**FINAL REPORT**

U. S. Department of Energy

# ADVANCED COMPUTATIONAL APPROACHES TO CHARACTERIZING STOCHASTIC CELLULAR RESPONSES TO LOW-DOSE, LOW-DOSE-RATE EXPOSURES


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## Executive Summary

This project final report summarizes modeling research conducted in the U.S. Department of Energy (DOE), Low Dose Radiation Research Program at the Lovelace Respiratory Research Institute from October 1998 through June 2003. The modeling research described involves critically evaluating the validity of the linear nonthreshold (LNT) risk model as it relates to stochastic effects induced in cells by low doses of ionizing radiation and genotoxic chemicals.

The LNT model plays a central role in low-dose risk assessment for humans. With the LNT model, any radiation (or genotoxic chemical) exposure is assumed to increase one's risk of cancer. Based on the LNT model, others have predicted tens of thousands of cancer deaths related to environmental exposure to radioactive material from nuclear accidents (e.g., Chernobyl) and fallout from nuclear weapons testing. Our research has focused on developing biologically based models that explain the shape of dose-response curves for low-dose radiation and genotoxic chemical-induced stochastic effects in cells. Understanding the shape of the dose-response curve for radiation and genotoxic chemical-induced stochastic effects in cells helps to better understand the shape of the dose-response curve for cancer induction in humans.

We have used a modeling approach that facilitated model revisions over time, allowing for timely incorporation of new knowledge gained related to the biological basis for low-dose-induced stochastic effects in cells. Both deleterious (e.g., genomic instability, mutations, and neoplastic transformation) and protective (e.g., DNA repair and apoptosis) effects have been included in our modeling. Our most advanced model, NEOTRANS$_2$, involves differing levels of genomic instability. Persistent genomic instability is presumed to be associated with nonspecific, nonlethal mutations and to increase both the risk for neoplastic transformation and for cancer occurrence. Our research results, based on applications of NEOTRANS$_2$, indicate that nonlinear **threshold-type, dose-response relationships** for excess stochastic effects (problematic nonlethal mutations, neoplastic transformation) should be expected after exposure to low linear energy transfer (LET) gamma rays or gamma rays in combination with high-LET alpha radiation. Similar thresholds are expected for low-dose-rate low-LET beta irradiation.

We attribute the thresholds to low-dose, low-LET radiation induced protection against spontaneous mutations and neoplastic transformations. The protection is presumed mainly to involve selective elimination of problematic cells via apoptosis. Low-dose, low-LET radiation is presumed to trigger wide-area cell signaling, which in turn leads to problematic bystander cells (e.g., mutants, neoplastically transformed cells) selectively undergoing apoptosis. Thus, this **protective bystander effect** leads to selective elimination of problematic cells (a tissue cleansing process *in vivo*). However, this protective bystander effects is a different process from low-dose stimulation of the immune system. Low-dose, low-LET radiation stimulation of the immune system may explain why thresholds for inducing excess cancer appear much larger (possibly more than 100-fold larger) than thresholds for inducing excess mutations and neoplastic transformations, when the dose rate is low.

For ionizing radiation, the current risk assessment paradigm is such that the relative risk (*RR)* is always ≥ 1, no matter how small the dose. Our research results indicate that for low-dose or low-dose-rate, low-LET irradiation, $RR < 1$ may be more the rule than the exception. Directly tied to the current *RR* paradigm are the **billion-dollar cleanup costs** for radionuclide-contaminated DOE sites. Our research results suggest that continued use of the current *RR* paradigm for which $RR \geq 1$ could cause more harm than benefit to society (e.g., by spreading unwarranted fear about phantom excess risks associated with low-dose low-LET radiation). Such phantom risks also may arise from risk assessments conducted for combined exposure to low- and high-LET radiations when based on the LNT or other models that exclude $RR < 1$.

Our results for high-LET radiation are consistent with the LNT hypothesis but only where there is no additional low-LET contribution (e.g., gamma rays) to the total dose. For high-LET neutron sources, gamma rays arise (especially *in vivo*) for large mammals such as humans from neutron interactions with tissue. The gamma rays might provide some protection from low-dose-related stochastic effects via inducing the protective bystander apoptosis effect that is considered to contribute to tissue cleansing via removal of problematic cells.

For astronauts exposed to combinations of high- and low-LET radiation during space exploration, one should consider the possibility that the low-LET component to their dose might also induce the protective bystander effect.

With regard to people of different ages, older individuals may benefit more form the protective bystander effect than younger individuals because problematic cells (e.g., mutants, neoplastically transformed cells, precancerous cells) increase with age.

People living in high background low-LET radiation areas also may benefit from unrecognized cancer risk reduction due to their radiation exposure. Our research results indicate that low-dose, and low-dose-rate, low-LET radiation possibly could be used in treating cancer successfully while minimizing damage to normal tissue. The protective bystander effect introduced could be turned on by low-dose gamma rays, X-rays, or beta radiation and operate against existing cancer cells as well as precancerous cells. Chemicals that initiate apoptosis (some are contained in foods) also could be used along with radiation. The low doses of radiation also may stimulate the immune system to provide additional pronounced protection against cancer. Thus, it is strongly recommended that new research initiatives in the field of low-dose, low-LET radiation therapy for cancer be supported by appropriate organizations, including the DOE.

## 1. Research Objectives (modified since project start date)

Our research, which has focused on mechanisms-based modeling of low-dose, radiation-induced stochastic effects, had the following main objective: to bring together and evaluate dosimetric (dose, dose rate), molecular (gene damage, repair, misrepair, mutation, genomic instability), and cellular (apoptosis, necrotic death, neoplastic transformation) information to better understand low-dose radiation risks. An intended outcome of our research was to establish a scientific basis for critically evaluating whether the linear nonthreshold (LNT) risk model (used for assessing cancer risks at low doses) is valid.

Research for this project was conducted at the Lovelace Respiratory Research Institute (LRRI), Albuquerque, NM, USA in the U.S. Department of Energy's (DOE's) Low Dose Radiation Research Program. A key interest has been clarifying the shape of dose-response relationships for radiation and genotoxic chemical-induced stochastic effects in cells. Our main focus has been on radiation-induced neoplastic transformation, which is considered an early step in cancer induction. Our initial research goals included studying genotoxic chemicals in addition to radiation. Guidance early on in the project from the DOE was to deemphasize the chemical research and focus mainly on radiation. Thus, only limited work has been carried out related to genotoxic chemicals. Therefore, this report focuses mainly on our radiation research.

The shape of the dose-response curve for low-dose, radiation-induced stochastic effects (mutations, neoplastic transformation, cancer) has been the topic of enormous debate for years; yet this debate continues (Crawford-Brown and Hofmann 1990, 1993; Chen and Wei 1991; Bond et al. 1995; Rossi and Zaider 1997; Becker 1998, 2002; Bogen 1998; Calabrese and Baldwin 1998, 1999; Calabrese et al. 1999; Kondo 2000; Pollycove and Feinendegen 1999; Brenner et al. 2001; Feinendegen and Pollycove 2001; NCRP 2001; Schöllnberger et al. 2001a, b, 2002). The key discussion relates to whether the LNT model for low-dose extrapolation of cancer risk is valid. This model is widely used by regulatory agencies and in radiation and

chemical protection. With the LNT hypothesis, risk progressively increases as dose increases. Any amount of carcinogen exposure increases one's risk of cancer. Thus, for radiation, any exposure is assumed to increase one's risk of cancer. Tens of thousands of cancer deaths in the U.S. have been calculated to arise from fallout from nuclear weapons testing (CDC/NCI 2001) and from nuclear accidents such as occurred at the Chernobyl plant in Russia.

Other possible dose-response curves (linear-threshold, sigmoid, U-shaped, etc.) are now considered to be more in line with known mechanisms of carcinogenesis (Feinendegen et al. 1999, 2000; Pollycove and Feinendegen 1999; Feinendegen and Pollycove 2001; Schöllnberger et al. 2002). The principal worker protection and public health implication is that if a threshold response were assumed, then exposures below the threshold value would be considered safe (Calabrese and Baldwin 1999). Such thresholds would have important implications for reducing cleanup costs for radionuclide-contaminated DOE and other sites in the U.S.

It is highly unlikely that use of the LNT model will be abandoned by regulatory agencies and in radiation/chemical protection unless substantial evidence of thresholds can be demonstrated from epidemiological studies and from mechanisms-based experimental and theoretical investigations. For years, the conventional wisdom has been that at low doses, risk will increase but that the increase will be too slight to be detected from epidemiological and animal studies. No consideration has been given to the possibility that risk could initially decrease, and that such a decrease might be statistically significant! Cancer induction dose-response relationships that initially decrease and then increase are called hormetic-type relationships (Calabrese and Baldwin 1998, 1999; Calabrese et al. 1999; Ducoff 2002).

Now there is growing evidence from epidemiological, experimental, and mathematical modeling studies indicating that in many cases hormetic-type dose response relationships may be more appropriate for central cancer risk estimation than the LNT model in many cases. Further, associated with hormetic-type dose-response relationships for cancer are thresholds for inducing excess cancers. In this report we summarize some of the growing evidence for possibly large threshold for induced excess cancers by low-LET or combined low- and high-LET irradiation.

Research for this project has lead to a biologically based model for low-dose radiation-induced stochastic effects in cells. The model is called NEOTRANS$_2$ and involves dose-related varying degrees of genomic instability. The biological effects considered include DNA damage induction, repair/misrepair, apoptosis, necrotic cell death, problematic mutations, and neoplastic transformation. Mainly we have applied the NEOTRANS$_2$ model to data for low-dose radiation-induced stochastic effects. Some limited applications also have been made to stochastic effects induced by genotoxic chemicals.

## 2. Methods and Results

Our project strives to develop improved understanding of cancer risks associated with low-dose radiation through conducting research specifically designed to better understand mechanisms associated with the underlying stochastic effects that take place in cells. Our approach to developing biologically based, dose-response models for the relevant stochastic effects was first to examine the state of knowledge related to mechanisms of radiation action. Key findings over the duration of the project related to mechanisms are summarized in Sections 2.1 through 2.6. Modeling methods and model applications are discussed in Section 2.7. Implications for low-dose risk assessment and low-dose cancer therapy are discussed in Section 3.

Our summaries about mechanisms of the action of low-dose radiation pertain to the following areas: (1) macromolecular changes induced by ionizing radiation, (2) genomic

instability and mutations, (3) apoptosis, (4) possible mechanisms for recognizing and selectively eliminating problematic cells, (5) cellular differentiation, and (6) deleterious bystander effects.

## 2.1 Macromolecular Changes Induced by Ionizing Radiation

Ionizing radiation induces a range of DNA damage similar to that which arises endogenously from reactive oxygen species generated as by-products of metabolism (Jeggo 2002). Daniel Billen (1990), in discussing the concept of negligible dose in the context of naturally occurring DNA damage and repair, has reported that thousands of spontaneous DNA damaging events occur in each cell each day. Robert Stewart (1999) reported an estimate (best estimate) numerically equivalent to $10^5$ spontaneous "locally multiple damage sites" (in particular, double strand breaks) occurring in DNA, per million cells, per day. These lesions are quickly repaired, essentially error free in most cases. It is highly plausible that adding a few tens or hundreds more of such lesions through low-dose radiation (especially low-LET radiation) or low-dose chemical exposure is unlikely to overwhelm the cell's highly efficient damage repair machinery. It is reasonable therefore that error-free repair could operate after very low doses of low-LET radiation or genotoxic chemical.

Numerous repair processes are now known and include nucleotide excision repair, base excision repair, transcription-coupled repair, mismatch repair, and nonhomologous end joining (Friedberg et al. 1995; Scicchitano and Mellon 1997; Hanawalt 2001). The indicated repair processes operate at the individual cell level and provide for individual cell resilience to vulnerable states. A complex cell-signaling network regulates the individual resilience system. Failure of this system can lead to repair errors, which in turn can lead to problematic lethal and nonlethal mutations (forms of genomic instability).

Operationally, two types of mutations (heightened vulnerability states) are used to classify genes: (1) those where a mutation causes a gain in function (proto-oncogene to oncogene change) and (2) those where mutations cause function loss (tumor suppressor genes). In the development of leukemia and lymphoma, the first step is considered to be activation of a proto-oncogene into an oncogene, which arises via a translocation of a promoter besides the active site of a normally repressed growth-promoting gene site (Young 1994).

In the case of thyroid cancer, specific genes are rearranged that involve activation of the *ret* proto-oncogene (Jacob et al. 1996; Rabes and Klugbauer 1998; Smida et al. 1999). Whereas oncogene activations are quite specific, for tumor suppressor gene mutations, random deletions of large amounts of DNA, large parts of a gene, an entire gene, or several genes could occur. For many solid tumors, the inactivation of a tumor suppressor gene is considered to be the first step in the cancer induction process and is commonly assumed to affect a tissue-specific "gatekeeper" (Sidransky 1996; Trott and Roseman 2000). After loss of the gatekeeper function, clonal expansion of tissue-specific stem cells is allowed (Sidransky 1996).

Radiation mutagenesis may proceed principally via DNA deletions through misrepair and misrecombination at DNA double-strand breaks (ICRP 1999; Trott and Roseman 2000). In our modeling of radiation-induced neoplastic transformation, mutations are assumed to arise from misrepair of DNA damage, and nonlethal mutations are assumed responsible for the initial persistent genomic instability. Here, we have not distinguished between misrecombination of DNA double-strand breaks, misrepair, or incomplete repair. Currently, we only distinguish between lethal and nonlethal mutations.

## 2.2 Genomic Instability and Mutations

The concept of genomic instability was introduced by W. F. Morgan and colleagues (1996) and is now widely accepted. Genomic instability can propagate over successive cell generations (Morgan et al. 1996; Wright 1998). We consider all mutations to represent genomic

instability. Problematic nonlethal mutations among dividing cells we consider to possess persistent problematic instability (PPI) transferable to progeny. Most radiation-induced mutations directly involve loss of large parts of the tested gene, leading to loss of heterozygosity (Trott and Roseman 2000). However, most radiation-induced mutations associated with genomic instability are point mutations and small deletions (Little 1999). In modeling radiation-induced genomic instability, we do not assume PPI to be associated with a specific type of mutation. We only distinguish between lethal and nonlethal mutations, and we assume that neoplastic transformation arises as a stochastic process among cells (including progeny) with PPI.

Some useful findings related to genomic instability have been reported in a study of 20 liver tumors, which were diagnosed in a cohort of people treated with thorotrast (Iwamoto et al. 1999). It was found that 95% of the cases showed p53 point mutations. Iwamoto et al. (1999) concluded that the relevant genetic alterations leading to liver cancer result from an induced genetic instability (indirect effect), rather than directly from radiation exposure. In our modeling of neoplastic transformation, we have characterized PPI as an indirect effect (arising via misrepair) of irradiation (or chemical exposure) that can be passed to cell progeny. We also have introduced a new class of genomic instability (transient) (Scott 1997), which is now modeled as a direct effect (hit hypersensitive cells) and indirect effect (including deleterious bystander effects) of irradiation.

The frequency of persistent genomic instability (PGI; expressed as stable chromosomal aberrations) in lymphocytes was evaluated for 79 plutonium workers from the Mayak Production Association (PA) plutonium production facility in Russia who were exposed to relative insoluble (low-transportable) compounds of $^{239}$Pu and external gamma rays (Okladnikova et al., manuscript being prepared under a different DOE project). Unstable aberrations also were evaluated and were presumed to reflect transient genomic instability (TGI) related to chronic exposure. The start of the occupational radiation exposure occurred more than 10 years before initiation of cytogenetic studies. The group average $^{239}$Pu body burden over the study population at the time of study initiation was estimated to be $1.23 \pm 0.26$ kBq. The group average absorbed alpha radiation dose to lymph nodes was estimated to be $2.2 \pm 0.7$ Gy. The group average total body, external gamma ray dose was estimated to be $0.076 \pm 0.009$ Gy. The indicated standard errors (±) reflect statistical error. Our multivariate, linear regression analysis revealed a significant positive correlation between PGI and TGI (abbreviated PGI/TGI) and increasing $^{239}$Pu incorporation. There also was a significant positive correlation between PGI/TGI and time since employment at the Mayak PA. External gamma ray doses to the total body over the range 0 to 0.29 Gy (average dose rate < 0.029 Gy/year) were not found to be correlated with PGI/TGI based on whole body doses delivered over 10 to 30 years. This is consistent with the emerging view of a possible threshold for the induction of excess lung cancer by low-dose rate gamma irradiation. Surprisingly, PGI/TGI was not found to be associated with smoking. No firm conclusions can be made at this time regarding the presence or absence of a threshold for alpha radiation-induced PGI or TGI.

### 2.3 Apoptosis: Protector of the Cell Community from Stochastic Effects

In contrast to the necrotic mode of cell death, apoptosis protects from problematic cells in the body via their elimination without causing inflammation (Mendonca et al. 1999). Strasser et al. (2000) summarized key points associated with apoptosis signaling as follows:

"Apoptosis, a physiological process for killing cells, is critical for the normal development and function of multicellular organisms. Abnormalities in cell death control can contribute to a variety of diseases, including cancer, autoimmunity, and degenerative disorders. Signaling for apoptosis occurs through multiple independent pathways that are initiated either from triggering events within the cell or from outside the cell, for instance, by ligation of death receptors."

New research results indicated that problematic cells in the body may be detected via molecular biological mechanisms and selectively eliminated via apoptosis to protect the cell community (Yang et al. 2000). A key assumption of the NEOTRANS$_2$ model to be introduced is that existing problematic cells (e.g., problematic mutants, neoplastically transformed cells) in the cell community can be signaled to undergo apoptosis and selectively eliminated via low-dose induced protective bystander mechanisms. These mechanisms of reduction in cell community vulnerability status we presume to explain, at least in part, reported low-dose hypersensitivity to cell killing among cancer cell lines (Joiner et al. 1999) as well as virally transfected cells (Seymour and Mothersill 2000). Thus, the NEOTRANS$_2$ model to be presented includes both deleterious and protective bystander effects.

## 2.4 Possible Mechanisms for Recognizing and Selectively Eliminating Problematic Cells

As indicated previously, we have hypothesized the existence of a protective apoptotic bystander effect for neoplastic transformation (also applies to problematic mutations). Such an effect is necessary to adequately explain existing data whereby risks for neoplastic transformation (Azzam et al. 1996; Redpath et al. 2001) and lung cancer (Rossi and Zaider 1997) decrease rather than increase at very low doses.

A crucial missing link related to our modeling is the identification of mechanisms whereby problematic cells already present in a population can be recognized and signaled to undergo apoptosis, while nearby normal cells are essentially unaffected. Some progress is being made by researchers to identify and characterize such a protective process for the cell community.

Cucinotta et al. (2002) point out that ionizing radiation produces DNA damage that causes protein fluctuations through binding damage recognition proteins to DNA breaks and subsequent downstream events. The type of fluctuations may depend on the type of DNA break such as simple or complex single-strand breaks and double-strand breaks or base damage (Cunniffe and O'Neil 1999).

Barcellos-Hoff and Brooks (2001) point out that bystander effects after low doses of radiation are extracellular signaling pathways that modulate both cellular repair and death programs. The authors also indicate that transforming growth factor $\beta$ (TGFB1) is known to be an extracellular sensor of damage. They further indicate that extracellular signaling relevant to carcinogenesis in normal tissue can eliminate abnormal cells or suppress neoplastic behavior.

