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STOCHASTIC THRESHOLDS: A NOVEL EXPLANATION OF NONLINEAR DOSE-RESPONSE RELATIONSHIPS FOR STOCHASTIC RADIOBIOLOGICAL EFFECTS

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□ New research data for low-dose, low- linear energy transfer (LET) radiation-induced, stochastic effects (mutations and neoplastic transformations) are modeled using the recently published NEOTRANS$_3$ model. The model incorporates a protective, stochastic threshold (StoThresh) at low doses for activating cooperative protective processes considered to include presumptive p53-dependent, high-fidelity repair of nuclear DNA damage in competition with presumptive p53-dependent apoptosis and a novel presumptive p53-independent protective apoptosis mediated (PAM) process which selectively removes genomically compromised cells (mutants, neoplastic transformants, micronucleated cells, etc.). The protective StoThresh are considered to fall in a relatively narrow low-dose zone (Transition Zone A). Below Transition Zone A is the ultra-low-dose region where it is assumed that only low-fidelity DNA repair is activated along with presumably apoptosis. For this zone there is evidence for an increase in mutations with increases in dose. Just above Transition Zone A, a Zone of Maximal Protection (suppression of stochastic effects) arises and is attributed to maximal cooperation of high-fidelity, DNA repair/apoptosis and the PAM process. The width of the Zone of Maximal Protection depends on low-LET radiation dose rate and appears to depend on photon radiation energy. Just above the Zone of Maximal Protection is Transition Zone B, where deleterious StoThresh for preventing the PAM process fall. Just above Transition Zone B is a zone of moderate doses where complete inhibition of the PAM process appears to occur. However, for both Transition Zone B and the zone of complete inhibition of the PAM process, high-fidelity DNA repair/apoptosis are presumed to still operate. The indicated protective and deleterious StoThresh lead to nonlinear, hormetic-type dose-response relationships for low-LET radiation-induced mutations, neoplastic transformation and, presumably, also for cancer.

Keywords: Low dose radiation, stochastic effects, nonlinearity

INTRODUCTION

The risk of induced stochastic biological effects in humans (e.g., mutations, neoplastic transformations, and cancers) after low doses of ionizing radiation of any type is generally assessed based on the linear-no-threshold (LNT) model whereby risk of harm increases linearly without a threshold (NRC 2005). The LNT model is used in establishing radiation protection guidelines for nuclear workers and the general public, and is often applied in epidemiological studies of radiation-induced cancer. In 2001, the National Council on Radiation Protection and Measurements

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published Report 136 entitled "Evaluation of the Linear-Nonthreshold Dose-Response Model for Ionizing Radiation" (NCRP 2001). A major but controversial conclusion expressed in the report was as follows:

> In keeping with previous reviews by the NCRP . . . , the Council concludes that there is no conclusive evidence on which to reject the assumption of a linear-nonthreshold dose-response relationship for many of the risks attributable to low-level ionizing radiation although additional data are needed. . . . However, while many, but not all, scientific data support this assumption . . . , the probability of effects at very low doses such as are received from natural background . . . is so small that it may never be possible to prove or disprove the validity of the linear-nonthreshold assumption.

I have omitted reference citations from the above quotation where ellipses appear. The above quote is a reflection of what I have called the low-dose extrapolation fallacy associated with the LNT model (Scott 2005a). Some radiation experts were quite surprised that such a conclusion could be made in light of the growing body of evidence against the general validity of the LNT model (Higson 2004; Tubiana *et al.* 2005). Evidence against the general validity of the LNT model has also been discussed in many publications (Ootsuymaya and Tanooka 1993; Howe 1995; Azzam *et al.* 1996, 2004; Rossi and Zaider 1997; Yamamato *et al.* 1998; Kondo 1999; Tanooka 2000; Yamamato and Seyama 2000; Redpath *et al.* 2001, 2003; Calabrese and Baldwin 2003a, 2003b; Mitchel *et al.* 2003; Scott *et al.* 2003, 2004; Chen *et al.* 2004; Feinendegen *et al.* 2004; Higson 2004; Ko *et al.* 2004; Scott 2004, 2005a, 2005b; Aurengo *et al.* 2005; Feinendegen 2005).

Don Higson in his recent article, "The Bell Tolls for LNT," wrote the following (Higson 2004):

> The linear no-threshold (LNT) model has been a convenient tool in the practice of radiation protection, but it is not supported by scientific data at doses less than about 100 millisievert or at chronic dose rates up to at least 200 millisievert per year. Radiation protection practices based on the LNT model yield no demonstrable benefits to health when applied at lower annual doses. The assumption that such exposures are harmful may not even be conservative and has helped to foster an unwarranted fear of low-level radiation.

The recent report by the Academy of Sciences, National Academy of Medicine (French) (Tubiana *et al.* 2005), written after an extensive review of available literature on radiation-induced stochastic biological effects, stated the following:

> In conclusion, this report raises doubts on the validity of using LNT for evaluating the carcinogenic risk of low doses (<100 mSv) and even more for very low doses (<10 mSv). The LNT concept can be a useful pragmatic tool for assessing rules in radioprotection for doses above 10 mSv; however since it is not based on biological concepts of our current knowledge, it should not be used without precaution for assessing by extrapolation the risks associated with low and even more so, with very low doses (<10 mSv) . . .

The French report also states the following:

> Decision makers confronted with problems of radioactive waste or risk of contamination, should re-examine the methodology used for evaluation of risks associated with very low doses and with doses delivered at a very low dose rate. The report confirms the inappropriateness of the collective dose concept to evaluate population irradiation risks.

