

## ADAPTION BY LOW DOSE RADIATION EXPOSURE: A LOOK AT SCOPE AND LIMITATIONS FOR RADIOPROTECTION

**Ron E. J. Mitchel** □ Radiological Protection Research and Instrumentation, Atomic Energy of Canada Ltd. Chalk River Nuclear Laboratories, Chalk River, ON, Canada, K0J1J0

□ The procedures and dose limitations used for radiation protection in the nuclear industry are founded on the assumption that risk is directly proportional to dose, without a threshold. Based on this idea that any dose, no matter how small, will increase risk, radiation protection regulations generally attempt to reduce any exposure to “as low as reasonably achievable” (ALARA). We know however, that these regulatory assumptions are inconsistent with the known biological effects of low doses. Low doses induce protective effects, and these adaptive responses are part of a general response to low stress. Adaptive responses have been tightly conserved during evolution, from single celled organisms up to humans, indicating their importance. Here we examine cellular and animal studies that show the influence of radiation induced protective effects on diverse diseases, and examine the radiation dose range that is effective for different tissues in the same animal. The concept of a dose window, with upper and lower effective doses, as well as the effect of multiple stressors and the influence of genetics will also be examined. The effect of the biological variables on low dose responses will be considered from the point of view of the limitations they may impose on any revised radiation protection regulations.

*Key words: Ionizing radiation; Adaptive response; Radiation protection; Protective thresholds*

### CURRENT STATUS FOR LOW DOSE RADIOPROTECTION

The fundamental assumption underlying all radiation protection regulations and operational procedures is that the risk arising from a radiation exposure increases linearly as a function of dose, without a threshold. This Linear No-Threshold (LNT) hypothesis is accepted by regulatory agencies worldwide, and applies to the protection of both humans and the environment (ICRP 2007). Since every radiation dose, no matter how low, is assumed to increase risk, and that those risks are additive, this assumption leads to practices such as ALARA (As Low As Reasonably Achievable) where large efforts are made to reduce dose below levels where there is no demonstrable risk, and even to levels below natural background. While current radiation protection practices recognize different risks per unit dose for different types of radiation and for different tissue types (using physical dose in Grays normalized with radiation and tissue weighting factors and expressed as Sieverts), they also assume that

Address correspondence to Ron E. J. Mitchel; Radiological Protection Research and Instrumentation, Atomic Energy of Canada Ltd. Chalk River Nuclear Laboratories, Chalk River, ON, Canada, K0J1J0; Phone: 613-584-8811 X44721; Fax: 613-584-8217; Email: mitchelr@magma.ca

the only factor affecting risk is the radiation itself (Mitchel 2007a). Aside from acknowledging that risk per unit dose can be reduced by reducing dose rate (which influences the balance between the physical damage input rate and the biological response/resolution rate of that damage), there is no other allowance that biological variables can influence the resulting risk. Risk estimates for humans are derived from human epidemiological data, with the Japanese A-bomb survivor data typically being the most important. Based on that and other data, it is generally accepted that no statistically significant risk has been demonstrated in humans at doses below about 100 mGy (Tubiana et al. 2005). Therapeutic exposures aside, typical public and occupational exposures are below that dose. In the discussion presented here, dose is expressed in Grays rather than Sieverts, because the weighting factors used for the normalization have been derived from high dose data and have not generally been verified after low dose exposure.

While radiation protection assumptions and practices assume essentially no biological modification of the risk from a radiation exposure (aside from dose rate effects), there is overwhelming evidence in the scientific literature that exposure to low doses of ionizing radiation induces a stress response in cells, that the stress response induces the elevation of protective mechanisms, and that those protective mechanisms reduce the risk of both spontaneous and radiation-induced disease. This response has been seen in every type of organism that has been examined, from single celled organisms up to humans, indicating a tight evolutionary conservation (Mitchel 2006). In mammals, induced protective effects typically occur at doses below the order of 100 mGy (Mitchel, 2006). While these radiation-induced protective effects have been known for decades, and to some extent their potential impact on radiation protection procedures discussed (for example, Averbeck 2009) recent research has produced an improved understanding of their complexity, which will further impact on any inclusion of this response in radiation protection standards and practices.

### **SCOPE OF PROTECTIVE EFFECTS FOR RADIATION PROTECTION**

As noted above, it has been known for a long time that, contrary to the LNT hypothesis, there is a dose threshold, above which high doses produce the familiar detrimental effects and increased risk but below which low doses produce protective adaptive effects which reduce risk. However, recent *in vivo* animal evidence indicates that there are actually two dose (stress) thresholds for protective adaptive responses to be initiated. One, the upper dose threshold where low radiation doses increase to the point where they no longer elicit protective responses has been well studied in many organisms, including humans, as described above. The other, is a lower dose threshold below which the dose is no longer able to initiate the cellular protective mechanisms seen at the higher low doses. This sub-crit-

ical dose was first reported by Zeng et al. (2006) looking at chromosomal inversions in cells taken from mice exposed to various X-ray doses. In that system, high doses elevated inversions, but these were suppressed below spontaneous levels as the dose decreased below the upper dose threshold. However, as the dose was further decreased, below about 0.01 mGy, the inversion rate again increased above the spontaneous rate, evidence that a second, lower dose threshold had been passed, below which translocations were elevated.

