

## Exposure to Chronic, Low-Dose Rate $\gamma$ -Radiation at Chernobyl Does Not Induce Point Mutations in Big Blue<sup>®</sup> Mice

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Empirical genetic effects resulting from low-dose rate irradiation and chronic, cumulative exposure are poorly characterized. Expected effects are based on epidemiological studies and downward, linear extrapolations from nonthreshold models derived from acute, high-dose exposures. These extrapolations and their associated risk coefficients have no experimental support, and because of their inherent uncertainty they are the subject of considerable debate. The expectation of deleterious genetic effects resulting from low-dose rate irradiation and chronic exposure is in need of empirical assessment because this type of exposure is typical of those encountered in occupational, residential, and environmental settings. Recent acute low-dose (<10 cGy) studies using cytogenetic and point mutation endpoints indicate that observed effects range from those lower than spontaneous to an increase in the frequency of

point mutations. Using the Big Blue<sup>®</sup> assay, we examined the ability of chronic, continuous  $\gamma$ -irradiation ( $2.3 \times 10^{-3}$  cGy/min) in the Chernobyl environment to induce point mutations. This system has demonstrated a significant point mutation sensitivity (4.5-fold increase) to acute, high-dose (1–3 Gy)  $\gamma$ -radiation. Mutant frequencies and the mutation spectra were examined in exposed and reference samples of Big Blue<sup>®</sup> mice following 90 days exposure (cumulative absorbed dose = 3 Gy) to the Chernobyl environment. No significant increase in the mutant frequency or bias in the mutational spectrum was observed in exposed individuals. This finding suggests that low-dose rate  $\gamma$ -irradiation at Chernobyl does not induce point mutations and that cumulative, chronically absorbed doses do not induce the same genetic effects as acute doses of the same magnitude. Environ. Mol. Mutagen. 42:11–18, 2003. © 2003 Wiley-Liss, Inc.

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### INTRODUCTION

The deleterious effects of exposure to high, acute doses of ionizing radiation are well documented [Auerbach, 1976]. In contrast, the effects of exposure to low doses are less well known and the effects of chronic, low-dose rate exposures are essentially untested. To accommodate this discrepancy, detrimental health effects (e.g., cancer) resulting from these low level and/or chronic exposures are extrapolated downward from models of high, acute exposure-induced effects in a simple, linear fashion. Two particularly noteworthy implications of this mathematical approach include the exclusion of a dose–response threshold and the expectation of equivalent effects regardless of the rate of dose administration. Although this provides the most conservative means by which to assign risk, these expectations are empirically unvalidated [Sinclair, 1998; Kondo, 1999; Kirsch-Volders et al., 2000].

Recent studies investigating the genetic effects of acute,

low-dose exposure, generally defined as <20 cGy, have revealed a variety of responses not typically observed in high, acute-dose studies. While acute, high-dose exposures induce a significant increase in easily detected chromosomal

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aberrations as well as point mutations, the spectrum of induced genetic effects resulting from acute, low doses does not include chromosomal aberrations. In fact, responses to these low doses have ranged from a significant increase in point mutations to a significant decrease in point mutations or an inverse dose–response relationship [Schwartz et al., 2000; Vilenchik and Knudson, 2000; Huo et al., 2001]. Fewer than expected neoplasia in cells acutely irradiated at low doses were found using neoplastic transformation as a biological endpoint [Redpath et al., 2001]. The validity of a nonthreshold linearity with respect to exposure and effects based on acute, low-dose research is questionable and actually appears to support the concept of an adaptive response [Wolff, 1996].