In the present article I present support for each of the following assertions:

- Stochastic thresholds (StoThresh (Scott *et al.* 2004)) can cause hormetic-type dose-response relationships for radiation-induced mutations, neoplastic transformation, and, likely, also cancer.
- At doses on the order of 1 mSv of low-LET radiation, neoplastic transformation and cancer risks are orders of magnitude more likely to decrease than to increase by an amount as would be expected based on the LNT model.
- Low doses of low-LET radiation may prevent cancer occurrence and extend life expectancy for adults that already have precancerous cells (e.g., heavy, long-time smokers).
- Low doses of low-LET radiation, when used in conjunction with apoptosis-sensitizing agents, could possibly cure cancer.
- Background low-LET radiation may be suppressing cancer occurrence and other mutations-related diseases among the human population.

RADIATION-RESEARCH RELATED CONCEPTS

Radiation Absorption in Tissue or Cell Cultures

X-rays, gamma rays, and beta radiation are examples of low-LET radiation. Neutrons, alpha particles, and heavy ions (which are encountered in space travel) are examples of high-LET radiation. High-LET radiation, such as alpha particles, does not penetrate very far in matter. For example, alpha particles can be stopped by a piece of paper. However, low-LET gamma rays

can penetrate the entire body of a human. Low-LET beta particles have an intermediate penetration between that for alpha particles and gamma rays. Low-LET X-rays and gamma rays have similar characteristics. Low-LET radiation is therefore more penetrating than high-LET radiation.

The absorption of ionizing radiation in biological tissue or cell cultures involves stochastic interactions with constituent atoms and molecules, and generates energy deposition (track) events accompanied by bursts of reactive oxygen species (ROS) (Feinendegen 2005). Induced radiogenic DNA damage increases as radiation dose increases. The ROS are similar to those that arise constantly by normal oxidative metabolism. Endogenous ROS alone induce about a million DNA oxyadducts per cell per day compared to 5×10^{-3} total DNA-damaging events per average cell per day from background radiation exposure at 1 mGy over a year (Feinendegen 2005).

In the case of penetrating low-LET radiation, particle tracks arise stochastically throughout the exposed tissue with relatively low density at low doses. For low doses of high-LET radiation, the distribution of ionized molecules and of ROS bursts is more heterogeneous (Feinendegen 2005). Intercellular communication after radiation damage probably depends on the spatial distribution of the hit cells as well as the cellular environment. Biological response to irradiation of humans is known to depend on the spatial distribution of the radiation hits in the body, the total radiation dose, and how rapidly the dose is delivered (i.e., dose-rate history).

Stochastic Radiobiological Effects

For ionizing radiation doses in the range 0 – 100 mGy, biological effects of interest include induced genomic instability, mutations, neoplastic transformation, and cancer (Scott 2004). These effects, along with genetic effects, are called stochastic, since their occurrence is governed by probabilistic considerations.

Three forms of genomic instability have been distinguished in the context of modeling low-dose radiation-induced stochastic effects (Scott 2004; Scott *et al.* 2004): normal minor instability (NMI), transient problematic instability (TPI), and persistent problematic instability (PPI). The transient instability can be eliminated via cooperative repair processes (e.g. high-fidelity, p53-dependent DNA repair/apoptosis). However, misrepair of damage can lead to PPI (mutant cells) which can be passed to cell progeny increasing their chances for undergoing spontaneous neoplastic transformation. These forms of instability are features of the NEOTRANS$_3$ model discussed later.

Protective and Deleterious Bystander Effects

Radiation hits in cells may cause non-hit neighboring cells to become affected by signaling substrates from hit cells (bystander effect) (Azzam *et*

al. 2004). The bystander effect can either be deleterious (enhancing the net biological damage) or protective (suppressing the net biological damage). Protective effects appear to predominate over the dose range of 1 – 100 mGy after brief exposure at a high rate to low-LET photon radiation. Three cooperative processes are now considered to contribute to protection in the indicated dose zone: activated presumptive p53-dependent high-fidelity DNA repair in competition with presumptive p53-dependent apoptosis, and the previously introduced novel presumptive p53-independent protective apoptosis mediated (PAM) process. The PAM process (a bystander effect) is thought to be mediated via ROS and cytokines such as transforming growth factor β, and selectively removes genomically compromised cells including mutants and neoplastically transformed cells (Bauer 1995, 1996; Scott 2004, 2005a, 2005b). The PAM process was first demonstrated experimentally by German researchers (Jürgensmeier *et al.* 1994; Bauer 1995, 1996, 2000; Langer *et al.* 1996; Hipp and Bauer 1997).

Deleterious bystander effects may predominate over the ultra-low-dose range of 0 – 0.01 mGy so far as inducing inversion mutation in mice (Hooker *et al.* 2004). This implicates an ultra-low-dose zone of activated low-fidelity DNA repair. The indicated inversion mutations results implicate a threshold on the order of 0.01 mGy for activating the cooperative protective processes. At doses from 250 – 1000 mGy and higher delivered at a high rate, there is no evidence for the PAM process (Scott 2004). It is either inhibited or is just not activated in the indicated dose range. Here the phrase "inhibition of the PAM process" is used in a very general way to simply imply that it does not occur since it is not clear whether a true inhibition is invoked.

When the dose rate is low and exposure time is spread over a large time interval, cooperative protective processes may occur over a much wider dose range, being continually activated (or reactivated) during the protracted exposure (Scott 2004, 2005b). In such cases the zone of suppression of stochastic effects may range from very low doses up to more than 400 mGy (Scott 2004, 2005b). Further, the magnitude of suppression may significantly increase after such protracted exposures (Scott 2004).

Adaptive Response

The classical two-dose, adaptive-response study involves exposing cell cultures or animals to a very small low-LET radiation dose and after a few hours or longer irradiating them with a much larger dose that is expected to produce easily measurable enhanced biological effects (Wolff 1998). For the comparison group, only the higher dose is given. The hypothesis is that the adapting low dose will protect the cells from damage from the subsequent high dose (e.g., by inducing enhanced repair capacity). Thus, if the frequency of biological effect (e.g., mutations, neoplastic transformations) is lower in the group that received both the

adapting and larger test dose than for the test dose alone, then one concludes that the small dose caused the cells to adapt so that they were less affected by the subsequent high dose.

