Res in press*.
- Cohen BL. 2008. The linear no-threshold theory of radiation carcinogenesis should be rejected. *J Am Physicians Surg* 13(3):70-76.
- de Toledo SM, Asaad N, Venkatachalam P, Li L, Howell RW, Spitz DR, and Azzam EI. 2006. Adaptive responses to low-dose/low-dose-rate gamma rays in normal human fibroblasts: the role of growth architecture and oxidative metabolism. *Radiat Res* 166(6):849-857.
- Dolling JA, Boreham DR, Brown DL, Raaphorst GP, and Mitchel REJ. 1997. Rearrangement of human cell homologous chromosome domains in response to ionizing radiation. *Int J Radiat Biol* 72:303-311.
- Dumont P, Leu JI, Della Pietra AC 3rd, George DL, and Murphy M. 2003. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. *Nat Genet* 33:357-365.
- Elmore E, Lao XY, Kapadia R, Giedzinski E, Limoli C, and Redpath JL. 2008. Low doses of very low-dose-rate low-LET radiation suppress radiation-induced neoplastic transformation *in vitro* and induce an adaptive response. *Radiat Res* 169(3):311-318.
- Feinendegen LE, and Neumann RD. 2005. Physics must join with biology in better assessing risk from low-dose irradiation. *Radiation Protection Dosimetry* 117(4):346-356.
- Feinendegen LE, Pollycove M, and Neumann RD. 2007. Whole-body responses to low-level radiation exposure: new concepts in mammalian radiobiology. *Exp Hematol* 35(4 Suppl 1):37-46.
- Hall EJ, and Brenner DJ. 2008. Cancer risks from diagnostic radiology. *Br J Radiol* 81(965):362-378.
- Harvey M, McArthur MJ, Montgomery Jr CA, Butel JS, Bradley A, and Donehower LA. 1993. Spontaneous and carcinogen-induced tumorigenesis in p53-deficient mice. *Nat Genet* 5:225-229.
- Hoel DG, and Li P. 1998. Threshold models in radiation carcinogenesis. *Health Phys* 75(3):241-250.
- Hooker AM, Bhat M, Day TK, Lane JM, Swinburne SJ, Morley AA, and Sykes, PJ. 2004. The linear no-threshold model does not hold for low-dose ionizing radiation. *Radiat Res* 162:447-452.
- ICRP. 2006. Low-Dose Extrapolation of Radiation-Related Cancer Risk. Publication 99, International Commission on Radiological Protection, Elsevier, Amsterdam.
- Ina Y, and Sakai K. 2004. Prolongation of life span associated with immunological modification by chronic low-dose-rate irradiation in MRL-lpr/lpr mice. *Radiat Res* 161:168-173.
- Ina Y, and Sakai K. 2005. Further study of prolongation of life span associated with immunological modification by chronic low-dose-rate irradiation in MRL-lpr/lpr mice: Effects of whole-life irradiation. *Radiat Res* 163:418-423.
- Iyer R, and Lehnert BE. 2002. Alpha-particle-induced increases in the radioresistance of normal human bystander cells. *Radiat Res* 157:3-7.
- Jaworowski Z. 2009. The paradigm that failed. *Dose-Response* 5(2):151-155.
- Joiner MC, Marples B, Lambin P, Short SC, and Turesson I. 2001. Low-dose hypersensitivity: current status and possible mechanisms. *Int J Radiat Oncol Biol Phys* 49(2):379-389.
- Joint Report No 2, Academie Nationale de Medecine, Institut de France—Academie des Sciences (2005) Tubiana M, Aurengo A, Averbeck D, Bonnin A, Le Guen B, Masse R, Monier R, Valleron AJ, de Vathaire F. Dose-effect relationships and the estimation of the carcinogenic effects of low doses of ionizing radiation. Edition Nucleon (Paris 2005)

Radiation-Induced Protective Adaptive Responses

- Lawson GW, Sato A, Fairbanks LA, and Lawson PT. 2005. Vitamin E as a treatment for ulcerative dermatitis in C57BL/6 mice and strains with a C57BL/6 background. *Contemp Top Lab Anim Sci* 44:18-21.
- Leonard BE., 2008. A review: Development of a microdose model for analysis of adaptive response and bystander dose response behavior. *Dose-Response* 6:113-183.