At present, biological effects resulting from chronic, low-dose rate exposures have not been experimentally investigated in the laboratory and risk assessment models do not attempt to account for this obvious temporal variable. Therefore, effects predicted from acute, high-dose exposures are expected to be the same for an equivalent cumulative exposure. For example, the biological effects resulting from an acute exposure of 1–3 Gy are expected following a chronic exposure of the same magnitude, even though the rate at which the dose is administered can be a small fraction of the acute administration [Eaton and Klaasen, 1996; Wickliffe et al., 2001]. Documentation and comparison of expected effects between acute and chronic exposures is particularly critical because absorbed radiation doses in occupational, residential, and environmental settings usually result from a chronic (continuous or fractionated) exposure administered at a low rate. Epidemiological studies on human exposures and environmental studies at Chernobyl represent some of the research directed at the questions regarding acute vs. chronic exposures and the effect of protracted low-level exposures. The body of research from Chernobyl has failed to unequivocally support the concept of effect equality between acute and chronic exposures [Baker et al., 1996, 1997, 1999a,b, 2001; Dubrova et al., 1996, 1997; Ellegren et al., 1997; DeWoody, 1999; Kovalchuk et al., 2000; Matson et al., 2000; Rodgers and Baker, 2000; Rodgers et al., 2001a,b; Wickliffe et al., 2002, 2003]. This is especially true of the research carefully documenting the highest instantaneous radioactive burdens observed in mammals and individual absorbed dose [Baker et al., 1996, 1997, 1999a,b, 2001; DeWoody, 1999; Chesser et al., 2000, 2001; Matson et al., 2000; Rodgers and Baker, 2000; Rodgers et al., 2001a,b; Wickliffe et al., 2002, 2003].

Therefore, we chose to experimentally evaluate the genetic response, in a point mutation mouse model, to chronic radiation exposure administered continuously in the Chernobyl environment [Kohler et al., 1991; Wickliffe et al., 2002]. This model, the Big Blue® Transgenic Rodent Mutation Assay System, has demonstrated a point mutation radiosensitivity to acute, high doses (1–3 Gy) of ionizing

radiation [Winegar et al., 1994; Hoyes et al., 1998]. A >4.5-fold increase in the frequency of point mutations was documented following acute exposure to  $\gamma$ -radiation administered at a rate of 11.5–50 cGy/min [Winegar et al., 1994; Hoyes et al., 1998]. These dose rates are from 5,000–20,000 times the estimated dose rate in the most radioactive portion of Chernobyl's Red Forest ( $2.3 \times 10^{-3}$  cGy/min) [Chesser et al., 2001]. Therefore, Big Blue® mice enclosed in this region of the Red Forest for 90 days (chronic exposure) should receive a cumulative, external dose of  $\sim 3.0$  Gy ( $^{137}\text{Cs}$ ). If absorbed dose alone is the primary factor determining genetic effects and biological impacts, rather than a variable associated with both the rate and magnitude of the absorbed dose, Big Blue® mice enclosed in the Chernobyl environment should exhibit a significant increase ( $\geq 4.5$ -fold) in the transgenic mutant frequency compared to unexposed mice.

The null hypotheses are that there will be no significant increase in the mutant frequency or bias in the mutational spectra of chronically irradiated mice. The most logical alternative hypothesis, with respect to mutant frequency, is that a chronic  $\gamma$ -radiation dose of 3.0 Gy will produce the same effect as that of an acute dose. In this case, the possible effects will be a significant 4.5-fold increase in the mutant frequency; however, there may also be a significant difference in the mutational spectra of the transgene between exposed and unexposed individuals. The mutational spectra from exposed and unexposed individuals will be investigated because radiation increases oxidative stress primarily through the ionization of  $\text{H}_2\text{O}$  and the formation of hydroxyl radicals. Oxidative stress is known to cause DNA base lesions which are differentially recognized and repaired by base excision repair (BER) enzymes [De Souza-Pinto et al., 2001; Grollman and Moriya, 1993]. This aspect of BER may alter the spectrum of induced mutations. Furthermore, it is possible that no significant increase in the mutant frequency of the transgene will be observed because of increased lesion repair (i.e., transcriptional response), but the mutational spectrum may still be altered because of a disproportionate number of specific base modifications.