Azzam *et al.* (1996) and Redpath *et al.* (2001) introduced a novel experimental single-dose protocol whereby only the small adapting dose was administered. The yield of biological effect (neoplastic transformation *in vitro*) was then compared to the spontaneous frequency for unirradiated cells. To the surprise of many, the adapting dose protected against spontaneous neoplastic transformation, yielding a decrease (rather than an increase) in the transformation frequency. Similar results were reported by Hooker *et al.* (2004) for inversion mutation induction in spleen of pKZ1 mice exposed *in vivo* to low doses of 250 kVp X-rays. However, their mutation data also showed an elevated mutation frequency at ultra low doses (< 0.01 mGy), suggestive of activation of low-fidelity DNA repair, rather than high-fidelity repair for this dose zone.

I have previously applied the NEOTRANS$_3$ model (Scott 2004) to neoplastic transformation data of Redpath *et al.* (2001). Here the model is applied to mutation data of Hooker *et al.* (2004) for inversion mutation in spleen of pKZ1 mice exposed to 250 kVp X-rays and also to data for 28 kVp X-ray- (Ko *et al.* 2004) and 60 kVp X-ray- (Redpath *et al.* 2003) induced neoplastic transformation *in vitro* of HeLa × skin fibroblast human hybrid cells.

The NEOTRANS$_3$ model and how it can be used to clarify issues related to the shape of the dose-response curves for specific stochastic effects at very low doses is discussed in the section that follows.

NONLINEAR DOSE-RESPONSE RELATIONSHIPS

Deterministic Threshold and Nonlinear Dose-Response Curves

The risk for specific radiation-induced deterministic effects (e.g., death from injury to the hematopoietic system) is known to be a nonlinear function of dose with an associated threshold (i.e., minimal tolerance dose for a population). Dose-response curves generally have a sigmoidal shape.

Stochastic Thresholds and Nonlinear Dose-Response Curves

Many stressor-induced biological processes likely have dose thresholds. It is a common view that DNA repair activation requires a damage threshold (Tubiana *et al.* 2005) which implicates a StoThresh. Because different individuals respond differently to a given amount of radiation, the indicated StoThresh likely varies for different individuals having a characteristic distribution that may depend on other covariates (e.g., age, gender, genetic background). Examples of molecular processes suspected of having StoThresh are oncogene activation, suppressor gene inactivation, stress protein induction, and induced activated p53-dependent high-fidelity DNA repair/apoptosis.

For replicate *in vitro* studies, conditions in the biological samples are not identical in every respect, thus local cellular environments would be expected to vary if only by small amounts. Further, radiation interactions with cells in cell cultures are stochastic. Because intercellular communication is likely influenced by the distribution of cells hit by radiation (Feinendegen *et al.* 2004), thresholds for activating some key biological effects (e.g., p53-dependent high-fidelity DNA repair/apoptosis) likely vary between different replicates. Such thresholds are therefore also stochastic and can be described using appropriate distributions.

The recently published NEOTRANS$_3$ model incorporates StoThresh for radiation-induced stochastic effects such as mutations and neoplastic transformation (an early step in cancer induction) (Scott 2004). The model has been used to characterize nonlinear (or linear) dose-response relationships for mutation or neoplastic transformation induction by low doses of ionizing radiation (Scott 2004). With the model, nonlinearity arises from protective and deleterious StoThresh. StoThresh distributions associated with specific radiation-induced stochastic effects (mutation and neoplastic transformation induction) are estimated in this paper.

NEOTRANS$_3$ Model and Stochastic Thresholds

The NEOTRANS$_3$ model is presented in Figure 1 and applies to low doses and to a small number of hypersensitive cells among a much larger number of resistant cells. The model features radiation-induced transitions between instability states NMI, TPI, and PPI. Doses above a threshold activate presumed p53-dependent, high-fidelity DNA repair/apoptosis and the presumed p53-independent PAM process. Features in Figure 1

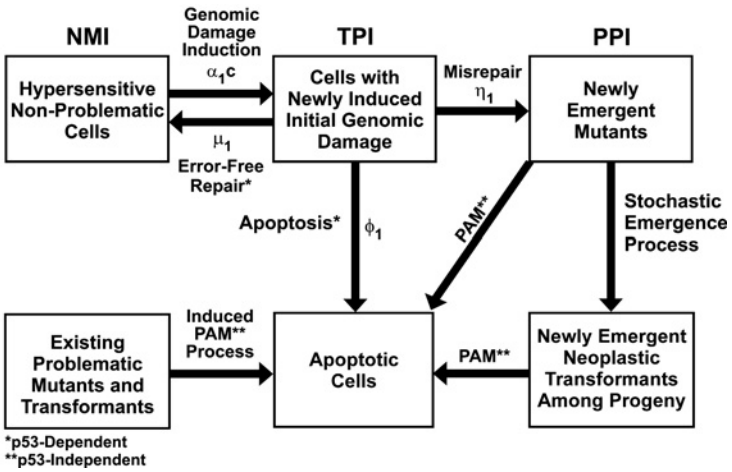

FIGURE 1 Modified NEOTRANS$_3$ model for low-dose-induced stochastic effects. See main text for more detail.

relate to dose ranges over which the indicated protective processes are activated. When activated the PAM process selectively removes genomically compromised cells (e.g. mutants and neoplastically transformed cells). In the present applications of the NEOTRANS$_3$ model, after ultra-low doses of low-LET radiation, high-fidelity repair/apoptosis is presumed not to be activated. Rather low-fidelity DNA repair/apoptosis is presumed activated. This facilitates characterizing the dose-response curve presented later for X-ray induced recombination mutations in mice.