A similar observation (Broome et al. 2002) has been reported for micronucleus (MN) formation in normal human fibroblasts following a high gamma-radiation dose. Doses from 500 mGy down to 1 mGy, given at a low dose rate 3 hours prior to a high dose, reduced the MN frequency compared to the high dose alone, indicating an elevated capacity for repair of DNA double strand breaks. However when the prior low dose was reduced to 0.1 mGy, no protective effects were seen, indicating that the dose was below the threshold stress required to induce the protective effects produced by slightly higher doses.

The observations of two dose thresholds seen in the cellular assays noted above have also been seen *in vivo* in mice for disease endpoints (Mitchel et al. 2008). Mice were exposed to 0.3 mGy/d, 5 days/week for 30, 60 or 90 weeks. In Trp53 normal mice, this very low chronic fractionated exposure accelerated the appearance of spontaneous lymphomas after exposure times up to 60 weeks, reducing average lifespan and indicating that a lower dose threshold for protective effects from this level of radiation stress had not been reached. However, extending the exposure to 90 weeks reversed this increased risk and induced protective effects that eliminated that risk of early death, indicating that the necessary protection inducing dose (stress) had been reached. Interestingly, when a different spontaneous tumor (tissue) type was examined (sarcomas), no increased risk was seen after 30 weeks exposure, and a strong protective effect was evident after 60 weeks exposure, but lost after 90 weeks exposure. These results indicate a strong tissue type dependence, as well as dose dependence, for the lower dose threshold for cancer risk. These experiments also provided evidence for a strong genetic influence on cancer risk at these low doses, since the same dose regime given to mice with reduced p53 function (Trp53 heterozygous) showed no increased or decreased risk at any dose in this 90 week chronic exposure regime. The net effect of these two dose thresholds is to define a dose window for radiation-induced protective effects, with the dose thresholds (i.e. the protective dose window) defined by tissue type and p53 functionality (Mitchel, 2010).

The chronic *in vivo* exposure noted above and implicating a low dose threshold for protective effects against cancer has recently been paralleled by a similar study monitoring DNA DSBs (Osipov et al. 2013). In that study, mice were chronically irradiated for 40, 80 or 120 days at a dose rate of

0.15mGy/h, and DNA DSBs were measured in blood leucocytes and splenocytes. An initial increase in the level of DNA DSBs after 40 days of exposure was observed compared to controls, followed by a subsequent drop after either 80 or 120 days of exposure for blood leucocytes and splenocytes. The DNA breaks level after both 80 and 120 days of exposure was lower than in control mice. Those results indirectly indicate that low level ionizing radiation *in vivo* may trigger inducible repair of endogenous DNA DSBs, and that there is a dose threshold for this inducible defence mechanism, below which it does not occur. The remarkable similarity in outcomes of the DNA break study and the cancer study may indicate a strong mechanistic link.

A further indication of the inherent biological complexities, and the resulting difficulties for radiation protection, was also provided in another report on the results of the above described long term chronic exposure (Mitchel et al. 2007). In that report, a different disease, acute ulcerative dermatitis, was monitored. This is a disease common in both old mice and humans, characterized by fragile skin, easily broken by mild trauma or abrasion. In the p53 normal mice, no protective or detrimental effects were seen after 60 weeks of exposure, but clear protective effects were evident after 90 weeks exposure. In contrast, for mice with reduced p53 function, the exposure resulted in increased disease severity for up to 60 weeks of exposure, and then protection after 90 weeks. While the results show that the lower dose threshold is again very different for different tissue types and diseases, they also demonstrate the completely opposite influences of reduced p53 function on the risk of these two different diseases (cancer and dermatitis) after a very low radiation exposure.

The influence of reduced p53 function was also evident in an investigation of the effects of a single low dose on the progression of atherosclerosis in mice prone to the disease (Mitchel et al. 2011, 2013). In general, in mice with fully functional p53, low doses given at low dose rate during either early- or late-stage disease were protective, slowing the progression of the disease. The results also showed that at early stage disease, reduced p53 function does not influence the protective effects against atherosclerosis of low doses given at low dose rate. However, in contrast, when exposed to the same doses at late stage disease, reduced p53 function produced detrimental effects, accelerating the disease rather than showing the protective inhibitory effects seen in Trp53 normal mice. In all cases, the effects were highly non-linear with dose.