## MATERIALS AND METHODS

### Animal Husbandry

Eight Big Blue® C57BL/6 hemizygous mice (five females, three males) were purchased from Stratagene (La Jolla, CA) and transported to Ukraine. Mice were kept in standard laboratory cages (International Radioecology Laboratory (IRL), Slavutych, Ukraine), for 5 days to allow for acclimation. Five mice (three females, two males) were placed in enclosures in the Red Forest, which is  $\sim 2$  km southwest (UTM (Universal Transverse Mercator): 36 295545 N (Northing) 5697040) of the Chernobyl Nuclear Power Plant-CNPP [Chesser et al., 2001; Rodgers et al., 2001a,b]. Chesser et al. [2001] estimated that the external  $\gamma$ -radiation ( $^{137}\text{Cs}$ ) dose rate in this area is  $\sim 2.3 \times 10^{-3}$  cGy/min. To accumulate a chronic, continuously administered dose of 3.0 Gy, individuals must be maintained in the enclosures in this

area for  $\geq 90$  days. Three mice (two females, one male) were placed in a reference enclosure in an uncontaminated site (external  $\gamma$ -radiation dose rate at background) in Slavutych, Ukraine ( $\sim 60$  km east of CNPP) [see Rodgers et al., 2001a,b; Chesser et al., 2001, for enclosure information]. The mice were fed sterilized laboratory chow (Ziegler Feed, Taconic Laboratories, Germantown, NY) and supplied clean water ad libitum. After 90 days within the enclosures, individuals were removed, taken to the IRL, and subjected to whole body counts to estimate internal radioactivity [Chesser et al., 2001]. Individuals were then sacrificed. Tissues (liver, heart, kidney, spleen, lung, brain, muscle, bone, intestine, testes, female reproductive tract) were immediately removed and flash-frozen in liquid nitrogen. Tissues were promptly returned to Texas Tech University (TTU) in a liquid nitrogen dewar shipper and transferred to a  $-80^\circ\text{C}$  freezer for either permanent storage or until assay processing (Natural Science Research Laboratory (NSRL), TTU Museum). All animal handling procedures followed those outlined in ACUC 01052X.

### DNA Isolation and Big Blue<sup>®</sup> Mutation Frequency Assay

Genomic DNA was isolated from liver tissue using a standard organic solvent extraction procedure [Strauss, 1994]. Intact, high molecular weight genomic DNA was verified using horizontal, submerged 1% agarose gel electrophoresis (Invitrogen, Carlsbad CA; Tris-EDTA-acetic acid). The procedures outlined in the Big Blue<sup>®</sup> Transgenic Rodent Mutagenesis Assay System instruction manual revision #027002 were followed. Genomic DNA was packaged using the Transpack<sup>®</sup> Packaging Extract according to the manufacturer's recommendations (Stratagene). Titering and plating of packaging reactions was performed according to the manufacturer's recommendations (Stratagene).

### Power Analyses—Estimating Experimental Limits

An initial power analysis using the GPOWER v. 2.0 software program was conducted prior to the analysis of mutant plaques [Faul and Erdfelder, 1992]. We used the spontaneous mutant frequency of  $4.3 \times 10^{-5}$  per animal estimated from intra- and interlaboratory comparisons of Big Blue<sup>®</sup> mice [Young et al., 1995]. A 4.5-fold increase in the spontaneous mutant frequency, such as that reported by Winegar et al. [1994] and Hoyes et al. [1998] following acute exposure to 1–3 Gy  $\gamma$ -radiation, would yield a mutant frequency  $19.4 \times 10^{-5}$  per animal. A standard deviation (SD) of  $2.5 \times 10^{-5}$  was used rather than the spontaneous value of  $0.85 \times 10^{-5}$  previously reported [Young et al., 1995]. This increase in variance was assumed to be reasonable because we were interested in reducing the effort associated with packaging phage, which would necessarily result in analyzing fewer plaques per animal. This would likely increase the variance in our study over that reported in previous studies examining spontaneous and mutagen-induced mutant frequencies. The power analysis indicated that a significant 3.25-fold increase in the mutant frequency could be detected by analyzing three animals per experimental group with a power of 0.99. Finally, by examining five animals in the radiation exposure group, we attempted to reduce a potential increase in effect variation often associated with exposure to mutagens.