The parameter α_1, in Figure 1, when multiplied by the radiation dose rate c, accounts for low-dose-induced genomic damage among the hypersensitive cells in the population. The parameter μ_1 governs the rate of commitment of damaged hypersensitive cells to the activated error-free repair pathway. This high-fidelity repair is presumed to be activated by radiation StoThresh and is presumed to depend on p53. The corresponding parameter for the misrepair pathway (which competes with the error-free pathway) is η_1. Misrepair (also a component of p53-dependent repair process) leads to a variety of viable mutations (cells with persistent problematic instability). The parameter ϕ_1 governs the rate of p53-dependent commitment of newly damaged cells (including lethal mutations) to apoptotic pathways. The p53-dependent high-fidelity repair and p53-dependent apoptosis when activated are in competition and the path selected depends on the nature of the DNA damage. The PAM process operates at low doses of low-LET radiation, while the p53-dependent high-fidelity repair/apoptosis may occur at low, moderate, and high doses as well as after high-LET irradiation. The PAM process appears not to occur after high-LET alpha irradiation (Scott *et al.* 2004). However, this view is based solely on one study. Thus, more research needs to be conducted related to addressing the question of whether the PAM process can be activated by alpha radiation.

Typical units for α_1 are mGy^{-1}. Typical units for μ_1, η_1, and ϕ_1 are min^{-1}. The parameter f_1, which represents the fraction of hypersensitive cells, is dimensionless. These parameters are stochastic (i.e., have distributions) and in some cases are now modeled as varying for different broad dose ranges (zones) to be introduced later.

With the NEOTRANS$_3$ model (as with its predecessor NEOTRANS$_2$, model) the p53-independent PAM process (Jürgensmeier *et al.* 1994; Hipp and Bauer 1997) and p53-dependent high-fidelity DNA repair/apoptosis, when jointly activated, work together in guarding against propagation of genomic instability and subsequent stochastic effects. The p53-dependent apoptosis removes damaged cells before mutations and neoplastic transformations arise and competes with error-free repair and misrepair pathways. When error-free repair occurs it eliminates DNA damage but not damaged cells. The PAM process (considered p53-independent) removes mutants and neoplastic transformants, as well

as other aberrant cells that are present. Thus, the indicated team of biological protectors when jointly activated provides powerful protection against adverse stochastic effects of exposure to genotoxic agents.

After moderate doses, protection is also presumed to be provided by high-fidelity p53-dependent DNA repair/apoptosis. Such protection could be revealed via inhibition of these protective processes, in which case the frequency of stochastic effects such as mutations and neoplastic transformation is expected to significantly increase.

Yield of Biological Effects after Low-Dose Irradiation

For brief exposure to small or moderate doses, D_L, of low-LET radiation, the expected frequency of biological effects (neoplastic transformations or mutations) per surviving cell is given in the context of NEOTRANS$_3$ by:

$$\begin{aligned} Y(D) &= Y_0 + [1 - Y_0]kD, \text{ for } D < D_{PAM}, \\ Y(D) &= (1 - f_0)\{Y_0 + [1 - Y_0]kD\}, \text{ for } D_{PAM} \le D < D_{off}, \\ Y(D) &= Y_0 + [1 - Y_0]kD, \text{ for } D \ge D_{off}, \end{aligned} \tag{1}$$

where the slope parameter k is given by:

$$k = f_1\alpha_1\eta_1\Omega/(\mu_1 + \eta_1 + \phi_1). \tag{2}$$

Note that for $D \ge D_{off}$, Equation 1 reduces to the classical moderate and high-dose LNT model which is currently considered to apply for alpha irradiation (Scott 2004). The notation used in this paper is different from that used in earlier publications. $Y(D)$ is used here for either mutations or for neoplastic transformation with the slope parameter k differing for these two endpoints. Parameters α_1, η_1, μ_1, and ϕ_1 are expected to take on the same value for neoplastic transformation and mutation induction. *However,* Ω differs depending on the biological endpoint considered. This makes it possible in theory to simultaneously fit the NEOTRANS$_3$ model to mutation induction and neoplastic transformation data.

The parameter f_1 is the fraction of hypersensitive cells among the target cell population. The parameter f_0 is the fraction of the spontaneously occurring, genomically compromised cells (e.g., spontaneous transformants) removed via the radiation-induced PAM process and has been given the special name "protection factor" (*PROFAC*) (Scott *et al.* 2004). For $f_0 = 0$ (i.e., no PAM), Equation 1 reduces to the LNT model for dose zones over which k is invariant.

The parameters Ω, μ_1, η_1, and ϕ_1, as well as the efficiency of the PAM process (*PROFAC*), are assumed to be influenced by genetic background. Evidence is presented later indicating that after ultra-low doses (< 0.01

mGy of low-LET radiation), the induced cooperative protective processes (PAM process, p53-dependent high fidelity DNA repair/apoptosis) may not be activated and that misrepair of DNA damage is much more likely because of activated low-fidelity DNA repair/apoptosis. Thus, the parameters μ_1, ϕ_1, η_1, and *PROFAC* are presumed to differ for this dose region than for the higher doses considered here. After very high doses μ_1 is expected to decrease, reflecting the induction of error-prone repair processes (Aurengo *et al.* 2005).

The parameter η_1 accounts for both unrepaired and misrepaired damage. Here, the fundamental model parameters discussed are allowed to change values over different dose zones. This is achieved via allowing the model parameter k (which depends on the indicated fundamental model parameters) to vary between different dose zones. This makes the $NEOTRANS_3$ model a very useful model for characterizing nonlinear dose-response relationships for radiation- (or other stressor-) induced mutations and neoplastic transformations.

For mutations induction, Ω represents the probability for the occurrence of mutation of interest among those induced by irradiation. For neoplastic transformation, Ω represents the probability that a cell with radiation-induced PPI (mutant cells) will produce transformed progeny during a specified follow-up time.