Dr. C.-R. Yang and colleagues (2000) at Case Western University have reported clusterin [CLU, a.k.a. TRPM-2, SGP-2, or radiation-induced protein-8 (XIP8)] to be implicated in apoptosis. In a recent study (Yang et al. 2000) they re-isolated CLU/XIP8 by yeast two-hybrid analyses, using the DNA double-strand break repair protein Ku70 as bait. They showed that low-dose, radiation-induced nuclear CLU/XIP8 protein coimmunoprecipitated and colocalized *in vivo* with Ku70/Ku80, a known DNA damage sensor and key double-strand break repair protein in human MCF-7:WS8 breast cancer cells. Their key finding was that enhanced expression and accumulation of nuclear CLU/XIP8-Ku70/Ku80 complexes appear to be an important cell death signal after irradiation. Further, their data suggest that CLU/XIP8 may play an important role in monitoring cells with genomic instability and/or infidelity, created by translesion DNA synthesis, by facilitating removal of genetically unstable cells as well as severely damaged cells. Yang et al. (2000) strongly suggest that the CLU/XIP8 protein is a general cell death signal, monitoring overall cell health.

Yang et al. (2000) point out in recent findings that Ku70 but not Ku80 knockout mice are cancer prone, and this appears consistent with the notion that formation of nuclear CLU/XIP8 with Ku70 may play an important role in eliminating carcinogenic initiated (problematic) cells.

Now it is known from *in vitro* studies of viral-induced neoplastic transformation (Bauer 1996) that:

- Increasing plating density reduces transformation frequency.
- Transformed cells are selectively killed via apoptosis.
- Cytokines and reactive oxygen produced by untransformed neighboring cells trigger apoptosis.
- TGFB1 enables untransformed cells to trigger apoptosis among transformed cells.

Given this information, we consider our key modeling assumption of the existence of an inducible protective bystander apoptosis effect whereby problematic cells are recognized (after signaling from other cells) and selectively eliminated from the cell community to be highly plausible. Another assumption we make is that neoplastic transformation is a necessary early step for cancer induction (a widely held view). Thus, demonstrating low-dose induced protection from neoplastic transformation *in vitro* is consistent with the possibility of low-dose-induced protection from cancer *in vivo*.

### 2.5 Cellular Differentiation

The current view is that some problematic cells may undergo differentiation (group resilience), and this also protects the cell community from propagating stochastic adverse effects. Currently, the NEOTRANS$_2$ model does not include this feature. We consider differentiation to be more important *in vivo* than *in vitro*. Our modeling applications presented in this report relate mainly to *in vitro* studies.

### 2.6 Deleterious Bystander Effects

Deleterious bystander effects (Ballarini et al. 2002) whereby unirradiated cells are damaged have been examined in two general types of cellular systems. In the first system, monolayer cultures have been exposed to very low fluences of alpha particles, either from an external source (Nagasawa and Little 1992; Azzam et al. 1998; Little et al. 2002) or focused microbeam (Hei et al. 1997; Prise et al. 1998). The second technique involves harvesting medium from irradiated cells and incubating it with unirradiated cells (Mothersill and Seymour 1997; Lyng et al. 2000). Both techniques have demonstrated that cells not being irradiated can still be damaged. Further, the bystander effect does not arise from simply irradiating media. Cell damage and intercellular signaling are essential.

We also allow for the possibility of deleterious bystander effects via model parameters that account for both direct and indirect deleterious radiation effects. Our modeling research focuses on characterizing excess stochastic effects (mutations, neoplastic transformations) after very low doses of radiation by using mechanisms-based models. While many *in vitro* experimental studies have been conducted on radiation-induced neoplastic transformation, only limited experimental data are available for doses < 100 mGy (Azzam et al. 1994, 1996; Redpath and Antoniono 1998; Redpath et al. 2001).

### 2.7 NEOTRANS$_2$ Model

In our early research, we introduced a class of models (that included NEOTRANS$_1$) for characterizing neoplastic transformation of cells that relate the probability of neoplastic transformation to the state of genomic instability (Scott 1997; Schöllnberger et al. 2001a; Scott et al. 2001). With NEOTRANS$_1$, the target cell population was modeled as heterogeneous with both hypersensitive- and resistant-cell subpopulations (considered the simplest case of heterogeneity). NEOTRANS$_1$ has now been refined, leading to the model called NEOTRANS$_2$ (Figs. 1 and 2) that includes apoptotic and necrotic death pathways. In this report, NEOTRANS$_2$ is applied to *in vitro* data for low-radiation dose-induced neoplastic transformation. We have focused only on data with several dose groups $\leq$ 100 mGy.

### 2.7.1 *Genomic Instability States Used in NEOTRANS$_2$*

Our use of terminology related to genomic instability is the same as used in earlier publications (Scott 1997; Schöllnberger et al. 2001a; Scott et al.2003). The expression "genomic instability state" refers to any spontaneous or toxicant-induced instability in the genome, including any initial transient instability as well as any persistent instability that can be passed to cell progeny. In addition to a stable (ST) genome, the NEOTRANS$_2$ model (as well as NEOTRANS$_1$) involves four types of genomic instability (Figs. 1 and 2): (1) *Normal-minor instability* (NMI), associated with normal cell function and normal genome status; (2) *Transient-minor instability* (TMI), associated with toxicant-induced genomic damage that is fully repairable (without any significant errors); (3) *Transient-problematic instability* (TPI), associated with genomic damage that may sometimes be fully repaired but can be misrepaired; and (4) PPI, which arises from misrepair that yields nonlethal mutations. Thus, PPI can be passed to progeny, increasing their potential for stochastic effects such as neoplastic transformation. We use the term "misrepair" in a broad sense as already indicated. We consider TPI and PPI to be vulnerability states (for additional deleterious stochastic effects).

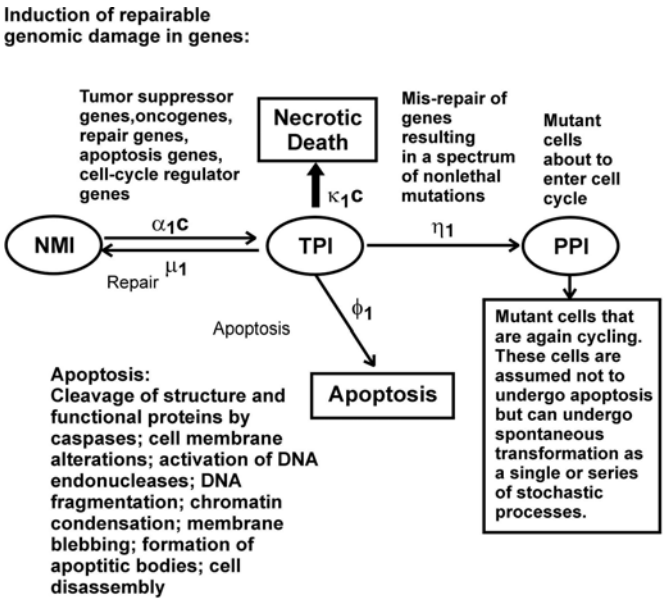


**Figure 1.** NEOTRANS$_2$ model, hypersensitive cells only. (Abbreviations are defined in the text.)

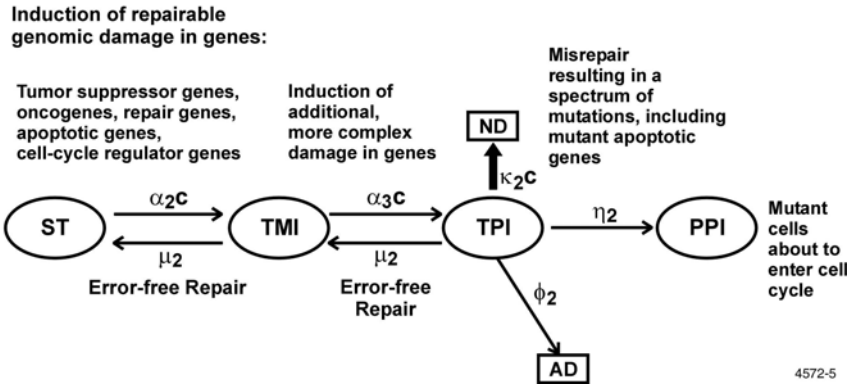


**Figure 2.** NEOTRANS$_2$ model, resistant cells only. ND = necrotic death, AD = apoptosis. (Other abbreviations are defined in the text.)

### 2.7.2 *Other Model Features*

With the NEOTRANS$_2$ model, a very small fraction, $T_0 << 1$, of the cell population is presumed to have already undergone neoplastic transformation. The discussion that immediately follows relates to the remaining vast majority ($1 - T_0 \approx 1$) of the cells. With both NEOTRANS$_2$ (Figs. 1 and 2) and NEOTRANS$_1$, only cells in the high vulnerability state PPI (viable mutants) can produce neoplastically transformed progeny. Only genomically ST cells, those with NMI and those with PPI, progress through the cell cycle and divide. Other cells are assumed arrested at cell cycle checkpoints (resilience facilitation) where genomic damage is repaired or misrepaired. Irradiation times were assumed to be quite short relative to cell cycle transit times, so that no equations were used to account for progression through the cell cycle during irradiation. Neoplastic transformations are assumed to occur as a stochastic process, and the transformed cells may have an altered cell cycle transit time distribution.

With NEOTRANS$_2$, target genes are specified (Figs. 1 and 2) and include tumor suppressor genes, oncogenes, repair genes, apoptosis genes, and cell-cycle regulator genes. Unlike NEOTRANS$_1$, with NEOTRANS$_2$ cell killing is explicitly addressed and not treated as independent of neoplastic transformation. Two modes of cell death are considered: *apoptosis* (assumed to predominate at very low doses) and *necrotic death* (assumed important only at moderate and high doses). Again, nonlethal mutations are assumed to arise via misrepair. Lethal mutations are assigned to the apoptosis pathway (including delayed lethal mutations). The analytical solutions presented here apply only to very low radiation doses where necrotic death can be assumed negligible.

Model parameters $\alpha_1$, $\alpha_2$, and $\alpha_3$, common to both NEOTRANS$_1$ and NEOTRANS$_2$, reflect genomic sensitivity to initial and higher levels of damage production and should be multiplied by the dose rate *c*. The parameters $\mu_1$ and $\mu_2$ are also common to both models and govern the commitment rate of damaged cells to an error-free repair pathway. In addition, the parameters $\eta_1$ and $\eta_2$ are common to both models and govern the commitment rate of damaged cells to a misrepair pathway that leads to nonlethal mutant cells (PPI cells).

In light of new evidence that protracted exposure to low-LET radiation can lead to large dose thresholds for cancer induction, we allow $\eta_1$ and $\eta_2$ to be step functions of dose rate. Below a critical dose-rate value *c** (currently undetermined), the parameters take on a value of zero. This dose-rate threshold is presumed to depend on the type of radiation and type of cancer. For dose rates above *c**, the parameters then take on fixed values > zero. The parameters $\phi_1$ and $\phi_2$ appear only in NEOTRANS$_2$ and govern the rate of commitment of damaged cells (including lethal mutations) to the apoptotic pathway. The parameters $\kappa_1$ and $\kappa_2$ (which are important only for moderate and high doses) appear only in NEOTRANS$_2$ and, when multiplied by dose rate, govern the rate at which already damaged cells enter the necrotic death pathway. Typical units for $\alpha_i$ and $\kappa_i$ are mGy$^{-1}$. Typical units for $\mu_i$, $\eta_i$, and $\phi_i$ are min$^{-1}$.

Parameters $\alpha_1$, $\alpha_2$, and $\alpha_3$ should be viewed as being comprised of two parts. (1) One part relates to direct damage to DNA; (2) the other part relates to indirect damage to DNA and includes deleterious bystander effects.

For very low radiation doses, only hypersensitive cells are assumed to be induced to transform (new transformations), and cells are modeled as being killed only via the apoptotic pathway. Thus, only Figure 1 applies for very low doses and to the hypersensitive subfraction, $f_1$, of cells at risk.

Further, with our current version of the NEOTRANS$_2$ model, a fraction $T_0$ (stochastic quantity) of cells at risk is assumed to have undergone spontaneous neoplastic transformation already, based on genomic alterations over their life history but prior to dosing with radiation (or chemicals). Because the life history of cells (over parent and daughter cells) spans a long time compared to the short time over which cells are irradiated during *in vitro* studies, our assumption is considered highly plausible when applying NEOTRANS$_2$ to data from *in vitro* irradiation studies. For *in vivo* exposure, additional protective mechanisms could be important (Stecca and Gerber 1998; Barcellos-Hoff and Brooks 2001).

### 2.7.3 *Model Solutions for Very Low Doses*

Evidence is strong now that death via apoptosis at low radiation doses can occur via a bystander mechanism (Mothersill and Seymour 1998a, b; Lyng et al. 2000; Belyakov et al. 2001a, b, 2002a, b; Prise et al. 2002). We consider the highly plausible possibility that a fraction $f_0$ of the $T_0$ cells already neoplastically transformed is killed via a bystander effect for apoptosis (a key modeling assumption). In such cases, the dose response at very low radiation doses could decrease rather than increase. Indeed, this type of dose response now has been demonstrated experimentally with $^{60}$Co-gamma irradiation of C3H 10T1/2 cells (Azzam et al.

1996) and with $^{137}$Cs-gamma irradiation of HeLa × skin fibroblast human hybrid cells (Redpath and Antoniono 1998; Redpath et al. 2001).

The following analytical solutions apply to very small dose increments. As indicated, a small fraction of $T_0$ cells in the population is modeled as already having the problem of interest (e.g., neoplastic transformation in this case; but, a similar equation would apply to nonlethal problematic mutations). At such doses, newly induced neoplastic transformations are modeled as arising from a small (in number), hypersensitive subfraction of the remaining $(1 - T_0)$ cells at risk. This hypersensitive subfraction is given by $f_1(1 - T_0) \approx f_1$.

From Figure 1 (which shows only hypersensitive cells), it can be seen that a very small dose increment $\Delta D$ (where $\Delta D = c\,\Delta t$, for a small time increment $\Delta t$) to the fraction $f_1(1 - T_0)$ of hypersensitive cells will lead to an expected fraction $f_1(1 - T_0)\alpha_1\Delta D$ of cells in the state TPI (assuming all hypersensitive cells are initially in the state NMI); for this fraction entering the transient state TPI, the conditional probability of subsequently undergoing misrepair (leading to PPI) is just $\eta_1/(\mu_1 + \eta_1 + \phi_1)$.

The dose-response function for radiation-induced, neoplastic transformations per surviving cell, TFSC($\Delta D$), at very low doses $\Delta D$ is thus given by the following:

$$\begin{aligned} &TFSC(\Delta D) = T_0, \text{ for } \Delta D = 0, \\ &TFSC(\Delta D) = (1 - f_0)T_0 + [(1 - T_0)f_1\alpha_1\eta_1\Omega/(\mu_1 + \eta_1 + \phi_1)]\,\Delta D, \text{ for } \Delta D > 0. \end{aligned} \quad (1)$$

For $\Delta D > 0$, Equation 1 has a fixed slope of $(1 - T_0)f_1\alpha_1\eta_1\Omega/(\mu_1 + \eta_1 + \phi_1)$. The parameter $\Omega$ is the proportion of the newly induced parental PPI cells that produce neoplastically transformed progeny. The parameter, $\Omega$, therefore, depends on follow-up time. It also is likely influenced by the signaling characteristic of the cellular community (Barcellos-Hoff and Brooks 2001). Equation 1 leads to the LNT model only when $f_0 = 0$ (i.e., when the protective apoptosis effect is absent) and $\eta_1 > 0$ (misrepair occurs).

Equation 1 is based on the assumption that the intercellular signaling that leads to the protective bystander apoptosis effect occurs without a radiation dose threshold. Data to be presented later (Azzam et al. 1996; Redpath et al. 2001) support this hypothesis for ionizing radiation. However, this may not be the case for genotoxic chemicals (Walker et al. 2003).

With Equation 1, the dose-response relationship is discontinuous at zero dose [steps down from $T_0$ to $(1 - f_0)T_0$]. The dose-response associated with Equation 1 is linear but with a zero-dose intercept of $(1 - f_0)T_0$ rather than $T_0$ when fitted to low-dose data with the zero-dose group excluded (see hypothetical dose-response curve in Fig. 3). As indicated in Figure 3, $T_0$ is stochastic.

The dose-response curve for TFSC will exceed $T_0$ (a random variable) only for $\Delta D$ in excess of a stochastic threshold (*StoThresh*) dose $D_{Th}$ (Fig. 3) given by:

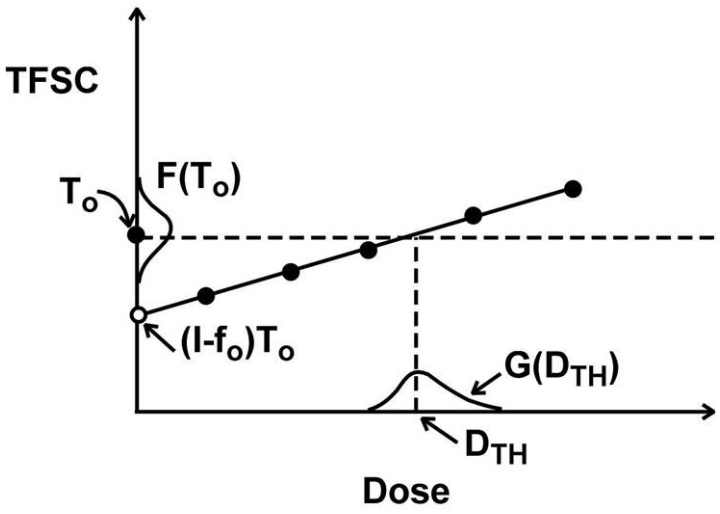


**Figure 3.** Hypothetical dose-response curve related to NEOTRANS$_2$ model. The parameter $T_0$ and the *StoThresh*, $D_{Th}$, have distributions $F(T_0)$ and $G(D_{Th})$, respectively.

$$D_{Th} = [f_0 T_0(\mu_1 + \eta_1 + \phi_1)]/[(1 - T_0)f_1\alpha_1\eta_1\Omega]. \quad (2)$$

Here, we assumed that cell survival is very near 100% at the very low doses considered and that $\eta_1$, $f_1$, $\alpha_1$, $f_1$, and $\Omega$ are all > 0. This is consistent with observations of Azzam et al. (1996). A *StoThresh* (as apposed to a deterministic threshold) is considered to occur because $T_0$ as well as all other model parameters are treated as stochastic.

Because $T_0$ is on the order of $10^{-4}$ to $10^{-5}$ for most *in vitro* studies of neoplastic transformation, selectively killing all $T_0$ cells (i.e., $f_0 = 1$) would still lead to a cell survival fraction > 0.999. Unfortunately, currently available data at low doses for which equations apply are inadequate to derive estimates for individual model parameters $\mu_1$, $\eta_1$, $\phi_1$, $f_1$, $\alpha_1$, $f_0$, and $\Omega$. However, more general forms of Equations 1 and 2 are derived and used in obtaining estimates of $f_0$, $T_0$, and $D_{Th}$. Since demonstrating that $D_{Th} > 0$ has important implications for radiation protection and radiation risk assessment, these more general solutions are quite useful.

Equation 1 can be rewritten in the more general form:

$$TFSC(\Delta D) = (1 - f_0)T_0 + (1 - T_0)k_T\Delta D, \quad (3)$$

where

$$k_T = f_1\alpha_1\eta_1\Omega/(\mu_1 + \eta_1 + \phi_1).$$

Equation 3 can be considered a generalized, three-parameter (stochastic parameters $f_0$, $T_0$, and $k_T$) form of the NEOTRANS$_2$ model for application to very low radiation doses. A corresponding equation also may apply to highly genotoxic chemicals, with $\Delta D$ then representing a very small dose of the agent of interest. For a constant exposure time (for a chemical), $\Delta D$ could be replaced by the concentration with the parameter $k_T$ redefined to include the exposure time in the numerator.