- Letters to the Editor. 2008. *Radiat Res* 169:245-248.
- Little MP, Wakeford R, Tawn EJ, Bouffler SD, and de Gonzalez AB. 2009. Risks associated with low doses and dose rates of ionizing radiation: Why linearity may be (almost) the best we can do. *Radiology* 251:6-12.
- Luckey TD. 1999. Nurture with ionizing radiation: a provocative hypothesis. *Nutrition and cancer* 34(1):1-11.
- Luckey TD. 2008. Atomic bomb health benefits. *Dose-Response* 6:369-382.
- Mitchel REJ. 2005. Radiation risk prediction and genetics: the influence of the Tp53 gene *in vivo*. *Dose-Response* 3:519-532.
- Mitchel REJ. 2006. Low doses of radiation are protective *in vitro* and *in vivo*: Evolutionary origins. *Dose-Response* 4:75-90.
- Mitchel REJ. 2007. Effects of ionizing radiation combined with other stressors, on non-human biota. Chapter 3 In *Multiple Stressors: A Challenge for the Future* (NATO Science for Peace and Security Series C: Environmental Security) (C. Mothersill, I Mosse and C. Seymour, eds), p31-38, Springer Publishing Co., The Netherlands.
- Mitchel REJ, Azzam EI, and de Toledo SM. 1997. Adaption to ionizing radiation in mammalian cells. In *Stress-Inducible Processes in Higher Eukaryotes* (T. Koval, Ed.), pp. 221-243. Plenum Press, New York.
- Mitchel REJ, Jackson JS, McCann RA, and Boreham DR. 1999. Adaptive response modification of latency for radiation-induced myeloid leukemia in CBA/H mice. *Radiat Res* 152:273-279.
- Mitchel REJ, Jackson JS, Morrison DP, and Carlisle SM. 2003. Low doses of radiation increase the latency of spontaneous lymphomas and spinal osteosarcomas in cancer prone, radiation sensitive Trp53 heterozygous mice. *Radiat Res* 159:320-327.
- Mitchel REJ, Jackson JS, and Carlisle SM. 2004. Upper dose thresholds for radiation-induced adaptive response against cancer in high-dose-exposed, cancer-prone, radiation-sensitive Trp53 heterozygous mice. *Radiat Res* 162(1):20-30.
- Mitchel REJ, Burchart P, and Wyatt H. 2007. Fractionated, low dose-rate ionizing radiation exposure and chronic ulcerative dermatitis in normal and Trp53 heterozygous C57BL/6 mice. *Radiat Res* 168:716-724.
- Mitchel REJ, Burchart P, and Wyatt H. 2008. A lower dose threshold for the *in vivo* protective adaptive response to radiation. Tumorigenesis in chronically exposed normal and Trp53 heterozygous C57BL/6 mice. *Radiat Res* 170:765-775.
- Morgan WF, and Sowa MB. 2007. Non-targeted bystander effects induced by ionizing radiation. *Mutat Res* 616:159-164.
- Mothersill C, and Seymour C. 2006. Radiation-induced bystander effects: evidence for an adaptive response to low dose exposures? *Dose-response* 4(4):283-290.
- Mullenders L, Atkinson M, Paretzke H, Sabatier L, and Bouffler S. 2009. Assessing cancer risks of low-dose radiation. *Nat Rev Cancer* 9(8):596-604
- National Research Council, Committee to Assess Health Risks from Exposure to Low Levels of Ionizing Radiation, Health Risks from Exposure to Low Levels of Ionizing Radiation: BEIR VII Phase 2. National Academies Press, Washington, DC, 2005.
- Portess DI, Bauer G, Hill MA, and O'Neill P. 2007. Low dose irradiation of nontransformed cells stimulates the removal of precancerous cells via intercellular induction of apoptosis. *Cancer Res* 67:1246-1253.
- Redpath JL, Liang D, Taylor TH, Christie C, and Elmore E. 2001. The shape of the dose-response curve for radiation-induced neoplastic transformation *in vitro*: evidence for an adaptive response against neoplastic transformation at low doses of low-LET radiation. *Radiat Res* 156(6):700-707.
- Rothkamm K, and Lobrich M. 2003. Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. *Proc Natl Acad Sci USA* 100(9):5057-5062.