With the current $NEOTRANS_3$ model, induced enhanced p53-dependent high-fidelity DNA repair/apoptosis can reduce the slope parameter k (see Equation 2) but cannot cause a negative slope in the dose response curve (i.e., a dose-response curve where the frequency of the effect goes down below the spontaneous level). However, because the p53-independent PAM process is also activated, already present spontaneous mutants and spontaneous neoplastic transformants can be eliminated and lead to a reduction in the frequency of these effects below the spontaneous level. However, this suppression below the spontaneous level is only likely at very low doses after brief exposure. Larger doses may be involved for protracted exposure at a low rate. This is because the *PROFAC* is expected to increase with decreasing dose rate and with increasing exposure time (Scott 2004).

Removal of precancerous cells via the PAM process would be expected to have the same impact on the cancer induction dose-response curve. Thus, for cancer induction, risk could decrease after low doses of low-LET radiation. A similar reduction may also occur after exposure to low doses of other toxic agents that activate the PAM process. Immune system stimulation may also contribute to the reduction.

Therefore, for protracted exposure of humans to low-LET radiation at very low rates over a prolonged period (e.g., years), the cancer incidence may be greatly reduced as has been reported (Chen *et al.* 2004). The title of the cited paper by Chen *et al.*, "Is Chronic Radiation an

Effective Prophylaxis against Cancer?", raises an important question to be considered by the scientific community. Other researchers have also reported reduced cancer risk in humans after chronic exposure at low rates (Aurengo *et al.* 2005). Living in areas of high background low-LET radiation appears also to provide protection against cancer.

Specifications of Dose Zones

To facilitate modeling of data for low-LET radiation-induced stochastic effects (mutations, neoplastic transformation), five dose zones are considered, as indicated below, with respect to brief high-rate or protracted low-rate exposure to low-LET radiation (e.g., X-rays, gamma rays).

Zone 1 (Ultra Low-Doses Zone)

After ultra low doses (close to daily natural background radiation), it is assumed that high-fidelity DNA repair is not activated. Rather low-fidelity, error-prone repair is presumed to occur in cooperation with apoptosis. This is based on inversion mutation data (Hooker *et al.* 2004) for brief exposure at a high rate to 250 kVp X-rays from doses near daily background up to 0.01 Gy. These data are presented later.

Zone 2 (Transition Zone A)

This is a relatively narrow low-dose zone just above Zone 1 in which StoThresh for activating the cooperative protective processes (p53-dependent high-fidelity DNA repair/apoptosis and the PAM process) are considered to occur. The indicated protective StoThresh is represented by the notation D_{PAM} (Scott 2004).

Zone 3 (Zone of Maximal Protection)

This dose zone is just above Zone 2 and is the zone where maximal protection against mutations and neoplastic transformation is afforded. For irradiation of animals or humans, each irradiated member has the cooperative protective processes activated. For *in vitro* replicate studies, each replicate has the cooperative protective processes activated.

Zone 4 (Transition Zone B)

This dose zone is just above Zone 3 and is the zone where the StoThresh for inhibiting the PAM process occur. The indicated deleterious StoThresh is represented by the notation D_{off} (Scott 2004).

Zone 5 (LNT Zone)

This dose zone is just above Zone 4 and is the zone where the PAM process is maximally inhibited. For irradiation of animals or humans, each individual does not have the PAM process activated. However, high-fidelity DNA repair/apoptosis is presumed to be activated in this zone as

well as in Zone 4. For doses in this zone, the LNT model is expected to adequately represent the frequency of stochastic effects.

Definitions of higher dose zones may also prove beneficial when studying high-dose effects. However, the focus here is on low to moderate doses. For the NEOTRANS$_3$ model applications to mutation induction and neoplastic transformation that follow, the slope parameter k is allowed to differ for Zone 1, where low-fidelity DNA repair occurs, as differentiated from the other zones (Zone 2 – Zone 5) where high-fidelity DNA repair/apoptosis are presumed to operate. Thus, for Zone 1, $k = k_1$ is used. For Zones 2 – 5, $k = k_2$ is used. The PAM process is considered to be operational only in Zone 2 (StoThresh occur in this zone), Zone 3 (PAM process activation maximal over this zone), and Zone 4 (PAM process inhibition threshold occurs over this zone).

The mutation data presented later span all five dose zones. The neoplastic transformation data presented later span Zones 3 – 5 but provide no information on the shape of the dose-response curve over Zones 1 and 2.

Impact of StoThresh on the Shape of the Dose-Response Curve for Mutation Induction

Figure 2 shows results of fitting the NEOTRANS$_3$ model to data of Hooker *et al.* (2004) for 250 kVp X-ray-induced inversion mutations in spleen of irradiated pKZ1 mice. The model has been applied using previously described Bayesian methods implemented via Markov Chain Monte Carlo to facilitate working with StoThresh (Scott 2004; Scott *et al.* 2004). The steep initial rise in the fitted curve in Figure 2 is due to the slope parameter $k = k_1$ having the very large posterior mean ± standard deviation of 0.03 ± 0.004 per mGy. In stark contrast, k_2 was estimated to

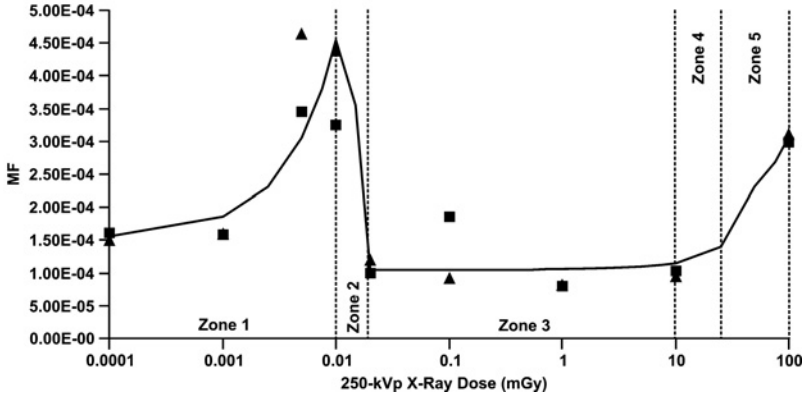

FIGURE 2 Application of the NEOTRANS$_3$ model to data of Hooker *et al.* (2004) for 250 kVp X-ray-induced inversion mutations in the spleens of irradiated mice. Dose zones are explained in the text.

be $1.5 \times 10^{-6} \pm 3.92 \times 10^{-7}$ per mGy. Thus, k_1/k_2 evaluated based on posterior means was approximately 20000, suggesting four orders of magnitude greater protection against stochastic effects associated with activated p53-dependent high-fidelity DNA repair/apoptosis than with the activated low-fidelity DNA repair/apoptosis at ultra-low doses.