Equation 2 also can be rewritten in the more general form:

$$D_{Th} = f_0 T_0/[(1 - T_0)k_T]. \quad (4)$$

Figure 3 shows a hypothetical mean dose-response curve based on Equations 3 and 4. In Figure 3, hypothetical distributions $F(T_0)$ (shown vertically) and $G(D_{Th})$ (shown horizontally) are presented for $T_0$ and $D_{Th}$, respectively.

For very low doses and in the framework of the NEOTRANS$_2$ model, it is possible that the protective bystander effect may predominate ($f_0 >> k_T\Delta D$) when the spontaneous frequency $T_0$ of transformation is relatively high and when $f_0 > 0$ and $\Delta D$ is very small (e.g., less than about 100 mGy low-LET radiation). Implied here is a relative small value for the slope parameter $k_T$ in combination with a small dose. In such cases, the data for radiation-associated neoplastic transformation (and for specific problematic nonlethal mutations) should be adequately represented by the relationships:

$$TFSC(\Delta D) = T_0, \text{ for } \Delta D = 0$$
$$= (1 - f_0)T_0, \text{ for } \Delta D > 0. \quad (5)$$

Further, $TFSC(\Delta D)$ should be uncorrelated with dose over the dose range for which Equation 5 applies. This requirement only applies to doses in excess of background. We later apply Equation 5 to two data sets for gamma-ray-induced neoplastic cell transformation for doses up to about 100 mGy.

We describe later how distributions for $T_0$ (stochastic), $D_{Th}$ (stochastic), and the slope parameter $k_T$ have been obtained for induced neoplastic transformation.

2.7.4 *Application to In Vitro Low Dose Radiation Data*

We fitted the protective bystander effects version of the model to available data for radiation-induced neoplastic transformation (two data sets) and low-dose apoptosis (one data set):

**Data Set 1** — Gamma-ray-induced neoplastic transformation data of Redpath et al. (2001) (delayed plating):

- HeLa × skin fibroblast human hybrid cells (delayed plating)
- $^{137}$Cs gamma rays
- Dose rate: 3.3 mGy/min for dose < 100 mGy; 41.3 mGy/min otherwise
- Doses: 0, 1, 5, 10, 50, 100, 300, and 500 mGy

**Data Set 2** — Gamma-ray-induced neoplastic transformation data of Azzam et al. (1996) (delayed plating):

- C3H 10T1/2 mouse embryo fibroblast clone 8 cells
- $^{60}$Co gamma rays
- Dose rate: 2.4 mGy/min
- Doses: 0, 1, 10, and 100 mGy

**Data Set 3** — Gamma-ray-induced cell killing (via apoptosis) data of Seymour and Mothersill (2000):

- Human keratinocytes (immortalized via viral transfection but not transformed)
- $^{60}$Co gamma rays
- Dose rate: 750 mGy/min
- Doses: 0, 10, 30, 50, and 100 mGy

For the narrow dose range (0 to 100 mGy), all data (for $\Delta D > 0$) for transformation and cell survival were uncorrelated with dose. This is in line with characteristics of the NEOTRANS$_2$ model that predicts that the largest effect at very low doses is the protective bystander apoptosis effect, which is modeled as being independent of dose.

For data in the dose interval 0 to 100 mGy (excluding the zero dose group) the parameter $f_0$ was evaluated for both the data of Redpath et al. (2001) and Azzam et al. (1996) as follows based on Equation 5. For $0 < \Delta D \leq 100$ mGy, $f_0$ for transformation was calculated as a function of the mean observed transformation frequency, TFSC, and reported mean for $T_0$ using the relationship:

$$f_0 = 1 - (TFSC/T_0). \quad (6)$$

Equation 6 was used for each dose in the dose range indicated, leading to different estimates of $f_0$, and corresponding values $(1-f_0)T_0$. Mean values for $(1-f_0)T_0$ and the associated standard deviation were obtained. Dose-response relationships (horizontal line) were based on these means and the associated 95% confidence intervals assuming a normal distribution.

Bayesian methods (Siva 1998) were used only for the neoplastic transformation data of Redpath et al. (2001) and only when doses over the wider range of 0 to 500 mGy were evaluated. For this dose range, Equations 5 and 6 do not apply. Equation 3 applies and therefore was used. WinBUGS software (Spiegelhalter et al. 1999) was used to carry out the

Bayesian inference via use of Markov Chain Monte Carlo (MCMC) analyses. Transformants were modeled as having poisson distributions. For the Bayesian analysis, the prior distribution for $k_T$ was uniform over the interval ($4 \times 10^{-8}$–$7.5 \times 10^{-8}$); for $f_0$, a uniform prior distribution over the interval 0 to 1 was used; for $T_0$, a normal prior distribution was used with a mean of $2.24 \times 10^{-5}$ and standard deviation of $2.8 \times 10^{-6}$ [same values as reported by Redpath et al. (2001)]. Five thousand MCMC iterations were first run. Auto correlations were then examined to judge how many additional iterations were needed for convergence. Fewer than 30,000 iterations (total) were found necessary to ensure convergence. Iterations were then increased so that the total was 60,000. These iterations were more than were needed, but they essentially guaranteed convergence of the Markov chains. The first 40,000 iterations were then discarded as burn-in. Analysis of posterior distributions was then based on the final 20,000 MCMC realizations (Scott et al., 2003).

Figure 4 shows results obtained for our analysis of the Azzam et al. (1996) data for gamma-ray-induced neoplastic transformation of C3H 10T1/2 cells *in vitro.* Only data in the very low dose range (0 to 100 mGy) where Equation 5 applies were used. For this dose range (with the zero dose group excluded), there was no significant correlation between transformation frequency and dose ($R^2 = 0.18$, $p > 0.5$).

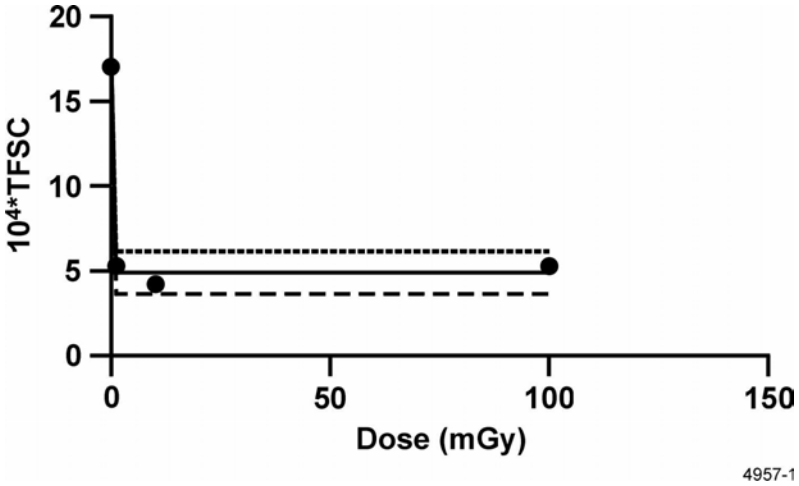


**Figure 4.** Application of the NEOTRANS$_2$ model to the Azzam et al. (1996) data (solid points) for gamma-ray-induced (*in vitro*) neoplastic transformation of C3H 10T1/2 cells for a dose range of 10 to 100 mGy. Model-associated means (central curve), 5% (percentile; lower curve) and 95% (upper curve) values are presented based on an assumed normal distribution.

The corresponding results for application of the NEOTRANS$_2$ model to the Redpath et al. (2001) data for gamma-ray-induced neoplastic transformation of HeLa × skin fibroblast cells are presented in Figure 5. Solid points in these figures represent the experimental data, and smooth and dashed curves represent model-associated results with means (central curve) and 95% confidence regions. For these data and for doses above zero, there was no significant correlation of transformation frequency with dose ($R^2 = 0.4$, $p = 0.2$).

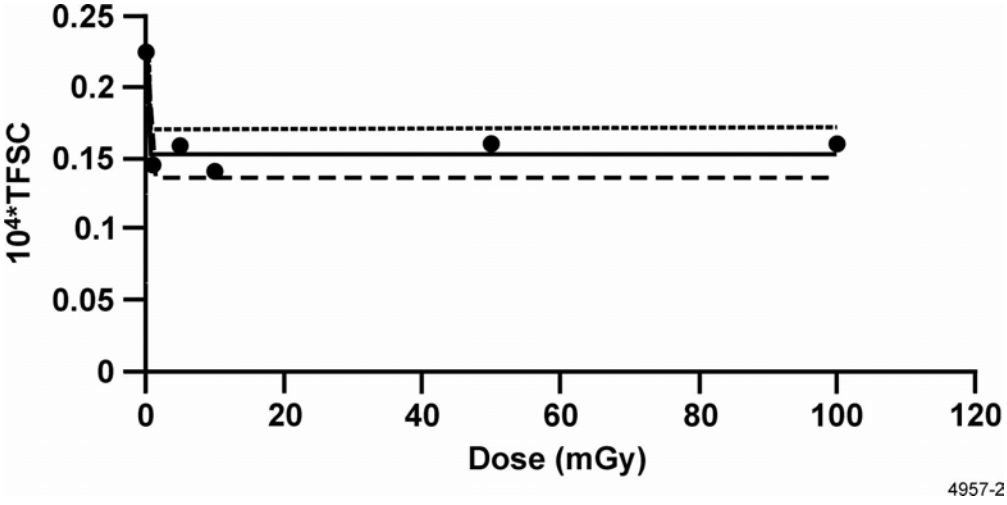


**Figure 5.** Application of the NEOTRANS$_2$ model to the Redpath et al. (2001) data (solid points) for gamma-ray-induced (*in vitro*) neoplastic transformation of HeLa × skin fibroblast human hybrid cells for the dose range 0 to 100 mGy. Model-associated means (central curve), 5% (percentile; lower curve), and 95% (upper curve) values are presented based on an assumed normal distribution.

In both Figures 4 and 5, the risk of neoplastic transformation clearly drops immediately below the spontaneous frequency to a fixed value independent of radiation dose, as predicted by the NEOTRANS$_2$ model (Equation 5).

The mean and standard deviations for $f_0$ were 0.32 $\pm$ 0.04 and 0.71 $\pm$ 0.04 for the data of Redpath et al. (2001) and Azzam et al. (1996), respectively. The parameter $f_0$ mean was therefore 2.2 times larger for the C3H 10T1/2 cells than for the HeLa $\times$ skin fibroblast human hybrid cells. Similarly, the spontaneous frequency mean was about 76 times larger for the C3H 10T1/2 cells than for the HeLa $\times$ skin fibroblast cells. These results suggest that $f_0$ may be correlated with genetic sensitivity, being larger (more protective) for the more sensitive target cells. However, what implication this has for sensitive individuals is unclear (Scott et al., 2003).

Figure 6 shows results of applying the NEOTRANS$_2$ model to a wider range of doses (0 to 500 mGy) based on the Redpath et al. (2001) data for gamma-ray-induced neoplastic transformation of HeLa $\times$ skin fibroblast human hybrid cells. Equation 3 was used in this analysis in conjunction with Bayesian methods. Transformants were modeled as having dose-dependent poisson distributions with the expected frequency given by Equation 3. Solid points in Figure 6 represent experimental data. Upper and lower lines drawn represent the upper and lower 95% credibility bands (from Bayesian posterior distribution), and the central line represents the posterior mean.

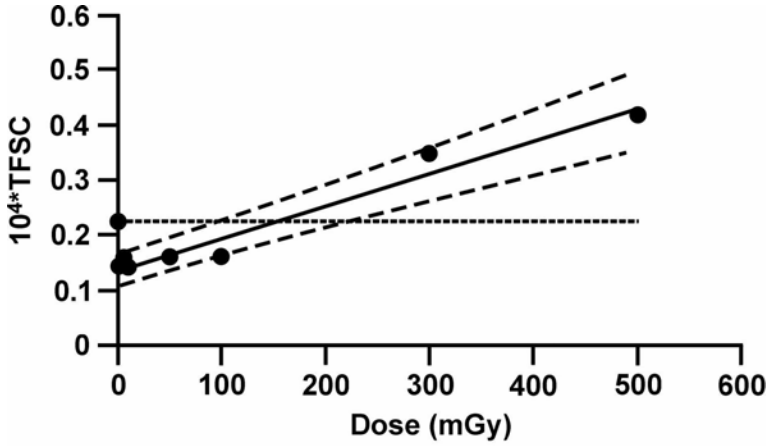


**Figure 6.** Application of the NEOTRANS$_2$ model to the Redpath et al. (2001) data (solid points) for gamma-ray-induced (*in vitro*) neoplastic transformation of HeLa $\times$ skin fibroblast human hybrid cells for the dose range 0 to 500 mGy. The central straight line is based on Bayesian posterior distribution mean for TFSC. Lower (5%; percentile) and upper (95%) values for the posterior distributions are shown also. The horizontal dash line is the posterior mean for $T_0$.

The central line in Figure 6 has been used to demonstrate a protective effect of low-dose radiation against neoplastic transformation. Figure 7 shows the benefit/harm ratio (expected number of spontaneous transformants eliminated/expected number of newly induced transformants). A benefit/harm ratio >> 1 demonstrates potential for possibly eliminating early stage cancer cells from the body via low-dose irradiation (e.g., from radon in the home, living at a high altitude where cosmic-ray doses are

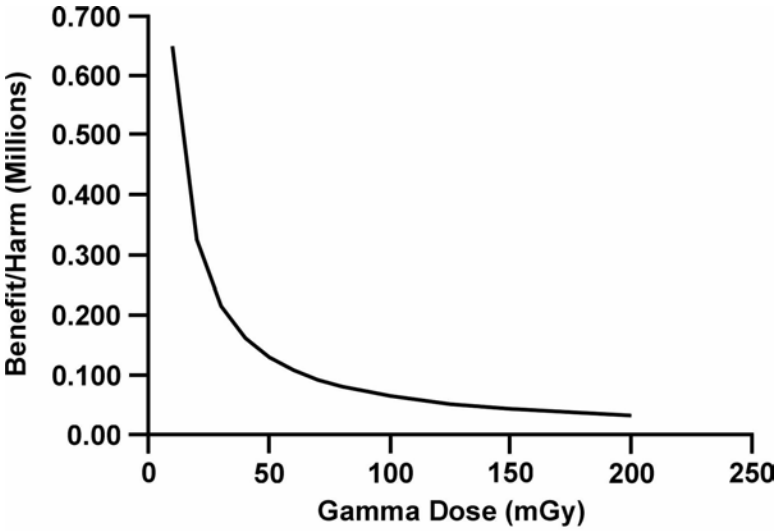


**Figure 7.** Benefit (spontaneous transformants eliminated) to harm (newly induced transformants) ratio based on the central line in Figure 6 for the neoplastic transformation data of Redpath et al. (2001).

higher, etc.). Similar potential protection also likely exists for other inducers of apoptosis signaling [e.g., apoptosis-inducing chemicals in food such as isothiocyanates (Yang et al. 2002)]. Note that the benefit/harm ratio increases steeply as the dose decreases below about 50 mGy. The lowest dose featured on the curve is 10 mGy. For this dose, the benefit/harm ratio exceeds 600,000. This means that on average, for each newly induced transformant, more than 600,000 assumed already present spontaneous transformants are eliminated via the presumed protective bystander apoptosis effect. This is a pronounced protective effect because relatively little harm to a human would be expected to be associated with a 10 mGy radiation dose, especially if protracted. Further, the benefit/harm ratio may increase as the period over which the dose is delivered increases because extending exposure also would be expected to prolong the period over which the protective bystander effect was operating.

Equally important, the indicated protective effect in Figure 7 possibly could operate against existing cancer cells through low-dose, low-dose-rate gamma ray (or X-ray, or beta radiation) therapy for cancer. There is strong evidence now that such a low-dose, low-dose-rate therapy can be quite effective in treating cancer, while greatly limiting radiation damage to normal tissue (J. M. Cuttler et al., Application of Low Doses of Radiation for Curing Cancer; paper available at: http://cnts.wpi.edu/RSH/Docs/byAuthor/Cuttler.htm#Technical).

Similar protection has been observed in *in-vitro* experiments on X-ray-induced mutations in mouse lung cells (T273; subclone of C10 cells), based on experiments conducted by one of the participants in this research project (D. Walker [DW], see submitted paper in Appendix A). DW adapted the *Hprt* assay developed by Dr. R. Albertini et al. (1982) and modified by Driscoll et al. (1995) for use with lung epithelial cells. The effect of low-dose X-rays on the *in vitro* induction of *Hprt* mutations and mutations in the presumptive mismatch repair (MMR) and apoptosis gene (Apop) systems (MMR/Apop gene systems) in mouse alveolar type II cells was investigated.

The C10 cells have two p53 alleles. However, the p53 protein has little to no activity. The T273 subclone used for these (preliminary) studies has a relatively high spontaneous mutation frequency, a wild-type *Hprt* gene, and is sensitive to the cytotoxic effects of 6-thioguanine. The high sensitivity to cell loss was interpreted to confirm the presence of functional MMR/Apop gene systems.

The T273 cells were exposed to 0, 100, or 1000 mGy of X-rays during log-phase growth. After exposure, the cells were grown for two weeks to allow phenotypic expression of treatment-related changes. The cells were then assayed for focus-forming mutations (transformed cells), mutations in the *Hprt* gene, and mutations in genes of the MMR/Apop systems, using a modification of the T-cell mutation assay. Results (*RR*) for the 0 and 100 mGy dose groups (dose-range of interest for this report) are presented in Table 1.

Table 1. Mutation frequency and associated relative risks for low-dose, X-ray-induced mutations among mouse lung cells (T273) exposed *in vitro*.

| | 0 mGy | 100 mGy | Relative Risk |
|---|---|---|---|
| *Hprt* | $(18.9 \pm 8.5)\times10^{-6}$ | $(11.0 \pm 7.0)\times10^{-6}$ | 0.58 ± 0.45 |
| MMR/Apop | $(37.9 \pm 13)\times10^{-6}$ | 0 | 0 |
| Focus-forming (transformed) | $(9.6 \pm 2.6)\times10^{-6}$ | 0 | 0 |
| Plating efficiency | 42.4 ± 7.8% | 36.5 ± 7.5% | |

Note the very dramatic protection afforded by the 100-mGy dose against the highly problematic mutations (MMR/Apop and focus-forming) in contrast to the modest protection suggested against the less problematic *Hprt* mutations; 100% protection was indicated against the problematic mutations. For the *Hprt* mutations, *RR* was not significantly less than one, although the data are consistent with a possible small decrease in *RR* with exposure to 100 mGy X-rays. These results suggest that an elaborate system of DNA damage detection and problematic cell mitigation may be operating with highly problematic cells efficiently recognized and removed when specific cell signaling associated with low-LET radiation-induced damage is turned on. Our views about possible mechanisms that would explain the differential levels of radiation-induced protection for the different mutation type are presented in the paper provided as Appendix A.

Similar protection also has been demonstrated in cancer chemoprevention studies where apoptosis-inducing isothiocyanates in the diet have prevented the occurrence of benzo(a)pyrene-induced lung tumors in mice (Yang et al. 2002).

Radiation also may induce the elimination of virally transfected cells via the protective bystander apoptosis effect. Figure 8 shows results obtained in modeling the cell-survival data of Seymour and Mothersill (2000) for gamma-ray-induced apoptosis in human Papillomavirus type 16 transfected (Pirisi et al. 1988) human keratinocytes. The cell killing for the dose range 0 to 100 mGy was modeled as arising from a protective bystander effect that was independent of dose for $\Delta D > 0$. As seen in Figure 8, the data are in excellent agreement with the modeling assumptions. For the indicated data and for doses > 0, there was no correlation between survival and dose ($R^2$ = 0.04, $p > 0.5$). The parameter $f_0$ (for removal of problematic cells) was found to have a mean and standard deviation of $0.37 \pm 0.0$ (i.e., 37% of problematic virally transfected cells are expected to be removed via a protective bystander apoptosis effect).