The ability of low doses of radiation to modify the risk of a variety of spontaneous and radiation induced diseases is part of a generalized response to low stress. Various other stressors, including exposure to heat or to various chemicals have also been reported to modify such risk. For example, exposure to mild heat stress (40 °C) increased the latency for high dose, radiation-induced myeloid leukemia in mice, restoring part of the lifespan otherwise lost (Mitchel et al. 1999). That result completely

paralleled a similar outcome when the mild stress was an exposure to a low dose (100 mGy) of radiation instead of to heat.

The existence of upper and lower thresholds which create a window for protective effects, and the ability of stressors other than radiation to induce protective effects raises the question of what happens to radiation risk after exposure to multiple stressors. In an experiment using fish cells and measuring unrepaired chromosome breaks (micronuclei) after a large radiation dose, a prior exposure to a low dose of radiation induced an adaptive response that protected the cells by increasing their ability to repair the broken chromosomes. Prior exposure of the cells to 15 mg/L of chlorine had no effect on the ability of the low dose to induce an adaptive response and increase cellular ability to repair broken chromosomes, indicating that the total stress was still within the protective dose (stress) window, i.e. between the upper and lower stress (dose) window. However, when the chlorine exposure was increased to 25 mg/L, the low radiation dose no longer induced an adaptive response, and provided no increased ability to repair chromosomes broken by a high radiation dose (Mitchel, 2007b). This result indicates that exposure to physically different stressors are not seen by the cells as independent events. Instead the cells somehow sum the different stresses, and the total, summed stress will then be either within or outside the protective dose (stress) window.

The above examples show that the impact on disease risk for low radiation dose-induced protective responses is very broad, and includes cancer, heart disease, birth defects and skin disease, whether or not these diseases are spontaneous or radiation-induced. Clearly then the demonstrated broad scope for the influence of low doses on risk should be considered in revisions to the current radiation protection assumptions and practices.

#### **LIMITATIONS OF PROTECTIVE MECHANISMS FOR RADIATION PROTECTION**

Any revision of the standards applied to radiation protection practices, if they are meant to properly reflect actual biological processes and their actual cancer or other risk outcomes, will need to consider a number of variables. Clearly, they would need to account for both upper and lower thresholds for induced protective effects. Additionally they will have to recognize and allow for tissue specific differences in both thresholds, as well as for p53 functional variations in the human population. Other genetic and epigenetic variations in the human population will likely also need to be considered. At our current knowledge level, these would certainly include those variations that affect DNA repair at low doses. Effectively, the procedures would need to predict whether the dose was or was not within the dose window for protective effects. Current radiation protection practices do none of this, and consequently, one practice, ALARA, could be viewed as potentially dangerous.

The responses to stressors other than radiation could also impact the outcome of any radiation protection procedures. For practical radiation protective considerations, a second stress in addition to a low radiation stress can change the protective effect (reduced risk) of a low dose of radiation into a detrimental effect (increased risk), by moving the response through the upper stress threshold and out of the protective window. Conversely, it could have the opposite effect on risk by moving a dose too low to be in the protective window, and that was either detrimental or had no effect, through the lower dose threshold and into the protective window. Consider, for example, an occupationally exposed person who performs work in a radiation field while wearing a plastic suit to prevent personal radioactive contamination. The likely increase in body temperature during the work shift could create a stress response that could move the radiation response (depending on the dose) either into or out of the dose window for protective effects. A similar situation could occur if the worker spent time in a hot tub either before or after the work related exposure. Such a response to a stressor like heat could make the risk of a radiation dose in an exposed person unpredictable. None of these effects are currently considered in radiation protection procedures. Unless revised radiation protection practices can somehow monitor all other physical stresses experienced by an individual, the impact on risk of a low dose can never be certain.

Induction of protective effects can also impact medical treatment of disease. Both radiation and the chemotherapeutic drug cisplatin are used to treat cancer. At high doses, cisplatin impairs human cellular ability to repair radiation induced DNA damage, a desirable outcome for disease treatment by radiotherapy. However, at lower doses, cisplatin apparently induces an adaptive stress response which accelerates the repair of radiation induced DNA damage, reducing the effectiveness of the radiation therapy (Dolling et al. 1998). Such a response could offer a means to protect normal tissue while maximizing the radiation effects on tumor tissue. Such a response could also influence the radiation protection measures applied to the surrounding normal tissue.

From the point of view of radiation protection, virtually nothing is known about the ability of various prescription and non-prescription drugs to induce a stress response in various human cells, and consequently to modify the risk of a radiation exposure in occupationally or publicly exposed individuals. Certainly, nothing is known about their dose thresholds for inducing a stress response or their effects above and below those thresholds. For radiation protection therefore, the actual risk of a radiation exposure is correspondingly uncertain in such individuals.

Given even the current level of knowledge of the biological complexity of responses to low doses and their influence on risk outcomes, it would seem very difficult for any revised radiation protection standards

to account for all of these biological variables. While it seems evident that low dose radiation protection standards must be revised, since the current LNT assumption is clearly inappropriate, defining a workable set of standards that actually reflect the biology may be difficult. An interim approach may be to simply institute a dose threshold, below which no action is taken.

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