The protective StoThresh, D_{PAM}, for activating the PAM process was modeled as uniformly distributed over the very narrow Zone 2 of 0.01 – 0.02 mGy, i.e., Transition Zone A. Essentially, the prior and posterior distributions were identical due to a lack of data over this zone. The StoThresh in Transition Zone A caused the dose-response curve to drop steeply over this range rather than to continue increasing as in Zone 1. Thus, the StoThresh for D_{PAM} invoked nonlinearity in the dose-response curve. For the dose zone 0.02 – 20 mGy, the mutation frequency is suppressed by roughly a constant amount due to maximal cooperative protection via p53-dependent high-fidelity DNA repair/apoptosis and the PAM process. Transition Zone B, where PAM inhibition thresholds occur, was modeled as spanning the dose range 20 – 50 mGy. Again, the uniform prior distribution and the posterior distribution obtained were essentially identical due to insufficient data over and near this dose range. The prior distribution was judgmental, based partly on Transition Zone B values for 28 kVp X-ray- and 60 kVp X-ray-induced neoplastic transformation *in vitro*. These neoplastic transformation data are discussed later. More experimental data are needed to refine the transition zone estimates. Similar inversion mutation data for the prostate are being generated by the same research group that published the spleen data. The spleen and prostate data might be modeled simultaneously with some NEOTRANS$_3$ model parameters common for both sites.

For doses in the range 50 – 100 mGy (portion of LNT zone; no PAM process), the LNT model applies and has a slope given by k_2.

Impact of StoThresh on the Shape of the Dose-Response Curve for Neoplastic Transformation

While the inversion mutation frequency data in Figure 2 span dose Zones 1 – 5, there are no data for neoplastic transformations with doses below 0.1 mGy except for control animals. Thus, it is not known if a similar curve shape below 0.1 mGy, as shown in Figure 2, also applies to low-LET radiation-induced neoplastic transformation. Previously the NEOTRANS$_3$ model was applied to data of Redpath *et al.* (2001) for gamma-ray induced neoplastic transformation of HeLa × skin fibroblast human hybrid cells exposed *in vitro*.

Figure 3 shows results of applying the NEOTRANS$_3$ model to data from Redpath *et al.* (2003) for 60 kVp X-ray induced neoplastic transformation of HeLa × skin fibroblast human hybrid cells. Bayesian methods as previously described (Scott 2004) were used to fit the data. Data only

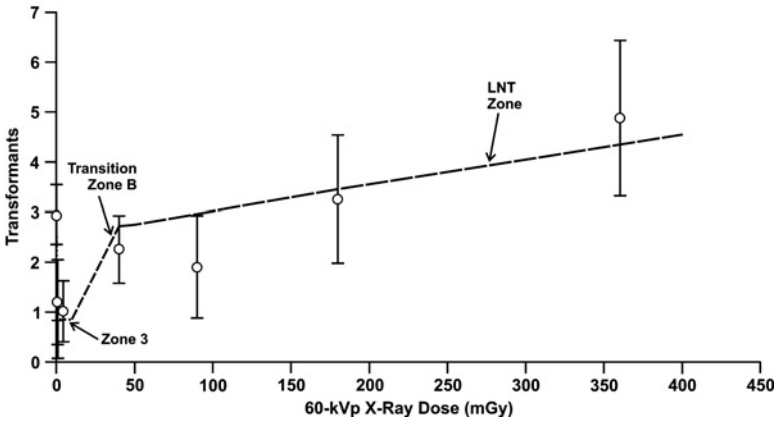

FIGURE 3 Application of the NEOTRANS$_3$ model to data of Redpath *et al.* (2003) for 60 kVp X-ray induced neoplastic transformation in HeLa × skin fibroblast human hybrid cells.

span Dose Zones 3 – 5. The shape of the dose-response curve over these zones is essentially the same as for the inversion mutations in Figure 2.

Figure 4 shows similar results for 28 kVp X-ray induced neoplastic transformation of HeLa × skin fibroblast human hybrid cells based on data from Ko *et al.* (2004). Posterior means and associated standard deviations for NEOTRANS$_3$ model parameters $k = k_2$, *PROFAC*, Y_0 (spontaneous frequency estimate), and lower and upper bounds for D_{off} (which define Transition Zone B) are provided in Table 1 for gamma rays, 60 kVp X-rays, and 28 kVp (mammographic-energy) X-rays.

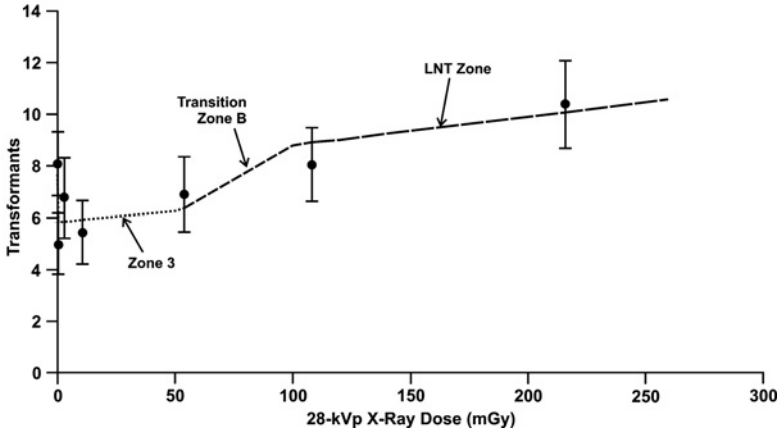

FIGURE 4 Application of the NEOTRANS$_3$ model to data of Ko *et al.* (2004) for 28 kVp X-ray-induced neoplastic transformation of HeLa × skin fibroblast human hybrid cell.