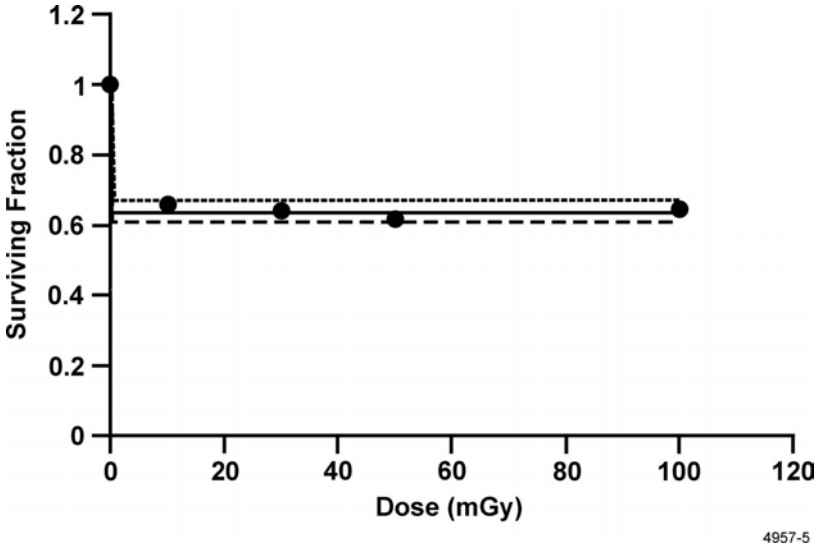


**Figure 8.** Observed and simulated survival of gamma irradiated human papillomavirus transfected keratinocytes based on the Seymour and Mothersill (2000) data (solid points). Analyses were performed for the dose range 0 to 100 mGy. Model-associated means (central curve), 5% (percentile; lower curve), and 95% (upper curve) values are presented based on an assumed normal distribution.

2.7.5 *Relative Risk Modeling for Neoplastic Transformation: Radiation and Chemicals*

Dr. Redpath and colleagues (2001) have shown that the dose-response relationship for the *RR* for low-dose, radiation-induced neoplastic transformation *in vitro* has a similar shape as for the *RR* for cancer induction in humans. This implies that dose-response functions for *RR* for neoplastic transformation (and possibly for problematic mutations) could be fitted to data for *RR* for cancer induction in humans, yielding more reliable characterization of *RR* at low doses. We assume this to be true for a single radiation, for combined exposure to different radiations, for combined exposure to radiation and genotoxic chemicals, and for combined exposure to different genotoxic chemicals. We provide results for combined exposure to high-LET alpha and low-LET gamma radiations that support our assumption.

2.7.5.1 *RR* for Low-Dose, Low-LET Radiation-Induced Stochastic Effects

Appendix A provides detailed information on our relative risk modeling for the endpoints neoplastic transformation and cancer induction that were obtained assuming that the shape of the dose-response curve was similar for both neoplastic transformation and cancer. Key results for low-dose gamma or alpha radiation and for combined exposure to low-dose alpha radiation and low-dose (or low-dose-rate) gamma rays follow. The results for combined exposure were obtained assuming independent action of the different radiations at low doses.

We use gamma rays to be representative of low-LET (L) radiation in general. For doses of low-LET gamma (or X-rays) in the range >0 to 100 mGy, *RR* for both radiation-induced neoplastic transformation and radiation-induced cancer is modeled as being fixed a value ($1-PROFAC_L$), where $PROFAC_L$ has replaced $f_0$ (see Equation 5) and indicates a protection factor against spontaneously occurring transformation (or precancerous cells). The dose-response curve for *RR* for neoplastic transformation *in vitro* or cancer induction in vivo by low-LET gamma (or X-ray) irradiation is then be characterized using

$$RR_L = 1\text{-}PROFAC_L, \quad (7)$$

for $0 < \Delta D_L \leq 100$ mGy

For high-dose-rate exposure to gamma (or X-ray) doses > 100 mGy but < about 500 mGy, the following equation applies (see Appendix A):

$$RR_L = 1\text{-}PROFAC_L + K_L \Delta D_L, \quad (8)$$

For cancer induction, the constant $K_L$ depends on the type of cancer and possibly on other factors such as age and health status. The slope parameter, $K_L$, represents the added *RR* per unit of dose of low-LET radiation and is assumed quite small in comparison to what would be expected for high-LET radiations such as alpha particles from inhaled plutonium-239 (Pu-239).

From Equation 8 it can be seen that the RR drops immediately from 1 for a very small dose increment about zero, and then increases linearly with dose as the dose increases further. This corresponds to the dose-response relationship presented in Figure 3.

2.7.5.2 *RR* for Low-Dose Alpha Radiation-Induced Stochastic Effects

For high-LET alpha irradiation, there appears to be essentially no induced protection (i.e., *PROFAC* = *0*) against spontaneous transformants, or the range of doses over which the protection occurs is too small to be detected from the available data. This is shown in Figure 9 where *RR* for neoplastic transformation among C3H 10T1/2 cells appears to increase in accordance to the LNT model, based on alpha radiation data of Bettega et al. (1992; see submitted paper in Appendix A). We speculate that for alpha irradiation (and possibly other high-LET radiation sources such as heavy ions encountered in space) the deleterious bystander effect predominates over the protective bystander effect.

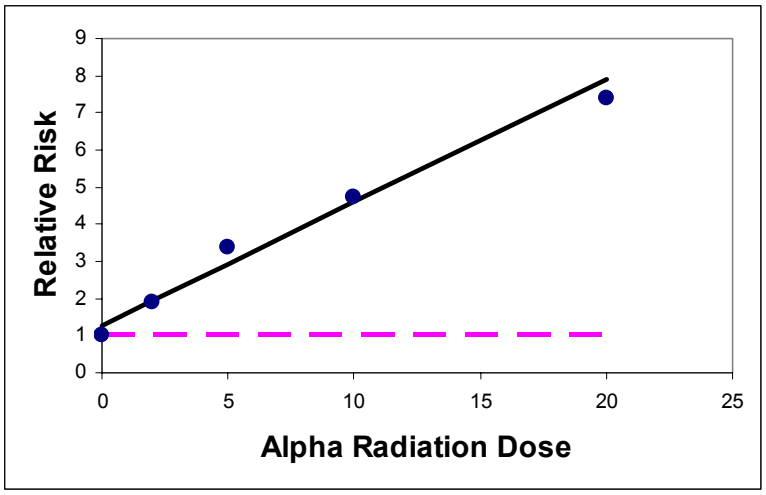


**Figure 9.** Relative risk for 4.3-MeV, alpha-particle-induced neoplastic transformation among C3H 10T1/2 cells based on data of Bettega et al. (1992). Data are consistent with the LNT hypothesis.

Because of the short range of alpha particles in tissue (only a few cell traversals), alpha radiation would be expected to be much less efficient in triggering widespread cell signaling related to the protective bystander effect.

We use alpha radiation to be representative of high-LET (H) radiations in general. The corresponding *RR* equation for low doses of only high-LET alpha radiation is:

$$RR_H = 1 + K_H \Delta D_H. \quad (9)$$

The slope parameter $K_H$ gives the added *RR* per unit of dose of the high-LET irradiation. Based on the indicated evidence, we have therefore assumed that no significant induced protection against spontaneous transformants is associated with the high-LET alpha radiation. This assumption also is made for cancer induction. We therefore consider Equation 9 to be applicable to both neoplastic transformation i*n vitro* and cancer induction *in vivo*. Equation 9 may not apply to *in vivo* exposure to neutrons, because neutrons produce gamma rays when interacting with body tissue of large mammals.

### 2.7.5.3 *RR* for Stochastic Effects of Combined Low- and High-LET Irradiation

For combined chronic low-dose-rate exposure to gamma (or X-rays) and alpha radiations, the appropriate equation for RR at low doses, assuming independent action (Scott 1984, 1986; Scott et al. 1990; Bukart et al. 1997) of the low- and high-LET radiations is given by:

$$RR_{L,H} = 1\text{-}PROFAC_L + K_H \Delta D_H, \quad (10)$$

for $\Delta D_H > 0$ and $\Delta D_L > 0$. Otherwise, $RR_{L,H} = 1$. Equation 10 was introduced in the paper presented in Appendix A and also may apply to neutron-induced cancer (at low doses) *in vivo* because of the large gamma ray component to the dose. Equation 10 also should apply to mixed high- and low-LET radiation fields encountered in space by astronauts.

### 2.7.5.4 Applications to Epidemiological Data

Equation 10 has been applied to data (Khokhryakov et al. 1996) for *RR* for lung cancer in humans (Mayak workers) after chronically exposed over years to alpha plus gamma radiations. Justification for use of Equation 10 is our assumption that the gamma ray component of the dose protected against both spontaneous and alpha radiation-induced lung cancers (see Appendix A for more details). The *RR* is plotted as a function of the estimated alpha radiation dose to the lung. The alpha irradiation arose from repeatedly inhaling plutonium-239 (Pu-239) in association with the production of weapons-grade plutonium (Pu). Results obtained (hormetic-type dose response) are presented in Figure 10 (note that RR < 1 at low doses). Initially, the slope parameter $K_H$ was found to be 0.01527 ± 0.00091 $mGy^{-1}$, with the intercept *(1-PROFAC$_L$)* not significantly different from zero (100% protection against spontaneous

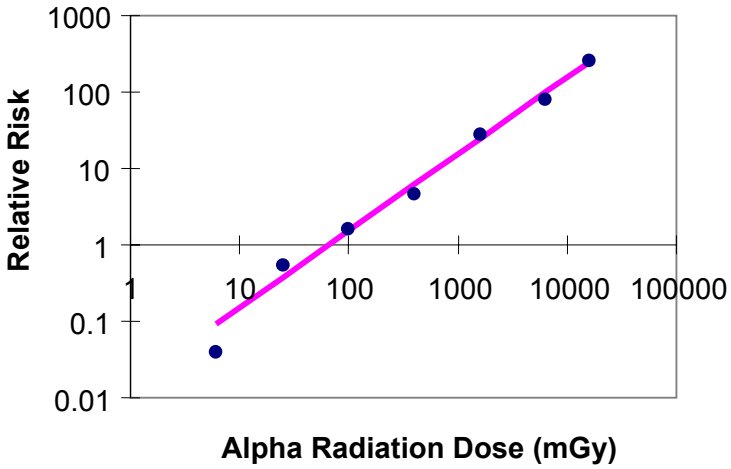


**Figure 10.** Relative risk for lung cancer induction in humans (Mayak workers) chronically exposed over years to alpha plus gamma radiations. The data points are estimates of relative risk based on published cancer incidence data (Khokhryakov et al. 1996). The smooth curve is based on fitting Equation 10 to the *RR* data by linear regression (with the zero dose group excluded).

cancers). The data were refitted with a zero intercept ($PROFAC_L$ = 1), yielding essentially the estimate for $K_H$ (i.e., 0.0153 ± 0.0005 $mGy^{-1}$). The central estimate of the threshold for excess lung cancer is 1/0.0153 mGy, which equals 65 mGy. Data consistent with a much higher threshold have been reported by others based on a case-control study (Tokarskaya et al. 2002) and suggest that there may be other gamma-ray-induced, protective processes (e.g., immune system activation) *in vivo*.

Equation 9 also was fitted to the Mayak worker data for high doses (1600, 6400, and 16000 $mGy^{-1}$) where risk was clearly elevated (Fig. 11). Figure 12 compares results based on Equation 10 to those obtained with extrapolating from high doses to low doses using the LNT model (Equation 9). Note that the chronic gamma irradiation appears to have protected against essentially all spontaneous lung cancers ($PROFAC_L$=1). Note also that the LNT model extrapolation from high to low doses leads to phantom excess risk based on the data of Khokhryakov et al. (1996)! The controls (zero-dose group) were based on national cancer statistics for the Russian population (Khokhryakov et al. 1996). In the case control study of Tokarskaya et al. (2002), internal controls (Mayak workers) were used, rather than Russian national cancer statistics.

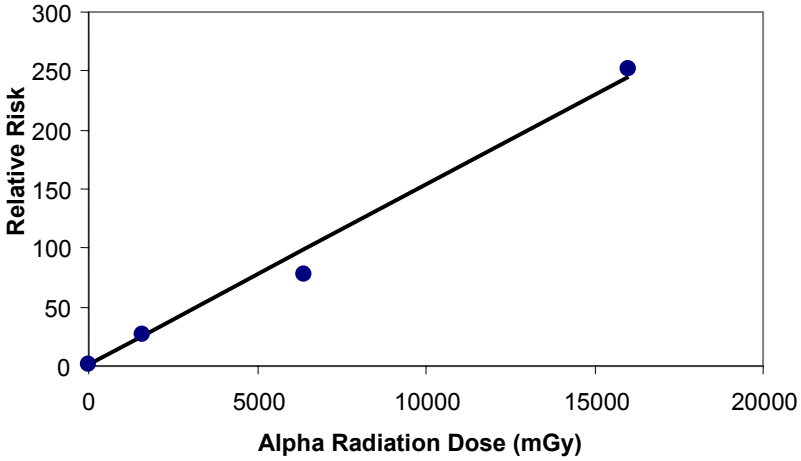


**Figure 11.** Relative risk for lung cancer induction in humans (Mayak workers) chronically exposed to alpha plus gamma radiations. Here only high-dose data are used in conjunction with the LNT model.

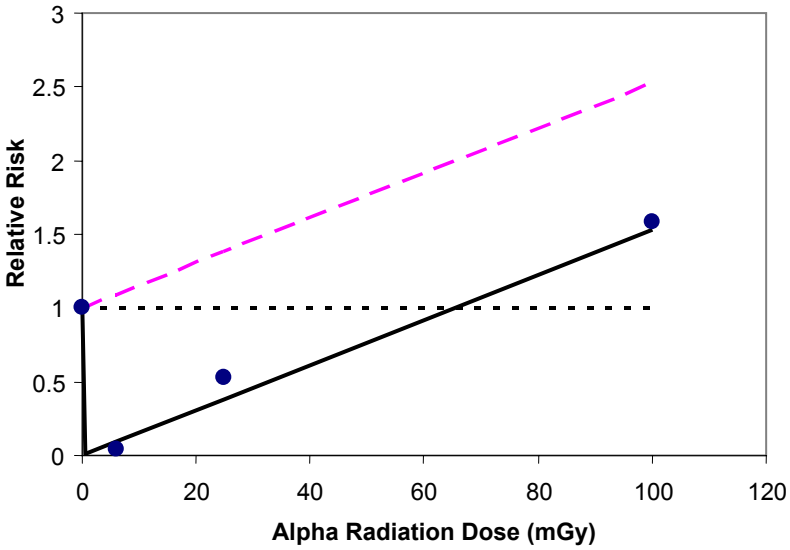


**Figure 12.** A comparison of the low-dose portions of Figures 10 and 11. The upper dashed curve is relative risk based on the LNT model extrapolated to low doses. The hormetic-type curve is based on Equation 10 fitted to all the presented data in Figure 10.

### 2.7.6 *Chemicals Evidence against the LNT Model*

We also have adapted the $NEOTRANS_2$ model to be applicable to mutation induction *in vivo* (in T lymphocytes in mice) by inhaled ethylene oxide (prototypic DNA-alkylating agent). The adapted model is called $NEOTRANS_2$-EO, and the ethylene oxide concentration was used as dose (corresponds to variable *c* in the $NEOTRANS_2$ model, when the exposure time is fixed). Details are described in Appendix A as well in a related paper (Walker et al. 2003).

Ethylene oxide is an immediate metabolite of ethylene, a normal body constituent and is genotoxic (Walker et al. 2003). It is classified as a Group 1 human carcinogen by the

International Agency for Research on Cancer, based on sufficient evidence in animals with strong evidence in humans of relevant mechanisms for carcinogenicity (IARC 1994).

Historically, risk assessment for genotoxic chemical carcinogens has been based on the assumption that any exposure carries a cancer risk no matter how small the dose. Using data for ethylene-oxide-induced mutations in T lymphocytes of B6CF31 mice exposed via inhalation, we have shown evidence against the validity of the LNT model so far as its application to low-dose-induced mutations *in vivo* (see Appendix A and Walker et al. 2003).

Briefly summarizing, two data sets were used to obtain the *RR* as presented in Figures 13 and 14 for mutation induction in B6C3F1 mice exposed via inhalation for 4 weeks (6 h/day, 5 days/week) to ethylene or EO (Walker et al. 2003). The ethylene-exposed mice had calculated equivalent doses of EO of 0.7, 4.4, and 8.6 ppm. For mice directly exposed to EO, the exposure concentrations were 50, 100, and 200 ppm. To adequately characterize the dose-response data in Figures 13 and 14, it was necessary to postulate a threshold EO exposure concentration $C_1$* (2.3 ± 1 ppm) for turning on the protective bystander apoptosis effect [estimated via Bayesian inference methods (Walker et al. 2003)]. Below this threshold, only error-free repair was assumed ($\mu_1 > 0$, $\eta_1 = 0$). This lead to a flat dose-response from 0 to the threshold EO concentration $C_1$* (Fig. 14, blow up of low-dose region). Above this threshold concentration, misrepair was presumed to occur ($\eta_1 > 0$) in competition with error-free repair ($\mu_1 > 0$), leading to newly induced mutations. The mutation frequency at 4.4 ppm EO equivalence was significantly different from the spontaneous frequency [p = 0.009, Mann-Whit U-statistics (Walker et al. 2003] and clearly below it, indicating presumed protection against spontaneous mutations. A second threshold $C_2$* (17 ± 11 ppm) is where the curve first increases above the spontaneous frequency (i.e., threshold for excess mutations; Figure 14).

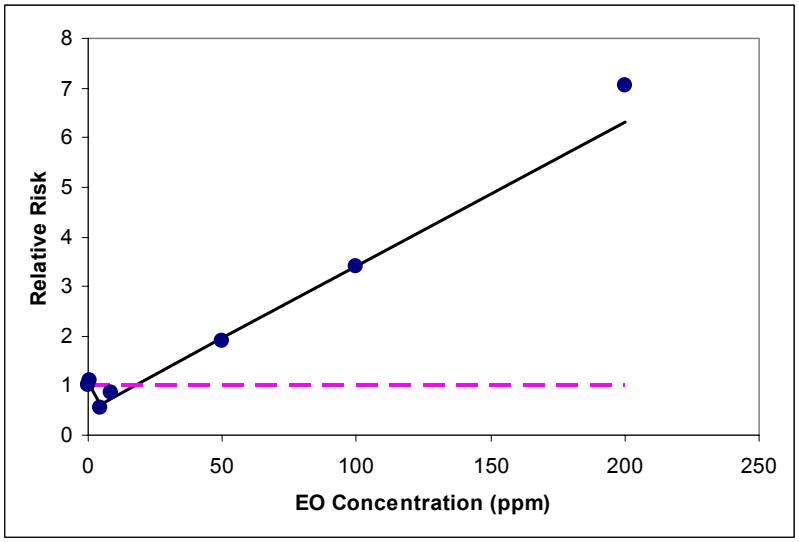


**Figure 13.** Relative risk for ethylene-oxide (metabolite of ethylene) induced *Hprt* mutations in T lymphocytes of B6C3F1 mice exposed via inhalation to ethylene (with associated low doses of EO: 0, 0.7, 4.4, or 8.6 ppm or high doses of EO: 50, 100, or 200 ppm) based on application of the NEOTRANS$_2$-EO model to data. Poisson regression implemented via Bayesian inference was used to fit the model to the data (Walker *et al.* 2003).