- Ryan LA, Seymour CB, Joiner MC, and Mothersill CE. 2009. Radiation-induced adaptive response is not seen in cell lines showing a bystander effect but is seen in lines showing HRS/IRR response. *Int J Radiat Biol* 85(1):87-95.

R. E. J. Mitchel

- Sanders CI. 2008. Prevention of cigarette smoke induced lung cancer by low LET ionizing radiation. *Nuclear Engineering and Technology* 40(7):539-550.
- Sasaki MS, Ejima Y, Tachibana A, Yamada T, Ishizaki K, Shimizu T, and Nomura T. 2002. DNA damage response pathway in radioadaptive response. *Mutat Res* 504:101-118.
- Sawant SG, Randers-Pehrson G, Metting NF, and Hall EJ. 2001. Adaptive response and the bystander effect induced by radiation in C3H10T(1/2) cells in culture. *Radiat Res* 156:177-180.
- Scott BR. 2008. It's time for a new low-dose-radiation risk assessment paradigm—one that acknowledges hormesis. *Dose-Response* 6:333-351.
- Scott BR, Sanders CL, Mitchel REJ, and Boreham DR. 2008. CT scans may reduce rather than increase the risk of cancer. *J Am Phys Surg* 13(1):8-11.
- Shadley JD, and Wiencke JK. 1989. Induction of the adaptive response by X-rays is dependent on radiation intensity. *Int J Radiat Biol* 56:107-18.
- Tubiana M, Aurengo A, Auerbeck D, and Masse R. 2006. Recent reports on the effect of low doses of ionizing radiation and its dose-effect relationship. *Radiat Environ Biophys* 44:245-251.
- Tubiana M, Feinendegen LE, Yang C, and Kaminski JM. 2009. The linear no-threshold relationship is inconsistent with radiation biologic and experimental data. *Radiology* 251:13-22.
- Tyner SD, Venkatachalam S, Choi J, Jones S, Ghebranious N, Igelmann H, Lu X, Soron G, Cooper B, Brayton C, Hee Park S, Thompson T, Karsenty G, Bradley A, and Donehower LA. 2002. P53 mutant mice that display early ageing-associated phenotypes. *Nature* 415:45-53.
- van Heemst D, Mooijaart SP, Beekman M, Schreuder J, de Craen AJ, Brandt BW, Slagboom PE, and Westendorp RG. 2005. Long Life study group. Variation in the human TP53 gene affects old age survival and cancer mortality. *Exp Gerontol* 40:11-15.
- Wang B, Ohyama H, Shang Y, Fujita K, Tanaka K, Nakajima T, Aizawa S, Yukawa O, and Hayata I. 2004. Adaptive response in embryogenesis: IV. Protective and detrimental bystander effects induced by X radiation in cultured limb bud cells of fetal mice. *Radiat Res* 161:9-16.
- Wolff S, Afzal V, Wiencke JK, Olivieri G, and Michaeli A. 1988. Human lymphocytes exposed to low doses of ionizing radiations become refractory to high doses of radiation as well as to chemical mutagens that induce double-strand breaks in DNA. *Int J Radiat Biol Relat Stud Phys Chem Med*. 53(1):39-47.
- Zablotska LB, Ashmore JP, and Howe GR. 2004. Analysis of mortality among Canadian nuclear power industry workers after chronic low-dose exposure to ionizing radiation. *Radiat Res* 161(6):633-641.
- Zeng G, Day TK, Hooker AM, Blyth BJ, Bhat M, Tilley WD, and Sykes PJ. 2006. Non-linear chromosomal inversion response in prostate after low dose X-radiation exposure. *Mutat Res* 602:65-73.
- Zielinski JM, Garner MJ, Band PR, Krewski D, Shilnikova NS, Jiang H, Ashmore PJ, Sont WN, Fair ME, Letourneau EG, and Semenciw R. 2009a. Health outcomes of low-dose ionizing radiation exposure among medical workers: a cohort study of the Canadian national dose registry of radiation workers. *Int J Occup Med Environ Health* 22(2):149-156.
- Zielinski JM, Ashmore PJ, Band PR, Jiang H, Shilnikova NS, Tait VK, and Krewski D. 2009b. Low dose ionizing radiation exposure and cardiovascular disease mortality: cohort study based on Canadian national dose registry of radiation workers. *Int J Occup Med Environ Health*. 22(1):27-33.