TABLE 1 Bayesian posterior means and standard deviations for NEOTRANS$_3$ model parameters and Transition Zone B boundary estimates

Radiation Type	Y_0	k_2 (mGy^{-1})	*PROFAC*	Transition Zone B Boundary Estimates (min, max), mGy
Gamma rays	$2.3(\pm0.3) \times 10^{-5}$	$4.1(\pm0.9) \times 10^{-8}$	0.4 ± 0.1	(150, 250)
60 kVp X-rays	$2.5(\pm0.2) \times 10^{-5}$	$5.0(\pm1.2) \times 10^{-8}$	0.67 ± 0.07	(10,40)
28 kVp X-rays	$7.7(\pm0.5) \times 10^{-5}$	$1.1(\pm0.4) \times 10^{-7}$	0.24 ± 0.06	(50,100)

Table 2 provides relative efficiencies of these radiation sources for inducing genomic instability (based on k_2) and the relative efficiency for activating the PAM process (based on the *PROFAC*). Note that the lower energy X-rays are more efficient in inducing genomic instability than gamma rays while the higher energy gamma rays and 60 kVp X-rays are more efficient in activating the PAM process than the 28 kVp X-rays. Presently, we do not have any evidence for the PAM process being activated by low doses of high-LET alpha radiation (Scott 2004; Scott et al. 2004). However, for combined low-dose rate exposure to gamma rays and alpha particles, there is evidence for the gamma rays activating the PAM process and protecting against the induction of cancer in humans by alpha radiation (Scott 2005b).

Similarity in Relative Risks for Neoplastic Transformation in Vitro and Cancer Induction in Vivo

Redpath and colleagues (2001) have shown that dose-response relationships for the relative risk (*RR*) for neoplastic transformation of HeLa × skin fibroblast cells *in vitro* and for cancer induction in humans (*in vivo*) are quite similar. This led to adapting *RR* dose-response functions for radiation-induced neoplastic transformation to be applicable to cancer *RR* evaluation in humans (Scott 2005b). Based on the indicated relationships, it can be shown that the ratio $PROFAC/k_2D$ for neoplastic transformation *in vitro* estimates the probability ratio $P_{decrease}/P_{increase}$, where $P_{increase}$ is the probability for an increase in the cancer incidence in humans after a dose, *D,* of low-LET radiation based on linear (LNT) extrapolation from moderate to low doses and $P_{decrease}$ is the probability for a suppression of

TABLE 2 Relative efficiency for different low-LET radiation sources for inducing stochastic effects in HeLa × skin fibroblast hybrid cells

Radiation Type	Genomic Instability	PAM Process
Gamma Rays	1.0	1.0
60 kVp X-Rays	1.23 ± 0.51	1.8 ± 0.53
28 kVp X-Rays	2.68 ± 1.12	0.6 ± 0.24

the cancer incidence below the spontaneous level. Here, it is assumed that the dose, *D*, falls in Dose Zone 3.

For gamma-ray induced cancer in humans, and for $D = 1$ mGy, the central estimate for $P_{decrease}/P_{increase}$ is 9.74×10^6 (Scott 2005a). For $D = 0.1$ mGy, the ratio would be tenfold higher. These results should be regarded as a rough approximation since they are based on a correspondence between *RR* for neoplastic transformation *in vitro* and *RR* for cancer induction in humans. However, it can be concluded that the odds for a decrease in cancer risk below the spontaneous level after a 1 mGy gamma-ray dose is orders of magnitude greater than for an increase as would be expected based on low-dose extrapolation using the LNT model.

Predictions for Protracted Exposure to Low-LET Radiation

During low-dose-rate protracted exposure to low-LET irradiation the PAM process can be repeatedly activated, thereby intensifying and prolonging its protective effect. For low-dose-rate exposure of humans over years, it is expected that the cancer incidence could be greatly suppressed below the level for unirradiated persons. The ratio $P_{decrease}/P_{increase}$ is therefore expected to be significantly larger for protracted exposure at low rates to low-LET radiation than for brief exposure at a high rate. Thus, for epidemiological studies of low-dose-radiation-induced cancers, one should include in the study design the ability to detect a reduction in the cancer incidence relative to the normal incidence rate for the population under study.

Regarding protracted exposure to gamma rays, Chen and colleagues' (2004) recently published a paper "Is Chronic Radiation an Effective Prophylaxis Against Cancer?", in which they described their study of cancer occurrence among approximately 10,000 residents of 180 apartment buildings in Taiwan that were built with cobalt-60 contaminated steel. Cobalt-60 is a gamma-ray source. The building inhabitants resided there from approximately 9 – 20 years, during which they unknowingly received radiation doses that averaged about 400 mGy (same as 400 mSv for gamma rays) to the total body. The cancer incidence among this population was reported by Chen *et al.* to be reduced by more than 95% below the expected level for the general population. Thus, as expected, the protracted exposure at low rates seems to have expanded the protective zone (over which deleterious stochastic effects are suppressed) to at least 400 mGy and to have enhanced the level of protection. Protracted doses as high as 4000 mGy also provided protection. The researchers (Chen *et al.* 2004) concluded the following:

> The experience of these 10,000 persons suggest that long-term exposure to radiation, at a dose rate of the order of 50 mSv (5 rem) per year, greatly reduces cancer mortality, which is a major cause of death in North America.