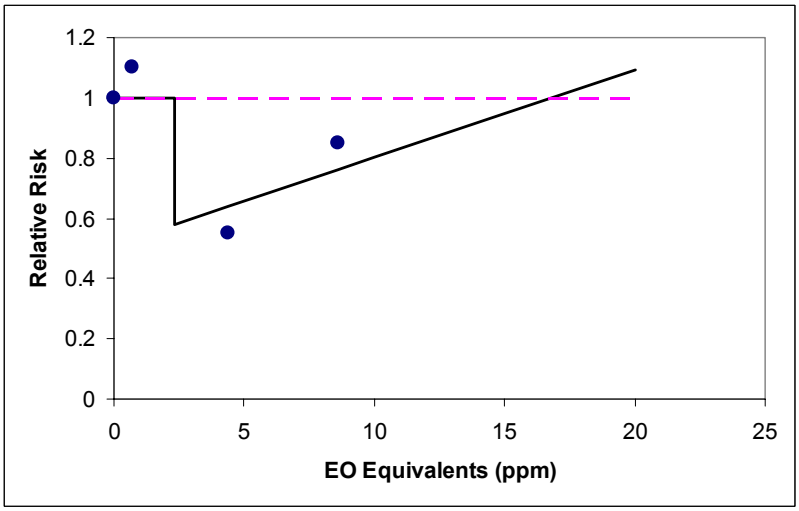


**Figure 14.** Low-dose portion of relative risk curve in Figure 13 for EO-induced *Hprt* mutations in T lymphocytes of B6C3F1 mice.

The indicated departure from the LNT model was demonstrated to be statistically significant (Walker et al. 2003).

Preliminary results obtained in a study at LRRI by V. E. Walker and D. M. Walker for propylene-induced *Hprt* mutations in splenic T-cells of male F344 rats are similar to the findings for ethylene oxide. Propylene is an atmospheric hydrocarbon and a major industrial intermediate to which humans are exposed by inhalation. Propylene is metabolized by mammalian cells to the genotoxic agent propylene oxide. Currently available data from studies using propylene were evaluated by the Walkers and were found to indicate that low-dose propylene dioxide induces significant protection against spontaneous lung and thyroid tumors (manuscript in preparation under a different project).

### 2.7.7 *Other Evidence against the LNT Model*

A hormetic-type dose-response relationship for lung cancer induction by low-LET photon radiation also has been reported by others. Rossi and Zaider (1997) critically reviewed the literature on radiogenic lung cancer and concluded that "at radiation doses generally of concern in radiation protection (< 2 Gy), protracted exposure to low LET radiation (X- or $\gamma$-rays) does not appear to cause lung cancer. There is in fact, indication of a reduction of the natural incidence."

As already indicated, with hormetic-type, dose-response relationships for cancer induction, there is a threshold for excess cancers. Results of earlier and recent case-control studies of lung, liver, and biliary tract cancer among Mayak workers are consistent with large thresholds for excess cancers for combined alpha and gamma irradiation (Tokarskaya et al. 1995, 1997, 2002, 2003). No association between chronic gamma irradiation and lung, liver, or biliary tract cancer was found by Dr. Tokarskaya and colleagues.

The recent Hanford Thyroid Disease Study (USDHHS 2002) did not find evidence of any excess risk for thyroid cancer induction for people living in the vicinity of the Hanford facility who were exposed to beta radiation from radioactive iodine released from the facility. For doses in the range of 0 to 100 mGy, risk was not correlated with dose and was less than for the control group based on people outside what was considered the irradiation zone. In addition, for several health effects, the mean slope of the risk versus dose relationship was negative (indicating possible hormetic-type dose-response relationships).

Rowland (1994) reported a large threshold for alpha radiation-induced bone cancer for female radium dial painters who ingested radium isotopes by licking their brushes to provide moisture. Based on the NEOTRANS$_2$ model, the threshold would be expected to be associated with induced protection related to the low-LET component of the dose, rather than with protection induction by alpha radiation.

Animal data also are consistent with thresholds for cancer induction by low-dose-rate, low-LET radiation (Yamamato et al. 1998; Kondo 2000; Tanooka 2000; Yamamato and Seyama 2000). In studies of Yamamato and Seyama (2000), female (C57BL/6N and C3H/He) $F_1$ mice were maintained for their entire lifespan (or different durations) on drinking water that contained different levels of tritiated water (a low-LET, beta radiation source). In their study, the dependence of the incidence of thymic lymphoma on the beta radiation dose and dose-rate was evaluated. They found both beta radiation dose and dose-rate thresholds for thymic lymphoma induction. The incidence of thymic lymphoma was decreased to zero by decreasing the total doses below 5 Gy or the dose rate below 12 mGy per day, respectively. Because the incidence was reduced to zero, it can be inferred that chronic, low-dose-rate beta irradiation protected against both spontaneous and newly induced thymic lymphoma.

Kondo (2000) pointed out that the major risks of low-dose photon radiation (X-ray, gamma rays) are mutagenesis, teratogenesis, and carcinogenesis. All three endpoints were found by Kondo (2000) to approach zero risk when the X-ray dose rate was reduced from 450 mGy/min

to 1.2 mGy/min in studies where mice were exposed at the embryonic age of 9.5 days (total dose = 2 Gy). The results were concluded to indicate low-dose rate photon radiation induced protection (via DNA repair and apoptosis) against stochastic effects. The studies by Kondo involved p53(+/+) mice (with the wild-type p53 gene) that were protected by the low-dose-rate exposure. However, the protection was not evident for p53(-/-) mice that were unable to carry out apoptosis.

R. E. J. Mitchell and colleagues (Atomic Energy of Canada Limited, Chalk River, ON Canada H0J 1J0) gave a presentation at the 2002 Annual Meeting of the Radiation Research Society (http://199.245.2000.45/pweb/document/?SOSIETY=rr&YEAR=2002&ID=792) entitled "Low Doses of Radiation Increases the Latency of Spontaneous Cancers in Cancer Prone Trp53 Heterozygous Mice." In their study, mice that are heterozygous for defects in Trp53 are known to be cancer prone, spontaneously developing a variety of fatal cancers. The effect of low doses of gamma rays (10 or 100 mGy) delivered at a low rate (0.5 mGy/min) on the latency of a variety of cancer was studied. As compared to unexposed mice that spontaneously developed lymphomas, hemangiosarcomas, spinal osteosarcomas, or undifferentiated sarcomas induced by physical injury, an exposure of 7- to 8-week old Trp53 (+/-) mice to either 10 or 100 mGy had no significant effect of tumor frequency. However, the 10 mGy exposure increased the mean tumor latency of all spontaneous cancers, as well as the cancers initiated by physical injury in these cancer-prone mice. The results were interpreted to indicate that the main *in vivo* effect of single low-dose, low-dose-rate exposure is reduced tumor risk resulting from a reduction in the rate at which initiated cells become genomically unstable. The indicated protective effect lasted for the entire lifespan of all the animals that developed tumors, effectively restoring a portion of the mean loss of lifespan attributed to Trp53 heterozygosity in the absence of radiation exposure. Increasing the dose 10-fold to 100 mGy produced variable results: increasing risk (decreased latency) for some tumors but increasing latency for others, indicating that the higher dose was in the transition zone between reduced (due to induced protection) and increased risk. This postulated zone is consistent with results presented in Figure 6 for gamma-ray-induced neoplastic transformation.

The observation that chronic low-dose photon or beta irradiation can induce pronounced and prolonged protection against both spontaneous and radiation-induced stochastic effects such as cancer are consistent with predictions of the NEOTRANS$_2$ model.

## 3. Relevance

The DOE through its Office of Environmental Management is responsible for cleaning up 114 sites that have been involved with research, development, and production, as well as testing of nuclear weapons (USDOE, 1996). Taken together, these sites encompass an area of over 2 million acres. At the beginning of fiscal year 2002, the DOE had completed active cleanup at 745 sites. However, the sites were small and the easiest to deal with. The remaining large sites present enormous challenges, and risk-based management decisions are likely to have a major role in total costs. Currently, risks are based on the LNT model.

The magnitude of the challenges facing EM can be appreciated by reported cleanup cost estimates. In February 2002 the estimate was $220 billion and could easily increase to $300 billion (DOE Office of Oversight, Safety and Health, 2000).

Currently, the high cleanup costs are tied to the current low dose risk assessment paradigm whereby the RR (i.e., relative risk) is always greater than 1, no matter how small the radiation dose, for all doses of radiation > 0. This RR paradigm arises from the LNT model, which assumes a linear increase in risk at low doses, irrespective of the type of radiation, type of tissue irradiated, dose rate, or other features of the radiation exposure. This RR paradigm is being sustained by those who have benefited from its use in risk assessment, for example in projecting thousands or tens of thousands of deaths arising from radiation exposure associated

with accidents such as occurred in 1976 at the Chernobyl plant and from fallout radioactivity from previous atmospheric nuclear tests. Their stated view is that at very low radiation doses, risk increases but to an extent too small to be demonstrated via epidemiological or animal studies. They do not consider the possibility that protective effects may be induced by low-dose, low-LET radiation (called "adaptation response" by some) leading to a significant drop (quantifiable) in risk.

Our research has revealed the existence of protective effects that can be induced by low doses and dose rates of low-LET radiation. Currently, the protective effect is considered to be mediated mainly by apoptosis (but induced DNA repair likely plays a role also). Because of the low-LET radiation induced protection, there is a threshold for induced excess cancers (relative to the spontaneous incidence). This threshold is expected to arise not only for exposure to low-LET radiation but also for combined exposure to low- and high-LET radiations. Thus, because of this protective effect, RR < 1 may be more the rule than the exception at low doses of low-LET radiation.

Regarding establishing future cleanup criteria for radionuclide contaminated DOE sites, there should be growing pressure placed on those who continue to advocate use of the low-dose RR paradigm for which RR is always ≥ 1, to also consider the possibility that RR < 1, especially in cases where humans are exposed to highly penetrating, low-LET radiation. Further, RR < 1 also can arise for combined exposure to low-LET and high-LET radiations.

For exposure only to high-LET alpha radiation, we have no evidence for induced protection (adaptation) against stochastic effects. For exposure of large mammals to neutrons, protection could arise from the gamma ray component of the absorbed dose that occurs through the interaction of neutrons with atoms in tissue.

Our research results also suggest that a new risk assessment paradigm also may be needed for assessing cancer risk to humans from combined exposure to radiation and genotoxic chemicals. For low-dose radiation plus chemical exposure, RR < 1 cannot be excluded and could turn out to be more the rule than the exception. This would have major implications for setting standards for worker and public exposures. Therefore, new research in this area is strongly needed and should be supported by the DOE as well as other agencies (e.g., Department of Defense and Environmental Protection Agency).

The DOE has for years sponsored research related to medical applications of radiation. Our research results indicate that low-dose, and low-dose-rate, low-LET radiation possibly could be used in successfully treating cancer while minimizing damage to normal tissue. The protective bystander effect introduced in this report could be turned on by low-dose gamma rays, X-rays, or beta radiation and operate against existing cancer cells as well as precancerous cells. Chemicals that initiate apoptosis (some are contained in foods) also could be used along with radiation. The low doses of radiation may stimulate the immune system to provide additional pronounced protection against cancer. Thus, it is strongly recommended that new research initiatives in the field of low-dose therapy for cancer be supported by appropriate organizations, including the DOE.

## 4. Project Productivity

Project annual reports were prepared in a timely manner. Numerous presentations were given at scientific meetings. Key research results were published in peer review journals.

## 5. Personnel Supported

This project involved numerous individuals, some of whom are no longer with the LRRI. Present and past co-investigators are as follows: Drs. H. Schöllnberger, Y. Tesfaigzi, .D. M. Walker, P. Gerde, and R. E. Neft. Past participating consultants are as follows: Drs. T. M. Koval

and T. E. Hanson. Mr. J. Aden participated as a graduated student (via a Research Associated position).

Dr. V. E. Walker also participated but via funding from other projects related to chemical toxicology. Drs. Z. B. Tokarskaya, G. Zhuntova, and N. D. Okladnikova also participated in the research but via funding from another DOE project in the Environmental Management Science Program.

## 6. Publications


1. Aden, J. and B. R. Scott. "Modeling variability and uncertainty associated with inhaled weapons grade $PuO_2$." *Health Physics* 84: 726-736, 2003.
2. Aden, J. and B. R. Scott. "Modeling variability and uncertainty associated with inhaled $PuO_2$ for the stochastic intake paradigm." Proceedings of the ANS Radiation Protection and Shielding Division $12^{th}$ Biennial Topical Meeting, Santa Fe, NM, April 14–18, 2002.
3. Gerde, P. and B. R. Scott. "A model for absorption of low-volatile toxicants by the airway mucosa." *Inhalation Toxicology* 13: 903-929, 2001.
4. Okladnikova, N. D., B. R. Scott, Z. B. Tokarskaya, G. V. Zhuntova, V. F. Khokhryakov, V. A. Syrchikov, and E. S. Grigoryeva. "Evaluation of genomic instability among Mayak workers that inhaled insoluble forms of Pu-239." *Medical Radiology and Radiation Safety Journal* (submitted, in Russian; English version in preparation under a different project).
5. Schöllnberger, H., B. R. Scott, and T. E. Hanson. "Application of Bayesian inference to characterize risks associated with low doses of low-LET radiation." *Bulletin of Mathematical Biology* 6: 865-883, 2001.
6. Schöllnberger, H., M. R. Mebust, D. J. Crawford-Brown, P. M. Eckl, and W. Hofmann. "The significance of cell-cycle delay, multiple initiation pathways, misrepair, and replication errors in a model of radiobiological effects." *International Journal of Radiation Biology* 77(4), 519-527, 2001.
7. Schöllnberger, H., J. Aden, and B. R. Scott. "Respiratory tract deposition efficiencies and evaluation of impacts from smoke released in the Cerro Grande forest fire." *Journal of Aerosol Medicine* 15 (4), 387-399, 2002.
8. Schöllnberger, H., B. R. Scott, M. Stafford, and S. V. Osovets. "Analytical solutions for mechanistic model for neoplastic transformation." (in Russian) *Radiation Safety Problems, Mayak Production Association Scientific Journal*, Russian Federation Ministry of Atomic Energy, No. 3:37-43, 2002.
9. Scott, B. R. "Transformation of C3H 10T1/2 cells by low doses of ionizing radiation." *Journal of Radiological Protection* 19(2):177-179 (Letter) 1999.
10. Scott, B. R. and Schöllnberger, H. "Introducing biological microdosimetry for ionizing radiation." *Radiation Protection Dosimetry* 91(4), 377-384, 2000.
11. Scott, B. R., D. M. Walker, Y. Tesfaigzi, H. Schollnberger, and V. Walker. "Mechanistic basis for nonlinear dose-response relationships for low-dose radiation-induced stochastic effects." *Nonlinearity in Biology, Toxicology and Medicine* 1 (1): 93-122, 2003.
12. Scott, B. R. and V. L. Peterson. "Risks estimates for deterministic effects of inhaled weapons grade plutonium." *Health Physics* (in press).
13. Scott, B. R., D. M. Walker, and V. E. Walker. Low-dose radiation and genotoxic chemicals protect against stochastic biological effects." *Nonlinearity in Biology, Toxicology and Medicine* (submitted for special issue publication).
14. Tokarskaya, Z. B., B. R. Scott, G. V. Zhuntova, N. D. Okladnikova, Z. D. Belyaeva, V. F. Khokhryakov, H. Schöllnberger, and E. K. Vasilenko: "Interaction of radiation and smoking in lung cancer induction among workers at the Mayak enterprise." *Health Physics* 83(6): 833-846, 2002.
15. Tokarskaya, Z. B., G. V. Zhuntova, B. R. Scott, V. F. Khokhryakov, Z. D. Belyaeva, and E. K. Vasilenko. "The influence of radiation and non-radiation factors on the incidence of

liver and biliary tract malignant tumors among Mayak PA workers." *Health Physics* (submitted).
16. Walker, D. M., V. E. Walker, and B. R. Scott. "Modeling of low dose stochastic effects for the prototypic DNA alkylating agent ethylene oxide: Improved characterization using non-linear models." *Toxicological Sciences* (submitted).
17. 7.0 Interactions
18. This project fostered numerous internal and external interactions with a variety of scientists around the globe. These interactions are reflected in the following presentations at scientific meetings and seminars where numerous discussions related to the implications of our research took place. :
19. Aden, J. and B. R. Scott. "Modeling variability and uncertainty associated with inhaled $PuO_2$ for the stochastic intake paradigm." ANS 12th Biennial Radiation Protection and Shielding Division Topical Meeting, Santa Fe, NM, April 14–18, 2002.
20. Henderson, R. and B. R. Scott. "Proteomics at LRRI." Joint seminar presentation at Lovelace Respiratory Research Institute, Albuquerque, NM, July 8, 2002.
21. Osovets, S. V. and B. R. Scott. "Modeling the dependence of the median effective dose on dose rate." 32nd Annual Meeting of the European Society for Radiation Biology, Liege, Belgiuim, September 4–7, 2002.
22. Schöllnberger, H., D. J.Crawford-Brown, P. M. Eckl, M. R. Mebust, and W. Hofmann. "Radioprotective Mechanisms and Dose-Response Plateau for Initiation in a State-Vector Model of Radiocarcinogenesis." Poster presentation (#214) given at the 47th Annual Meeting of the Radiation Research Society, Albuquerque, NM, April 29–May 3, 2000.
23. Scott, B. R., Y. Tesfaigzi, H. Schöllnberger, and P. Gerde. "Advanced Computational Approach for Characterizing Stochastic Cellular Responses to Low-Dose, Low-Dose-Rate Exposures." Poster presentation given at the U.S. Department of Energy Low Dose Radiation Program Workshop I, Washington, DC, November 10–12, 1999.
24. Scott, B. R., Y. Tesfaigzi, and H. Schöllnberger. "Mechanistic Models for Radiation-Induced Neoplastic Transformation." Poster presentation (#53) given at the 47th Annual Meeting of the Radiation Research Society, Albuquerque, NM, April 29–May 3, 2000.
25. Scott, B. R., H. Schöllnberger, Y. Tesfaigzi, T. E. Hanson, S. V. Osovets, C. Schmitt, and J. Aden "Genomic Instability State Models for the Induction of Neoplastic Transformation by Low Radiation Doses." DOE/NASA Radiation Investigators Workshop, Arlington, VA, Book of Abstracts, p. 263, June 2001, http://www.orau.gov/doenasa/postedabstracts.htm.
26. Scott, B. R., Y. Tesfaigzi, J. Aden, H. Schöllnberger, and D. Walker. "Thresholds for radiation-induced mutations and neoplastic transformation could arise from apoptosis and error-free repair." DOE Low Dose Radiation Research Program Investigators Workshop III, Rockville, MD, March 25–27, 2002.
27. Scott, B. R., D. Walker, and V. E. Walker. "Low dose extrapolation: Evidence against the validity of the linear nonthreshold hypothesis." Presented to American Conference of Governmental Industrial Hygienist (ACGIH) representatives, Lovelace Respiratory Research Institute, Albuquerque, NM April 13, 2002.
28. Scott, B. R., D. M. Walker, V. Walker, J. Aden, and Y. Tesfaigzi. "Low-dose protective mechanisms: Implications for risk assessment." BELLE Conference, Non-linear Dose-response Relationships in Biology, Toxicology and Medicine, University of Massachusetts, Amherst, MA, June 11–13, 2002.
29. Scott, B. R. and V. L. Peterson. "Use of NUREG/CR-4214 models to estimate risks for deterministic health effects of inhaled weapons grade plutonium." American Radiation Safety Conference and Exposition, Health Physics Society's 47th Annual Meeting, Tampa, Fl. June 16–20, 2002.
30. Scott, B. R. and D. M. Walker. "Research on stochastic biological effects of low doses." Joint seminar presented at Lovelace Respiratory Research Institute, August 5, 2002.