### Mutant Plaque Verification and DNA Sequencing

Single letter designations for nucleotides and amino acids follow IUPAC guidelines. The complete DNA sequence from each *lacI* transgene from all observed mutant (blue) plaques was determined to verify mutant status and characterize the nature of each mutation. Mutant plaques were cored using a sterilized wide-bore pipette and expelled in 100  $\mu\text{l}$  of sterilized water. This suspension was then denatured at  $100^\circ\text{C}$  for 10 min and vortexed

briefly in a manner similar to Stiegler and Stillwell [1993]. The *lacI* transgene was then directly amplified using the polymerase chain reaction (PCR) with the primers (Integrated DNA Technologies (IDT), Coralville IA) *lacI*-FOR (5'-GTA TTA CCG CCA TGC ATA CTA G-3') and *lacI*-REV (5'-CGT AAT CAT GGT CAT AGC TGT T-3'). Specifically, 3  $\mu\text{l}$  of the  $\lambda$  DNA suspension were used in a 50  $\mu\text{l}$  PCR consisting of 0.2 mM each dNTP (Perkin Elmer Biosystems, Foster City CA), 3 mM  $\text{MgCl}_2$  (Promega, Madison WI),  $1 \times (5 \mu\text{l}$  of  $10 \times$ ) *Taq* DNA polymerase buffer (Promega), 0.5 mM each PCR primer, 1.5 U of *Taq* DNA polymerase (Promega), and sterilized, reverse-osmosed water. Column-purified amplicons (Qiagen, Valencia CA) were then cycle-sequenced according to the manufacturer's recommendations using the ABI Big Dye<sup>®</sup> v. 2 and 3 Terminator chemistry (Applied Biosystems (ABI), Foster City, CA) with each of the following primers (IDT): *lacI*120F (5'-TAT GCC GGT GTC TCT TAT CAG-3'), *lacI*131F (5'-TTA TCA GAC CGT TTC CCG-3'), *lacI*279F (5'-TTG CCA CCT CCA GTC TG-3'), *lacI*323F (5'-TTG TCG CGG CGA TTA AAT CTC G), *lacI*881R (5'-TGT CTT CGG TAT CGT CGT ATC), *lacI*975R (5'-CCT GAG AGA GTT GCA GA AG-3'). The numbers in each of the primer names correspond to their respective nucleotide position (extreme 3') in the *lacI* gene relative to nucleotide position  $-38$  following the sequence designation of Farabaugh [1978]. Primers suffixed with an "F" anneal to the antisense strand and are used to generate the sense DNA sequence, while primers suffixed with an "R" anneal to the sense strand and are used to generate the antisense DNA sequence. Fluorescent sequence chromatograms were generated using a rapid sequencing protocol on an ABI310 Prism<sup>™</sup> Genetic Analyzer (ABI). DNA sequence chromatograms were proofed and aligned with an accessioned (GenBank, National Center for Biotechnology Information, Bethesda MD) wildtype, *lacI* gene (AF031088). Four control (clear) plaques (two from the provided control template (Stratagene), two from mouse samples generated in this study) were also sequenced to verify this approach. Sequences for the *lacI* gene from each control and mutant plaque were compared and translated in the MEGA v. 2.1 software program [Kumar et al., 2001]. All recovered mutants were compared to the database of known *lacI* mutants maintained by Johan de Boer [1995]. These data were also accessioned into a database (<http://eden.ceh.uvic.ca/bigblue.htm>).

### Statistical Analysis

A Fisher's Exact Test (FET; SPSS for Windows v. 11.0, SPSS, Chicago, IL) was used to test for significant differences in the mutant frequencies. The mutational spectra from the exposed and reference groups were compared with one another and with the spontaneous mutation spectrum estimated for liver tissue in this Big Blue<sup>®</sup> substrain by de Boer et al. [1997] using the Likelihood Ratio Test (LRT; SPSS). Differences were considered significant at an  $\alpha$  of 0.05.