Background Radiation Considerations

We are all exposed to background radiation. The average dose from background radiation is about 3 mSv annually. The dose unit mSv is used for mixed high- and low-LET radiations. For low-LET X-rays, gamma rays, and beta particles, 1 mSv = 1 mGy. However, for alpha particles and neutrons, this is not the case. For a 1 mGy alpha or neutron dose, the corresponding dose in mSv is significantly larger than 1 mSv. Here, our focus is on low-LET radiations such as gamma rays and beta radiation. Background doses of low-radiation > 0.01 mGy could activate the PAM process. If so, mutant and neoplastically transformed cells already present in the body could be triggered to undergo apoptosis via the PAM process.

This can be seen from results of applying the NEOTRANS$_3$ model to a two-dose exposure scenario involving 250 kVp X-ray irradiation of pKZ1 mice. Here, the 250 kVp X-rays are considered as representative of a low-LET environmental radiation source (e.g., beta or gamma radiation). For this evaluation, the first dose is any of the doses along the dose axis in Figure 2. The second dose is fixed at 1 mGy and is given 4 hours after the first dose. The second dose (representative of background radiation after having had previous exposure to another radiation source) is expected to activate the PAM process irrespective of whether it was previously activated or inhibited by the first dose. Thus, the frequency for inversion mutations in the indicated two-dose group would be expected to be less than for a group receiving only the first dose. The predicted reduction is indicated in Figure 5, where a second dose-response curve (two-dose study) has been added to the results presented in Figure 2. Thus, subsequent

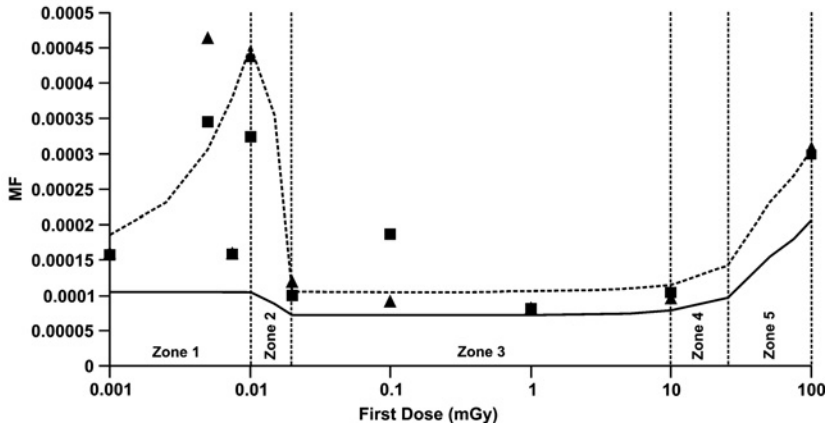

FIGURE 5 A comparison of dose-response curves for a two-dose and single-dose induction of inversion mutations in 250 kVp X-ray exposed pKZ1 mice. The prediction for the two-dose study (lower curve) is based on the NEOTRANS$_3$ model. The single-dose results (upper curve) are the same as for the fitted curve in Figure 2. Doses are in mGy. Dose zones are explained in the text.

exposure to elevated background low-LET radiation after first exposure to a mutation-inducing dose would be expected to suppress (via activation of the PAM process) the initial rise in the dose-response curve presented in Figure 2. This has been reported for the pKZ1 mouse inversion mutation assay (P. Sykes *et al.*, this issue).

The PAM process, if activated by background radiation, should also protect from low-dose high-LET radiation and chemically induced stochastic effects (Scott 2005b). These results have interesting implications for low-dose cancer risk, pointing to a possible major role of background radiation in suppressing cancer occurrence. Background radiation may also suppress other mutation-related diseases.

Cancer Prevention Possibilities

Consider the hypothetical case of a 25-year smoker who based on a computed tomography (CT) scan has precancerous (neoplastically transformed) cells in their lung due to their smoking. Could a little low-LET X- or gamma rays to the lung protect from cancer via activating the PAM process? Possibly, if not likely! Repeated exposures to low doses of low-LET radiation in Dose Zone 3 could possibly amplify the level of protection. Induced immune system stimulation would be expected to add additional protection against cancer. Note that the indicated form of cancer prevention if successful would eliminate the need for surgery (which is currently controversial for small precancerous lesions). Agents other than ionizing radiation may also activate the PAM process and stimulate the immune response (e.g., sunlight, environmental chemicals, workplace chemicals, cosmic rays [low-LET component] received by airline flight attendants, etc).

Low Dose Cancer Therapy Implications

The PAM process also could be exploited related to developing a novel, low-dose therapy for cancer (Scott 2005b). Cancer cells are considered to be resistant to undergoing the PAM process. However, new research may lead to the discovery of apoptosis sensitizing agents (e.g., arsenite in small amounts has this characteristic). If so, then use of such sensitizing agents in combination with low-dose (or low-dose-rate) low-LET radiation could lead to eliminating some cancers.

Cautionary Note

Not everyone may benefit from the PAM process. Persons not bearing significant numbers of genomically compromised cells (e.g., mutation-bearing cells, neoplastically transformed cells, micronucleated cells, etc.) would not be expected to benefit from the PAM process in normal circumstances. Whether the PAM process would remove cells with signifi-

cant mitochondrial DNA damage is unclear. This is an area where new research may prove fruitful.

CONCLUSIONS

- Stochastic thresholds D_{PAM} and D_{off} cause hormetic-type dose-response relationships for radiation-induced mutations, neoplastic transformation, and likely, also cancer.
- At doses on the order of 1 mSv of low-LET radiation, neoplastic transformation and cancer risks are orders of magnitude more likely to decrease than to increase by an amount as would be expected based on the LNT model.
- Low doses of low-LET radiation may prevent cancer occurrence and extend life expectancy for adults that already have precancerous cells (e.g., heavy, long-time smokers).
- Low doses of low-LET radiation, when used in conjunction with apoptosis sensitizing agents, could possibly cure cancer.
- Background low-LET radiation may be suppressing cancer occurrence and other mutations-related diseases among the human population.

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