31. Scott, B. R. and D. M. Walker. "Have we been misinformed about low dose radiation being harmful?" Annual Meeting of the Environmental Mutagen Society, Miami, FL, May 10–14, 2003.
32. Scott, B. R., D. M. Walker, and V. E. Walker. "Low dose radiation and genotoxic chemicals protect against stochastic biological effects." BELLE Conference on Non-Linear Dose-Response Relationships in Biology, Toxicology and Medicine, University of Massachusetts, Amherst, MA, May 28–30, 2003.
33. Tokarskaya, Z., G. Zhuntova, B. Scott, V. Khokhryakov, and E. Vasilenko. "Influences of radiation and non-radiation factors in the occurrence of liver and biliary tract malignancies among plutonium production workers". American Radiation Safety Conference and Exposition, Health Physics Society's 47th Annual Meeting, Tampa, Fl. June 16–20, 2002.
34. In addition, key project findings have been made available to the scientific community via our web site (http://www.radiation-scott.org) jointly supported by the DOE Office of Environmental Management and Office of Science. The indicated site also has educational materials related to radiation posted for the public.
35. 8.0 Transitions
36. Our research findings related to low-dose, low-LET radiation-induced protection against stochastic effects has provided and explanation for the observed hormetic-type dose response for lung cancer among Mayak worker exposed to low-dose-rate gamma rays in combination with alpha radiation form inhaled plutonium-239. Our research results also provide an explanation for the observed hormetic-type dose response for lung cancer induction in humans by low-dose-rate photon radiation. The results argue strongly for the inclusion of the possibility for RR < 1 for exposure of humans to low doses and low dose rates of low-LET radiation. These findings should aid DOE in revising cleanup criteria for radionuclide-contaminated DOE sites. Currently used criteria are based on the high-cost relative risk paradigm where any radiation exposure leads to an increase in risk of cancer. Revising the risk assessment paradigm applicable to radionuclide contaminated DOE sites to allow for RR < 1 should be given high priority by both the DOE Office of Science and Office of Environmental Management.
37. Our research findings also have very important implications for cancer therapy. The research findings strongly indicate that low doses of photon radiation (e.g., gamma rays) could be very effective in treating cancer while not producing much harm to normal tissue. Currently, such therapy is not being adequately researched in the U.S. The DOE should increase sponsorship for low dose cancer therapy research.

## 7. Patents: None

## 8. Future work

Work in this project has been completed. However, additional modeling of low-dose radiation-induced stochastic effects will be continued through a new project in the DOE Low Dose Radiation Research Program. The focus will be on adapting the NEOTANS$_2$ model to be applicable to three-dimensional tissue *in vivo*.

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## Appendix

Appendix A: Paper by B. R. Scott et al. submitted to *Nonlinearity in Biology, Toxicology and Medicine*

**Appendix A**

**Paper Status: Submitted to Nonlinearity in Biology, Toxicology and Medicine**

# Low-Dose Radiation and Genotoxic Chemicals Protect against Stochastic Biological Effects

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Running Head: Low-dose radiation and chemicals protect

**Abstract**–The linear nonthreshold (LNT) model plays a central role in low-dose risk assessment for stochastic biological effects (e.g., problematic mutations, neoplastic transformation, and cancer) associated with radiation and genotoxic chemical exposures of humans. With the LNT model, the risk increases linearly with dose and without a threshold. Using the LNT model, others have "calculated" tens of thousands of deaths related to environmental exposure to radioactive material from radiological incidents (e.g., Chernobyl). Here, we present biologically based models for low-dose-radiation- and genotoxic-chemical-induced stochastic effects (mutations and neoplastic transformation) that lead to nonlinear, hormetic-type relationships between the risk for specific stochastic effects and dose. We provide modeling, experimental, and epidemiological evidence that low-dose and low-dose-rate gamma radiation can protect from spontaneous problematic mutations, neoplastic transformation, and cancer. The protection is attributed to low-dose-induced, selective removal of problematic bystander cells via apoptosis. We provide modeling and experimental evidence that the prototypic DNA-alkylating agent ethylene oxide (EO) can also induce selective removal of spontaneous *Hprt* mutations, suggesting that DNA-alkylating agents might also protect against spontaneous cancers. For gamma radiation, the protective process does not appear to have a threshold. For EO, a threshold for turning on the protective process is implicated.

Key Words: Low-dose, radiation, ethylene oxide, risk assessment, threshold

INTRODUCTION

The shape of the dose-response curve for stochastic effects (mutations, neoplastic transformation, and cancer) of exposure to low doses of ionizing radiation or genotoxic chemicals has been the topic of continuous debate (Pollycove 1995; Rossi and Zaider 1997; Calabrese and Baldwin 1999, 2003a,b; Joiner *et al.* 1999; Pollycove and Feinendegen 1999, 2001; Feinendgen and Pollycove 2001; Schöllnberger *et al.* 2001a,b,c, 2002). The key discussion relates to whether the linear nonthreshold (LNT) model for low-dose extrapolation of cancer risk is valid (Rowland 1994; NCRP 2001). The LNT model is widely used by regulatory agencies and in radiation and chemical protection.

With the LNT hypothesis, any amount of carcinogen exposure increases one's risk of cancer. Based on this hypothesis, tens of thousands of cancer deaths in the U.S. have been calculated to arise from fallout from nuclear weapons testing (CDC/NCI 2001).

Now there is growing evidence from epidemiological, experimental, and mathematical modeling studies that does not support use of the LNT model for central estimation of cancer risks at low doses (Hoel and Anderson 1983; Bond *et al.* 1987; Feinendegen *et al.* 1999, 2000; Pollycove and Feinendgen 1999; Feinendegen and Pollycove 2001; Cucinotta *et al.* 2002; Schöllnberger *et al.* 2002; Scott *et al.* 2003). Instead, the results support the existence of thresholds (quite large in some cases) for induced "excess cancers," possibly in association with complex dose-response relationships (e.g., u-shaped). The u-shaped dose-response relationship is well known among researchers of hormesis (Calaberse and Baldwin 2003a,b).

EPA's 1996 proposed revision of the carcinogen risk assessment guideline suggests the most appropriate model(s) for risk extrapolation to be used to incorporate the existing understanding of mechanisms, and indicates a preference for biological-based dose-response models (Wiltse and Dellarco 1996). The use of mode of action information in low-dose risk characterization facilitates reducing uncertainties (Butterworth and Bogdanffy 1999).

Here, we present two biological-based models for low-dose-induced stochastic effects that lead to hormetic-type, dose-response relationships. The first model is called NEOTRANS$_2$ and relates to low-dose radiation. The second model is an adaptation of NEOTRANS$_2$ for application to a prototypic DNA-alkylating agent, ethylene oxide (EO). The adapted model is called NEOTRANS$_2$-EO.

Currently, the low-dose, relative-risk (*RR*) paradigm whereby *RR* is always $\geq 1$ is used in regulating low-dose exposure of humans to radiation and genotoxic chemicals. Using the NEOTRANS$_2$ and NEOTRANS$_2$-EO models, we show evidence that this paradigm needs revision so as to include $RR < 1$ (due to low-dose-induced protective effects).

**Low-Dose-Related Stochastic Processes**

In developing models for use in low-dose, radiation, and genotoxic chemical risk assessment for humans, one has to consider the related key stochastic processes involved:

- One key stochastic process is associated with the occurrence of genomic instability (Little 1985, 1999; Little *et al.* 1990; Mauder and Morgan 1993; Martins *et al.* 1993; Kennedy *et al.* 1996; Kadhim *et al.* 1996, 1998; Morgan *et al.* 1996; Mothersill and Seymour 1998b; Wright 1998).
- A second key stochastic process relates to the occurrence of deleterious bystander effects (Hei *et al.* 1997; Mothersill and Seymour 1997, 1998a; Azzam *et al.* 1998; Prise *et al.* 1998, 2002; Lyng *et al.* 2000; Seymour and Mothersill 2000; Brenner *et al.* 2001; Goldberg and Lehnert 2002; Iyler and Lehnert 2002a,b; Little *et al.* 2002; Nagasawa *et al*. 2002).

- A third key stochastic process relates to the occurrence of a protective bystander effect, thought to be mediated via apoptosis (Bauer 1996; Barcellos-Hoff 2001;Barcellos-Hoff and Brooks 2001; Belyakov *et al.* 2001a,b, 2002a,b,c, 2003; Scott *et al.* 2003).
- A fourth key stochastic process relates to the occurrence of problematic mutations (Kent *et al.* 1994; Kiefer *et al.* 1999; Tates *et al.* 1999; Scott *et al.* 2003).
- A fifth key stochastic process relates to the occurrence of neoplastic transformation (and early step in cancer occurrence) (Little 1985; Azzam *et al.* 1994, 1996; Scott 1997; Redpath *et al.* 1998, 2001; Medonca *et al.* 1999; Schöllnberger *et al.* 2001b; Scott *et al.* 2003).

- The overall stochastic process of usual interest, which is cancer (Armitage and Doll 1954; Barrett and Wiseman 1987; Portier 1987; Thorslund *et al.* 1987; Portier *et al.* 1990; Tan 1991; Moolgavkar *et al.* 1993; Portier and Sherman 1994; Luebeck *et al.* 1996; Hoel and Li 1998; Trott and Roseman 2000; Mebust *et al.* 2002) involves the general stages of initiation, promotion, and progression and relates to the other key stochastic processes indicated.

In modeling these key stochastic processes, it is very important to account for repair and misrepair of DNA damage (Friedberg *et al.* 1995; Stewart 1999; Thompson and Schild 1999, 2001; Hanawalt 2001; Leskov *et al.* 2001a,b; Jeggo 2002; Plotkin and Nowak 2002). Induced adaptation could also be important *in vivo* (Mitchel 1995; Mitchel *et al.* 1997; Stecca and Gerber 1998; Belyakov *et al.* 2002b).

**Deleterious Bystander Effects**

In modeling cancer induction by low-dose radiation and genotoxic chemicals, one has to account for both protective and deleterious bystander effects (Scott *et al.* 2003). Past focus has been on the deleterious bystander effects, with very little attention to the very important protective effects.

Deleterious bystander effects whereby unirradiated cells are damaged have been examined in two general types of cellular systems. In the first, monolayer cultures have been exposed to very low fluences (particles/unit area/unit time) of alpha particles either from an external source (Azzam *et al.* 1998; Little *et al.* 2002; Nagasawa *et al.* 2002) or focused microbeam (Hei *et al.* 1997; Prise *et al.* 1998). The second technique involves harvesting medium from irradiated cells and incubating it with unnirradiated cells (Mothersill and Seymour 1997; Lyng *et al.* 2000). Both techniques have demonstrated that cells not being irradiated can still be damaged. Further, the bystander effect does not arise from simply irradiating media. Cell damage and intercellular signaling are essential (Mothersill and Seymour 1997, 1998a).

**Protective Bystander Effects**

Evidence is now strong that death via apoptosis at low radiation doses can occur via a bystander mechanism (Mothersill and Seymour 1998a,b; Lyng *et al.* 2000; Belyakov *et al.* 2001a,b, 2002a,b,c, 2003; Prise *et al.* 2002). Barcellos-Hoff and Brooks (2001) point out that bystander effects *in vivo* after low doses of radiation are extracellular signaling pathways that modulate both cellular repair and death programs. The authors also indicate that transforming growth factor β is an extracellular sensor of damage. They further indicate that extracellular signaling relevant to carcinogenesis in normal tissue can eliminate abnormal cells or suppress neoplastic behavior.

Dr. C.-R. Yang and colleagues (Yang *et al.* 2000a,b; Davis *et al.* 2001) at Case Western University have reported clusterin [CLU, a.k.a. TRPM-2, SGP-2, or radiation-induced protein-8 (XIP8)] to be implicated in selective removal of problematic cells via apoptosis. Their key finding was that enhanced expression and accumulation of nuclear CLU/XIP8-Ku70/Ku80 complexes appear to be an important cell death signal after irradiation. Further, their data suggest that CLU/XIP8 may play an important role in monitoring cells with genomic instability and/or infidelity (e.g., created through translesion DNA synthesis), by facilitating removal of genetically unstable cells as well as severely damaged cells.

It is now also known from in vitro studies of viral-induced neoplastic transformation (Bauer 1996) that:

- Bystander transformed cells (i.e., existing problematic cells) are selectively killed via apoptosis.
- Cytokines and reactive oxygen produced by nontransformed neighboring cells trigger apoptosis in bystander transformed cells (protective bystander effect called group adaptation [Scott *et al.* 2003]).
- Transforming Growth Factor β1 enables nontransformed cells to trigger apoptosis among transformed cells.

The indicated research findings support the view by some scientists that the bystander apoptosis effect serves to rid tissue of problematic cells (e.g., mutants, neoplastically transformed cells, badly damaged cells) and therefore protect from cancer induction.

It is now recognized that radiation-induced cell signaling can trigger cell differentiation (including bystander cells). The current view is that some problematic cells (including bystander cells) may be triggered to undergo differentiation and this could protect the cell community from the emergence of new adverse stochastic effects such as neoplastic transformation and cancer (Belyakov *et al.* 2002b).

Thus, in modeling low-dose, radiation-induced, stochastic effects such as problematic nonlethal mutations, neoplastic transformation, and cancer, one has to account for both deleterious and protective effects. This was done in developing the NEOTRANS$_2$ model for low-dose, radiation-induced, stochastic effects (Scott *et al.* 2003). The current form of the model is briefly described in the section that follows.

## NEOTRANS$_2$ MODEL

### Low-Dose Radiation

In our earlier research, we introduced models that relate neoplastic transformation potential to genomic instability status of cells. The models were given the general name "genomic instability state" (GIST) models (Scott 1997; Scott *et al.* 2001). The expression genomic instability state refers to any spontaneous or toxicant-induced instability in the genome, including any initial transient instability, as well as any persistent instability that can be passed to cell progeny. Our current GIST model for characterizing stochastic effects of low-dose radiations is called NEOTRANS$_2$. In addition to a stable (ST) genome for resistant cells, the NEOTRANS$_2$ involves three types of genomic instability considered to be important among hypersensitive cells that respond to low radiation doses: (1) Normal-minor instability (NMI), associated with normal cell function and normal genome status; (2) Transient-problematic instability (TPI), associated with genomic damage that may sometimes be fully repaired but can be misrepaired; and (3) Persistent-problematic instability (PPI), which arises from misrepair that yields nonlethal mutations. Thus, PPI can be passed to progeny, increasing their potential for neoplastic transformation.

With NEOTRANS$_2$, mainly hypersensitive cells respond after very low radiation doses (e.g., 0–100 mGy of gamma rays). Radiation-associated transitions among hypersensitive cells in the NEOTRANS$_2$ are summarized in Figure 1.

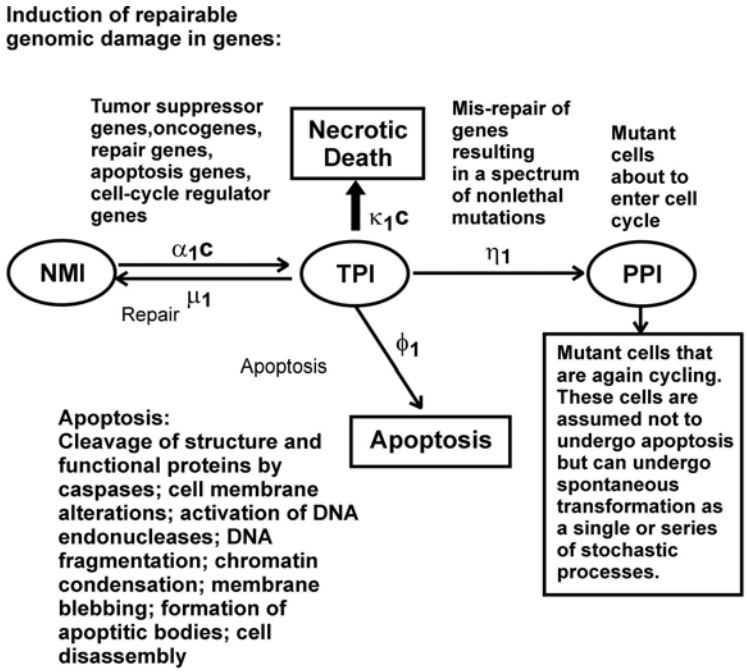


Figure 1: NEOTRANS$_2$ model transitions for hypersensitive cells that respond to low-dose radiation. Genomic instability states NMI, TPI, and PPI are explained in the text (Scott *et al.* 2003).

With the NEOTRANS$_2$ model, a very small fraction, $T_0 << 1$ (not shown in Figure 1) of the cell population is presumed to have already undergone neoplastic transformation over their life history. Only hypersensitive cells in the high vulnerability state PPI (viable mutants) can produce neoplastically transformed progeny.

Target genes for the hypersensitive cells (Figure 1) include tumor suppressor genes, oncogenes, repair genes, apoptosis genes, and cell-cycle regulator genes. With NEOTRANS$_2$, two modes of cell death are considered: apoptosis (assumed to predominate at very low doses) and necrotic death (assumed important only at moderate and high doses). Nonlethal mutations are assumed to arise via misrepair. Lethal mutations are assigned to the apoptosis pathway.

The NEOTRANS$_2$ model presented in Figure 1 applies only to low radiation doses and to the hypersensitive sub-fraction, $f_1$, of cells at risk (excluding cells already transformed). The model parameter $\alpha_1$, when multiplied by the dose rate, accounts for low-dose induced genomic damage among the hypersensitive cells in the population. Thus, damage induction is dose-rate dependent. The parameter $\alpha_1$ is comprised of two parts: (1) one part relates to direct damage to DNA; (2) the other part relates to indirect damage to DNA and includes deleterious bystander effects.

The parameter $\mu_1$ governs the rate of commitment of damaged hypersensitive cells to the error-free repair pathway. The corresponding parameter for the misrepair pathway is $\eta_1$. Misrepair leads to a variety of viable mutations (PPI cells). The parameters $\phi_1$ govern the rate of

commitment of newly damaged cells (including lethal mutations) to the apoptotic pathway. The parameters $\kappa_1$ (important only for moderate and high doses) when multiplied by dose rate govern the rate at which damaged hypersensitive cells enter the necrotic death pathway.

Typical units for $\alpha_1$ and $\kappa_1$ are $mGy^{-1}$. Typical units for $\mu_1$, $\eta_1$, and $\phi_1$ are $min^{-1}$. The parameter $f_1$ is dimensionless. These parameters are stochastic (i.e., have distributions) but currently are not time-dependent.

Analytical solutions for the NEOTRANS$_2$ model that apply to *in vitro* data for very low radiation doses, $\Delta D$, were developed elsewhere (Scott *et al.* 2003). The steady-state solution for neoplastic transformation frequency (per surviving cell) after a small dose $\Delta D$ is given by the following:

$$TFSC(\Delta D) = T_0 \text{ , for } \Delta D = 0,$$
$$TFSC(\Delta D) = (1-f_0)T_0 + [(1-T_0)]k\Delta D, \text{ for } \Delta D > 0, \quad (1)$$

where

$$k_T = f_1\alpha_1\eta_1\Omega/(\mu_1 + \eta_1 + \phi_1). \quad (2)$$

The parameter $\Omega$ is the probability that a cell with induced PPI will produce neoplastically transformed progeny at some point during the follow-up period of interest. The term $f_0$ is the fraction of the spontaneous transformants $T_0$ removed via the radiation-induced protective bystander apoptosis effect. It has been given the special name protection factor (PROFAC). Induced intracellular signaling is assumed important for the protective effect. Presently, the $f_0$ is assumed to be non-zero only during radiation-induced signaling to bystander cells. The parameter $\phi_1$ accounts for removal via apoptosis of cells with radiation-induced TPI. The threshold dose (stochastic and called *StoThresh* [Scott *et al.* 2003]) for excess neoplastic transformants is given by

$$D_{Th} = f_0T_0/[(1-T_0)k_T]. \quad (3)$$

For very low doses of low-LET radiation, the predominate term in Equation 1 is $(1-f_0)T_0$ for $\Delta D > 0$ (Scott *et al.* 2003). This leads to an initial drop in the dose-response relationship from $T_0$ down to $(1-f_0)T_0$. For low-LET, gamma-ray doses in the range >0–100 mGy, the dose-response curve appears independent of dose remaining at the value $(1-f_0)T_0$ (Scott *et al.* 2003). For this range, the *RR* for neoplastic transformation is approximately $(1-f_0)$ for all doses. This occurs only because gamma rays are not very effective in producing new transformants (e.g., as compared to alpha particles). Large doses of gamma rays are needed to produce lots of transformants.

This is shown in Figures 2 and 3 where *RR* is presented for gamma-ray-induced neoplastic transformation of C3H 10T1/2 cells based on data of Azzam *et al.* (1996) and for transformation of HeLa x skin fibroblast human hybrid cells based on data of Redpath *et al.* (2001). For both data sets, there is pronounced protection against spontaneous transformations associated with the gamma-ray exposure.

Similar results have been observed in *in-vitro* experiments on x-ray-induced mutations in mouse lung cells (T273; subclone of C10 cells), based on experiments conducted by one of this papers authors (D. Walker [DW]). DW adapted the *Hprt* assay developed by Dr. R. Albertini *et al.* (1982) and modified by Driscoll *et al.* (1995) for use with lung epithelial cells. The effect of low-dose x rays on the *in vitro* induction of *Hprt* mutations and presumptive mutations in the mismatch repair (MMR) and apoptosis gene (Apop) systems (MMR/Apop gene systems) in mouse alveolar type II cells was investigated.

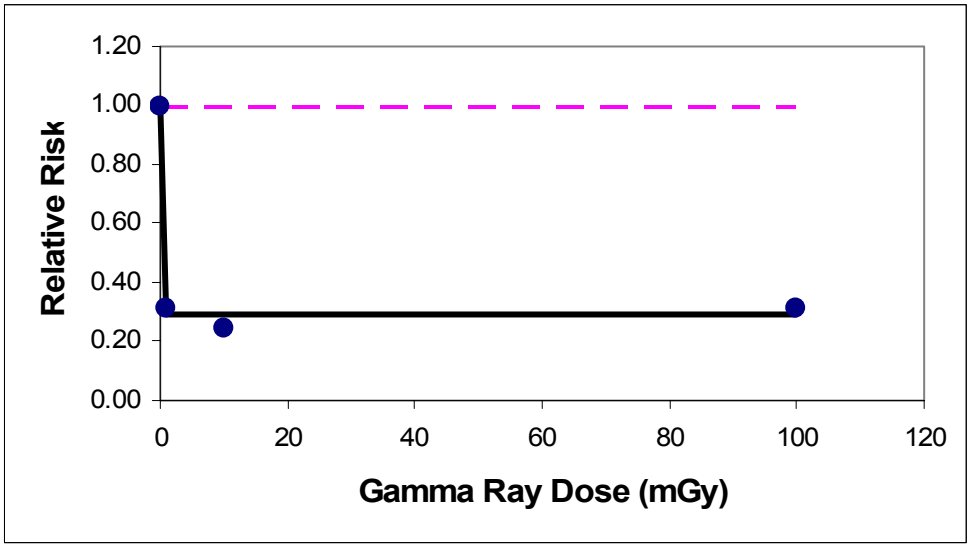


Figure 2: Relative risk for gamma-ray induced neoplastic transformation of C3H 10T1/2 cells based on data of Azzam *et al.* (1996). The data were fitted with Equation 4. The parameter $f_0$ (which equals $PROFAC_L$) was previously estimated to be 0.71 ± 0.04 (Scott *et al.* 2003).

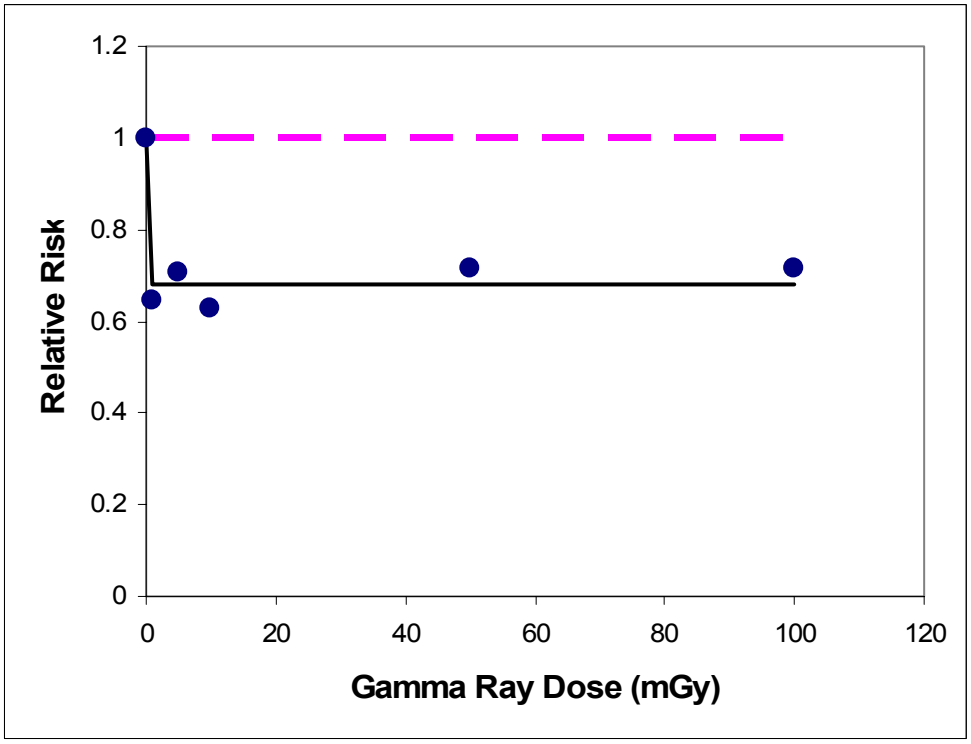


Figure 3. Relative risk for gamma-ray induced neoplastic transformation of HeLa x skin fibroblast human hybrid cells based on data of Redpath *et al.* (2001). The data were fitted with Equation 4. The parameter $f_0$ (which equals $PROFAC_L$) was previously estimated to be 0.32 ± 0.04 (Scott *et al.* 2003).

The C10 cells have two p53 alleles. However, the p53 protein has little to no activity. The T273 subclone used for these studies (preliminary) has a relatively high spontaneous mutation frequency, a wild type *Hprt* gene, and is sensitive to the cytotoxic effects of 6-thioguanine. The high sensitivity to cell loss was interpreted to confirm the presence of functional MMR/Apop gene systems.

The T273 cells were exposed to 0, 100, or 1000 mGy of X rays during log-phase growth. After exposure, the cells were grown for 2 weeks to allow phenotypic expression of treatment-related changes. The cells were then assayed for focus-forming mutations (transformed cells), mutations in the *Hprt* gene, and mutations in genes of the MMR/Apop systems, using a modification of the T-cell mutation assay. Results (*RR*) for the 0 and 100 mGy dose groups (dose-range of interest for this paper) are presented in Table 1.

Table 1. Mutation frequency and associated relative risks for low-dose, x-ray-induced mutations among mouse lung cells (T273) exposed *in vitro*.

| | 0 mGy | 100 mGy | Relative Risk |
|---|---|---|---|
| *Hprt* | $(18.9 \pm 8.5)\times10^{-6}$ | $(11.0 \pm 7.0)\times10^{-6}$ | $0.58 \pm 0.45$ |
| MMR/Apop | $(37.9 \pm 13)\times10^{-6}$ | 0 | 0 |
| Focus-forming (transformed) | $(9.6 \pm 2.6)\times10^{-6}$ | 0 | 0 |
| Plating efficiency | $42.4 \pm 7.8\%$ | $36.5 \pm 7.5\%$ | |

Note the very dramatic protection afforded by the 100 mGy dose against the highly problematic mutations (MMR/Apop and focus-forming) in contrast to the modest protection suggested against the less problematic *Hprt* mutations; 100% protection was indicated against the problematic mutations. However, the sensitivity of the assay was not sufficient for detecting extremely small frequencies of mutations. For the *Hprt* mutations, *RR* was not significantly less than one, although the data are consistent with a possible small decrease in *RR* with exposure to 100 mGy x rays. These results suggest that an elaborate system of DNA damage detection and problematic cell mitigation may be operating with highly problematic cells efficiently recognized and removed when specific cell signaling associated with low-LET radiation-induced damage is turned on. As already indicated, there is growing evidence for such a signaling system (Bauer 1996; Barcellos-Hoff and Brooks 2001; Belyakov *et al*. 2002a,b; Davis *et al.* 2001; Yang *et al.* 2000a,b). Less problematic cells (e.g., *Hprt* mutants) may be less efficiently recognized or may be considered by homeostatic mechanisms as posing little threat to the cellular community. If so, then *RR* based on MMR/Apop system mutations and focus-forming (transformed) cells is likely to be more relevant to cancer risk assessment than is *RR* based on *Hprt* mutations. If so, the *Hprt* mutations would be a better marker of biologically relevant dose, while MMR/Apop gene system mutations would be a better marker of biological effect.

Our views about possible mechanisms that would explain the differential levels of radiation-induced protection for the different mutation type are briefly stated as follows. The *Hprt* gene in both humans and rodents is X-linked. Thus, target cells for mutation induction at this locus have only one functional copy of the gene (in females, one allele is silenced, effectively rendering the *Hprt* gene hemizygous). Genes identified in the MMR and apoptosis systems as probable targets of mutational events associated with the assay used are autosomal; thus, each cell has two copies of each gene. If one assumes that mutations can be induced in a cell at any phase of the cell cycle, and using the example of a single point mutation in a single gene of the MMR system, such a mutation would result in a local region of mismatch (discontinuity) between the two alleles. This mismatch should stimulate DNA damage recognition processes. However, even error-free repair could not remove preexisting mutant cells, where both alleles contained the same mutation (autosomal genes) or where one mutated gene was present (X-linked genes).

Among the control group, approximately 40 out 1 million cells had mutations in the MMR/Apop gene systems. Thus, it is considered highly plausible that a significant proportion of these 40 mutants were already present at the start of the irradiation. That none was detected after irradiation suggests that apoptosis was a key player in the protection process. Thus, the internally generated signaling associated with recognition of the discontinuity between two alleles, where one bears a mutation, may have resulted in stimulation of apoptosis and removal of the problematic cells from the population. Such signaling would be continuous throughout all phases of the cell cycle in the case of mutated autosomal genes, but limited (i.e., reduced signaling time) to the specific phase of the cell cycle during cell replication where two gene copies are present for X-linked genes. This difference in signaling duration may account for observed differences in the degree of radiation-induced protection against mutations.

Although we have explained the low-dose protection as being due to apoptosis (consistent with the NEOTRANS$_2$ model), we cannot not rule out the possibility that at least some protection may be associated with induced DNA repair. Actually the NEOTRANS$_2$ model incorporates both modes of protection (via repair parameter $\mu$ and apoptosis parameter $\phi$). However, additional studies are needed to resolve the mechanistic basis for the low-dose induced protection.

For high-LET alpha irradiation, there appears to be essentially no induced protection (i.e., $f_0 = 0$) against spontaneous transformants, or the range of doses over which the protection occurs is too small to be detected from the available data. This is shown in Figure 4 where *RR* for neoplastic transformation among C3H 10T1/2 cells appears to increase in accordance to the LNT model, based on data of Bettega *et al.* (1992). We speculate that for alpha irradiation (and possibly other high-LET radiation sources such as heavy ions encountered in space) the deleterious bystander effect predominates over the protective bystander effect. Because of the short range of alpha particles in tissue (only a few cell traversals), alpha radiation would be expected to be much less efficient in triggering widespread cell signaling related to the protective bystander effect.

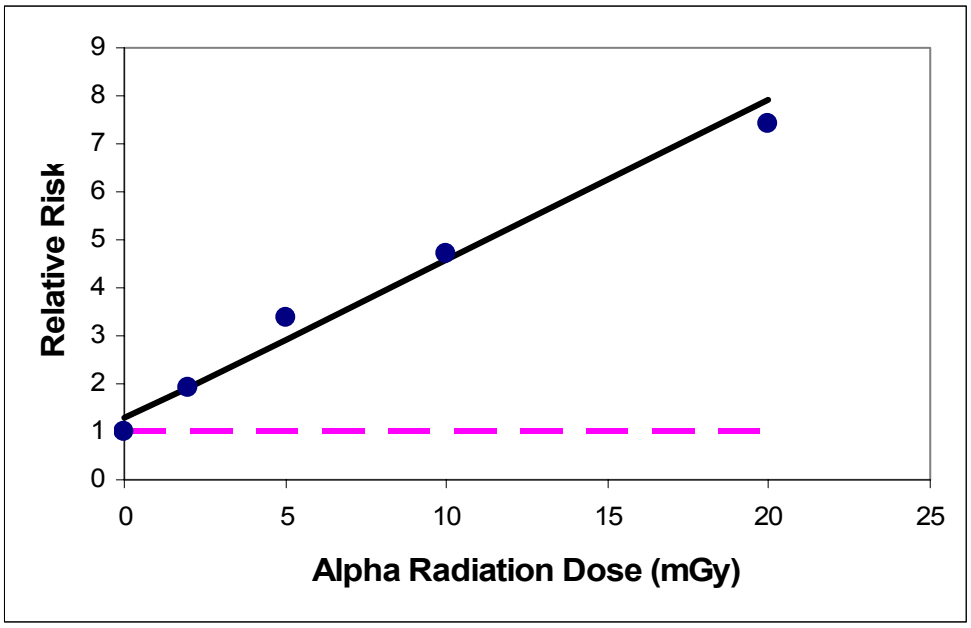


Figure 4. Relative risk for 4.3-MeV, alpha-particle-induced neoplastic transformation among C3H 10T1/2 cells based on data of Bettega *et al.* (1992). Data are consistent with the LNT hypothesis.

**Adapting NEOTRANS$_2$ Model for Application to Prototypic Chemical**

We have now adapted the NEOTRANS$_2$ model to be applicable to mutation induction *in vivo* (in T lymphocytes in mice) by inhaled EO (prototypic DNA-alkylating agent). The adapted model is called NEOTRANS$_2$-EO, and the EO concentration was used as dose (corresponds to variable c in the NEOTRANS$_2$ model, when the exposure time is fixed). Details are described in a separate paper (Walker *et al.* 2003) along with evidence for nonlinearity in the dose-response curve for EO-induced DNA adducts in B6C3F1 mice and F344 rats.

EO is an immediate metabolite of ethylene, a normal body constituent (Walker *et al.* 1990). It is classified as a Group 1 human carcinogen by the International Agency for Research on Cancer, based on sufficient evidence in animals with strong evidence in humans of relevant mechanisms for carcinogenicity (IARC 1994). EO has caused dose-related increases in the incidence of mononuclear cell leukemias, gliomas, and peritoneal mesotheliomas in F344 rats and lymphomas and tumors of the uterus, lung, Harderian gland, and mammary gland of B6C3F1 mice (EPA 1990; IARC 1994; Their and Bolt 2000). However, there is little information on health risk to humans from exposure to very low doses of EO.

Historically, risk assessment for genotoxic chemical carcinogens has been based on the assumption that any exposure carries a cancer risk no matter how small the dose (Butterworth and Bogdanffy 1999). Using data for EO-induced mutations in T lymphocytes of B6CF31 mice exposed via inhalation, we have shown evidence against the validity of the LNT model so far as its application to low-dose-induced mutations *in vivo* (Walker *et al*. 2003).

Briefly summarizing, two data sets were used to obtain the results presented in Figures 5 and 6 for mutation induction in B6C3F1 mice exposed via inhalation for 4 weeks (6 h/day, 5days/week) to ethylene or EO (Walker *et al.* 2003). The ethylene-exposed mice had calculated

equivalent doses of EO of 0.7, 4.4, and 8.6 ppm. For mice directly exposed to EO, the exposure concentrations were 50, 100, and 200 ppm. To adequately characterize the dose-response data in Figures 5 and 6, it was necessary to postulate a threshold EO exposure concentration $C_1$* (2.3 ± 1 ppm) for turning on the protective bystander apoptosis effect [estimated via Bayesian inference methods (Walker *et al.* 2003)]. Below this threshold, only error-free repair was assumed ($\mu_1 > 0$, $\eta_1 = 0$). This lead to a flat dose-response from 0 to the threshold EO concentration $C_1$* (Figure 6; blow up of low-dose region). Above this threshold concentration, misrepair was presumed to occur ($\eta_1 > 0$) in competition with error-free repair ($\mu_1 > 0$), leading to newly induced mutations. The mutation frequency at 4.4 ppm EO equivalence was significantly different from the spontaneous frequency [p = 0.009, Mann-Whit U-statistics (Walker *et al.* 2003)] and clearly below it, indicating presumed protection against spontaneous mutations. A second threshold $C_2$* (17 ± 11 ppm) is where the curve first increases above the spontaneous frequency (i.e., threshold for excess mutations; Figure 6). The indicated departure from the LNT model was demonstrated to be statistically significant (Walker *et al*. 2003).

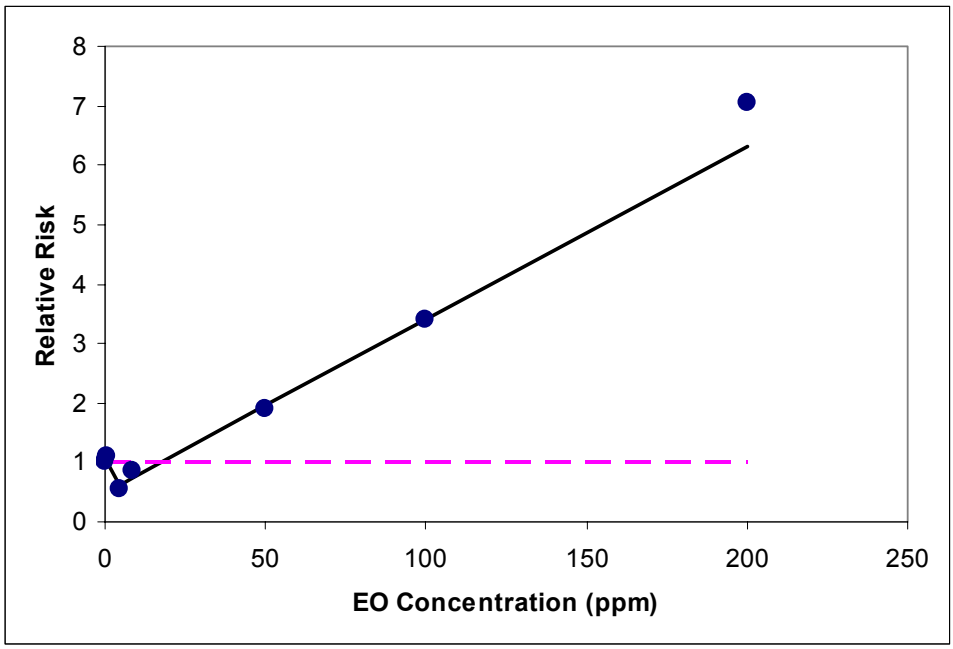


Figure 5. Relative risk for ethylene-oxide (metabolite of ethylene) induced Hprt mutations in T lymphocytes of B6C3F1 mice exposed via inhalation to ethylene (with associated low doses of EO: 0, 0.7, 4.4, or 8.6 ppm or high doses of EO: 50, 100, or 200 ppm) based on application of the NEOTRANS$_2$-EO model to data. Poisson regression implemented via Bayesian inference was used to fit the model to the data (Walker *et al.* 2003).

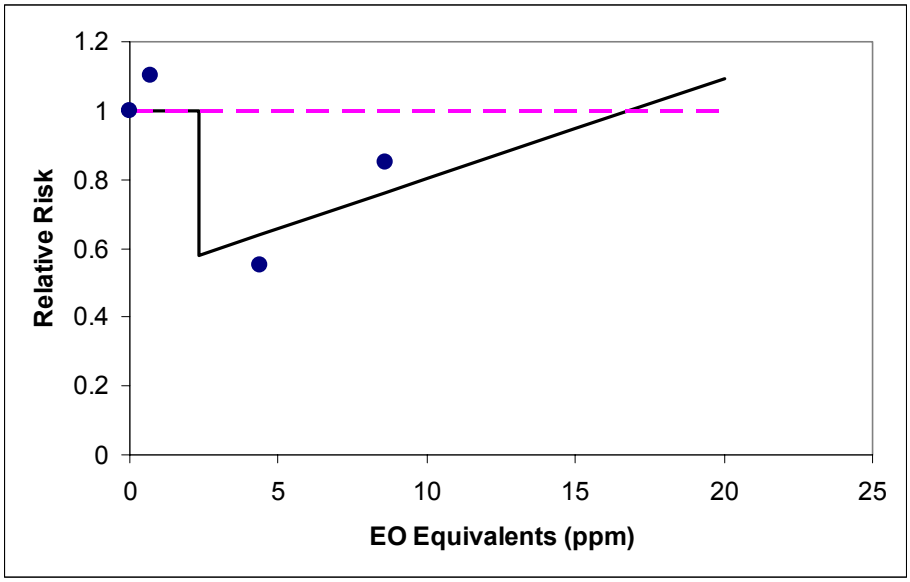


Figure 6. Low-dose portion of relative risk curve in Figure 5 for EO-induced Hprt mutations in T lymphocytes of B6C3F1 mice.

## APPLICATIONS TO RISK ASSESMENT FOR HUMANS

### Similarity in Relative Risk for Transformation and Cancer

Dr. Redpath and colleagues (2001) have shown that the dose-response relationship for the *RR* for low-dose, radiation-induced neoplastic transformation *in vitro* has a similar shape as for the *RR* for cancer induction in humans. This implies that dose-response functions for *RR* for neoplastic transformation (and possibly for problematic mutations) could be fitted to data for *RR* for cancer induction in humans yielding more reliable characterization of *RR* at low doses. We assume this to be true for a single radiation, for combined exposure to different radiations, for combined exposure to radiation and genotoxic chemicals, and for combined exposure to different genotoxic chemicals. We provide results for combined exposure to high-LET alpha and low-LET gamma radiations that support our assumption.

### *RR* for Cancer Induction by Low-Dose Gamma Rays

We use gamma rays to be representative of low-LET (L) radiation in general. For doses of low-LET gamma (or X rays) in the range 0–100 mGy, *RR* for cancer is therefore modeled as being fixed a value ($1\text{-}PROFAC_L$), where $PROFAC_L$ has replaced $f_0$ and indicates a protection factor against spontaneously occurring cancer. The dose-response curve for *RR* for cancer induction in humans by low-LET gamma (or X-ray) irradiation could then be characterized using

$$RR_L = 1\text{-}PROFAC_L, \tag{4}$$

for $0 < \Delta D_L \leq 100\ mGy$, based on results obtained for neoplastic transformation (assuming dose-response relationships for *RR* have similar shapes for both neoplastic transformation and cancer).

The equation applies to both *in vitro* data for neoplastic transformation and epidemiological data for cancer incidence or mortality. The subscript "L" is used to indicate low-LET radiation.

For chronic, low-dose-rate exposure to low-LET radiation, the range of doses over which Equation 4 applies may be greatly extended (due to prolongation of damage-related signaling associated with the protective bystander effect) possibly to doses in excess of 1000 mGy (1 Gy). This speculation is consistent with observations of lung cancer after chronic photon irradiation of humans (Rossi and Zaider 1997) where protracted x-ray or gamma radiation doses up to 2000 mGy (2 Gy) did not cause excess cancers and appear to have protected against spontaneous cancer.

For high-dose-rate exposure to doses > 100 mGy but < about 500 mGy, the following equation is recommended based on our previous results (Scott *et al*. 2003) when Equation 1 was fitted to data of Redpath *et al.* (2001) over the dose range >0 – 500 mGy:

$$RR_L = 1\text{-}PROFAC_L + K_L \Delta D_L, \tag{5}$$

where $K_L$ is a constant (slope parameter) that depends on the type of cancer and possibly on other factors such as age and health status. The slope parameter, $K_L$, represents the added *RR* per unit of dose of low-LET radiation and is assumed quite small in comparison to what would be expected for high-LET radiations such as alpha particles and heavy ions encountered in space travel.

### *RR* for Cancer Induction by Low-Dose Alpha Radiation

We use alpha radiation to be representative of high-LET (H) radiations in general. The corresponding equation for exposure to only high-LET alpha radiation is:

$$RR_H = 1 + K_H \Delta D_H, \tag{6}$$

where the subscript "H" is used to indicate high-LET radiation. The slope parameter $K_H$ gives the added *RR* per unit of dose of the high-LET radiation. Here, it has been assumed that no significant induced protection against spontaneous cancers is associated with the high-LET alpha radiation dose as illustrated in Figure 4. Equation 6 may not apply to *in vivo* exposure to neutrons, because neutrons produce gamma rays when interacting with body tissue of large mammals. This is especially true for humans who have large body masses. The gamma rays could trigger the protective bystander effects *in vivo* after exposure to low doses of neutrons. Bremsstrahlung radiation (e.g., encountered in space vehicles from the interaction of electrons with vehicle shielding material) could trigger the protective bystander effect. If so, the equation presented in the following section for the RR would be expected to apply to combined high- and low-LET irradiations.

### *RR* for Combined Exposure to Low- and High-LET Radiations

For combined chronic low-dose-rate exposure to gamma (or x rays) and alpha radiations, the appropriate equation, assuming independent action (Scott 1984, 1986; Scott *et al.* 1990; Burlkart *et al.* 1997) of the low- and high-LET radiations at low doses, is given by

$$RR_{L,H} = 1\text{-}PROFAC_L + K_H \Delta D_H. \tag{7}$$

Equation 7 may also apply to neutron-induced cancer (at low doses) *in vivo*. Equation 7 should also apply to mixed high- and low-LET radiation fields encountered in space travel by astronauts, so long as doses are reasonably low. In a similar manner, *RR* relationships could be constructed for combined exposure to radiation and genotoxic chemicals and for combined exposure to different genotoxic chemicals.

**Applications to Epidemiological Data**

Equation 7 has been applied to data (Khokhryakov *et al.* 1996) for *RR* for lung cancer in humans (Mayak workers) after chronically exposed over years to alpha plus gamma radiations. The alpha irradiation arose from repeatedly inhaling plutonium-239 (Pu-239) in association with the production of weapons-grade plutonium (Pu). The production facility is called the Mayak Production Association. Starting in the late 1940s, workers were chronically exposed to alpha and gamma radiations (Tokarskaya *et al.* 1995, 1997, 2002). Gamma-ray doses were likely much higher than 100 mGy but were generally delivered at low rates so that Equation 7 is assumed here to still apply. Gamma-ray doses were not reported by Khokhryakov *et al*. (1996), but it is known that some were as high as several Gy (Tokarskaya *et al.* 2002). Results obtained (hormetic-type dose response) are presented in Figure 7 for alpha radiation doses from 0 to tens of thousands of mGy. Initially, the slope parameter $K_H$ was found to be 0.01527 ± 0.00091 $mGy^{-1}$, with the intercept $(1\text{-}PROFAC_L)$ not significantly different from zero (100% protection against spontaneous cancers). The data were refitted with a zero intercept ($PROFAC_L = 1$) yielding essentially the estimate for $K_H$ (i.e., 0.0153 ± 0.0005 $mGy^{-1}$).

Equation 6 was also fitted to the Mayak worker data for high doses (1600, 6400, and 16000 $mGy^{-1}$) where risk was clearly elevated (Figure 8). Figure 9 compares results based on Equation 7 to those obtained with extrapolating from high doses to low doses using the LNT model. Note that the chronic gamma irradiation appears to have protected against essentially all spontaneous lung cancers ($PROFAC_L$=1). Note also that the LNT model extrapolation from high to low doses leads to phantom excess risk based on the data of Khokhryakov *et al.* (1996)! The controls (zero-dose group) were based on national cancer statistics for the Russian population (Khokhryakov *et al.* 1996), to which some researchers have objected (Kreisheimer *et al.* 2000).

Kreisheimer *et al.* (2000) did not find evidence for low-dose-induced protection in their study of lung cancer among Mayak workers but rather found the LNT model to describe risk adequately. However, we think this is because of their choice of controls. In their study, the baseline lung cancer mortality rate was not taken from national statistics but was derived from the cohort of Mayak workers (all of whom were likely irradiated). Even family members residing in of the city of Ozyorsk (where the workers resided), who had no association with the Mayak facility, were not free of irradiation. Thus, it is likely that there were no unirradiated members of the Mayak worker population present during its early years of existence. Workers exposed to low-level gamma radiations (or possibly beta radiation from releases to air of beta-emitting radionuclides from the Mayak facility) could have had induced protection against spontaneous cancers.

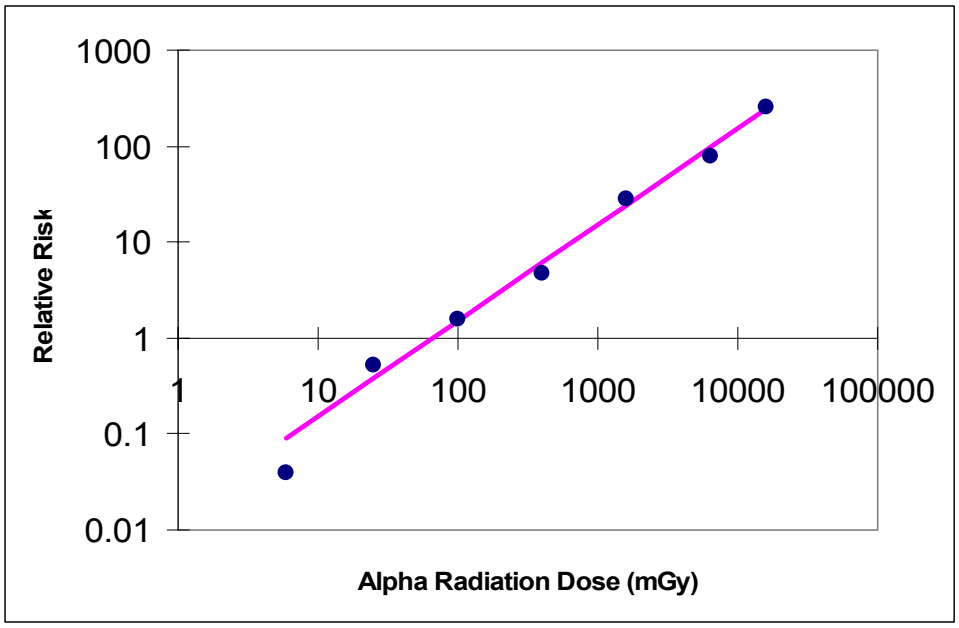


Figure 7. Relative risk for lung cancer induction in humans (Mayak workers) chronically exposed over years to alpha plus gamma radiations. The data points are estimates of relative risk based on published cancer incidence data(Khokhryakov *et al.* 1996). The smooth curve is based on fitting Equation 7 to the *RR* data by linear regression (with the zero dose group excluded).

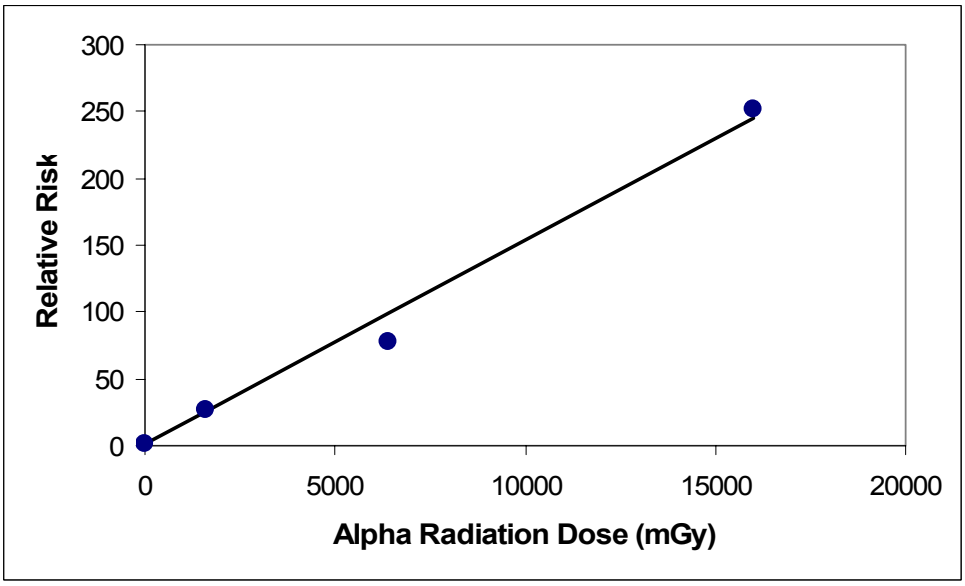


Figure 8. Relative risk for lung cancer induction in humans (Mayak workers) chronically exposed to alpha plus gamma radiations. Here only high-dose data are used in conjunction with the LNT model.

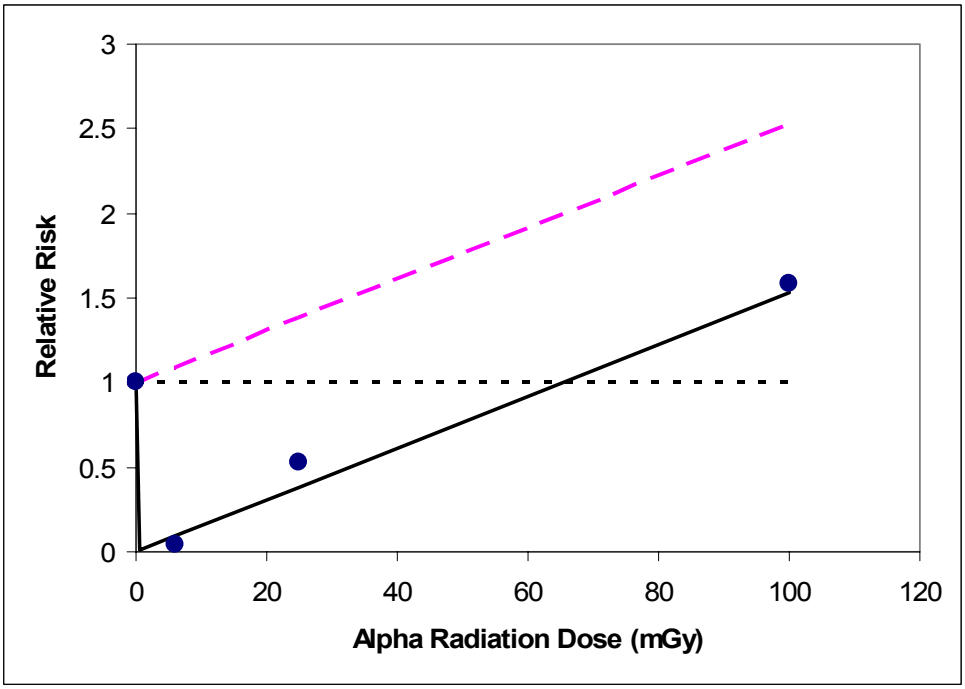


Figure 9. A comparison of the low-dose portions of Figures 7 and 8. The upper dashed curve is relative risk based on the LNT model extrapolated to low doses. The hormetic-type curve is based on Equation 7 fitted to all the presented data in Figure 7.

**Other Evidence against the LNT Model**

A hormetic-type dose-response relationship for lung cancer induction is not unique to Mayak workers. Rossi and Zaider (1997) critically reviewed the literature on radiogenic lung cancer and concluded that "at radiation doses generally of concern in radiation protection (< 2 Gy), protracted exposure to low LET radiation (x- or γ-rays) does not appear to cause lung cancer. There is in fact, indication of a reduction of the natural incidence."

With such hormetic-type, dose-response relationships, there is a threshold for "excess cancers!" Results of earlier and recent case-control studies of lung, liver, and biliary tract cancer among Mayak workers are consistent with large thresholds for excess cancers for combined alpha and gamma irradiation (Tokarskaya *et al.* 1995, 1997, 2002, 2003).

The recent Hanford Thyroid Disease Study did not find evidence of any excess risk for thyroid cancer induction for persons living in the vicinity of the Hanford facility who were exposed to beta radiation from radioactive iodine released from the facility (USDHHS 2002). For doses in the range of 0–100 mGy, risk was not correlated with dose and was less than for the control group based on persons outside what was considered the irradiation zone. In addition, for several health effects, the mean slope of the risk vs. dose relationship was negative (indicating a possible hormetic-type dose-response relationship).

Animal data are also consistent with thresholds for cancer induction by low-dose-rate, low-LET radiation (Yamamato *et al.* 1998; Kondo 1999; Tanooka 2000; Yamamato and Seyama 2000).

## CONCLUSIONS

Currently, in health risk assessment for combined exposure to low doses of radiation and genotoxic chemicals, one generally assumes either additive or synergistic interactions. However, results presented here suggest two points: low-dose radiation could protect from both spontaneous and chemical-induced stochastic effects (problematic mutations, neoplastic transformation, and cancer); and chemicals that trigger apoptosis signaling might also protect from both spontaneous and radiation-induced effects.

The current *RR* paradigm where *RR* is always $\geq 1$ no matter how small the dose cannot be considered valid in light of the research results described here. This paradigm is used in establishing human exposure standards for both radiation and genotoxic chemicals and in evaluating possible harm to humans from environmental and workplace exposure to these agents. It appears that the continued use of a risk assessment paradigm for which $RR \geq 1$ could cause more harm than benefit to society by spreading unwarranted fear about phantom excess risks associated with low-level exposure to low-LET radiation or combinations of low- and high-LET radiations. Such phantom risk may also arise in risk assessment conducted for combined exposure to low doses of low-LET radiation and genotoxic chemicals or combinations of genotoxic chemicals when based on the LNT or other models that exclude $RR < 1$.

Our results for high-LET radiation are consistent with the LNT hypothesis but only where there is no low-LET component (e.g., gamma rays) to the total dose. For high-LET neutron sources, gamma rays arise (especially *in vivo*) for large mammals such as humans from their interaction with tissue. The gamma rays might provide some protection from low-dose-related stochastic effects via inducing the protective bystander apoptosis effect discussed here.

For astronauts exposed to combinations of high- and low-LET radiations in space exploration, one should consider the possibility that the low-LET component to their dose might induce some protection from spontaneous and high-LET-induced stochastic effects.

Persons living in high background radiation areas may benefit from unrecognized cancer risk reduction due to their radiation exposure.

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