## RESULTS

Mice were successfully maintained in the enclosures for 90 days (five mice, two males, three females) in the Red Forest; three mice (one male, two females) in the reference area. The mice housed in the Red Forest received a cumulative external radiation dose of  $\sim 3.0$  Gy [Chesser et al., 2001]. Based on whole body counts, the average cumulative dose resulting from the internal deposition of radionuclides in mice housed in the Red Forest was  $8.1 \pm 0.3 \times 10^{-5}$  (standard error; SE) Gy [Chesser et al., 2001]. The mice housed in the reference enclosure did not receive a detectable external radiation dose above background. Based on

whole body counts, mice housed in the reference enclosure received an average internal, cumulative dose of  $0.4 \pm 0.2 \times 10^{-5}$  Gy.

### Post-Hoc Power Analysis

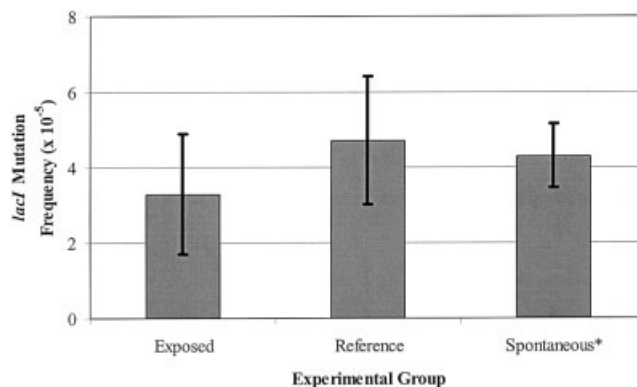
Following the examination of an average of 71,380  $\pm$  2,216 plaques per animal, a post-hoc power analysis was conducted [Faul and Erdfelder, 1992]. The goal of this exercise was to determine if the desired amount of data for adequate statistical testing had been collected while attempting to minimize the cost of the analyses. The mutant frequency estimated from the reference mice and largest estimated variance in either group was to be used for this power analysis. The estimated mutant frequency in the reference mice was  $4.72 \times 10^{-5}$  (standard deviation; SD =  $2.99 \times 10^{-5}$ ). The largest estimate of variance was from the exposed mice (SD =  $3.49 \times 10^{-5}$ ). As in the previous power analysis, the goal of this power analysis was to determine if we could still statistically distinguish the previously estimated 4.5-fold increase in the mutant frequency ( $19.4 \times 10^{-5}$  from above) from the “spontaneous” mutant frequency of  $4.72 \times 10^{-5}$  now estimated from our reference mice. Thus, we used a slightly larger estimate of the spontaneous mutant frequency ( $\sim 1.1\times$ ) and a larger estimate of the standard deviation ( $\sim 4.1\times$ ) in this power analysis with a minimum of three animals per test group. The power analysis indicated that we could detect a significant 3.5-fold increase in the mutant frequency with a power of 0.96.

### Mutant Frequency

A total of 26 putative mutant plaques were observed. Four (three from the exposed mice, one from the unexposed mice) of these presented as possible *ex vivo* mutations. Sequence analysis indicated 100% nucleotide identity with the wildtype *lacI* sequence, so these four events were subsequently reclassified as nonmutants. Twelve verified mutant plaques were observed in the animals ( $n = 5$ ) chronically exposed to the Chernobyl environment, which results in a mutation frequency of  $3.29 \times 10^{-5}$  ( $\pm 1.56 \times 10^{-5}$ ; SE). Ten verified mutant plaques were observed in animals ( $n = 3$ ) from the uncontaminated, reference enclosure, which results in a mutation frequency of  $4.72 \times 10^{-5}$  ( $\pm 1.72 \times 10^{-5}$ ; SE). The estimated transgene mutation frequencies are presented in Figure 1. Mutation frequencies were not significantly different (one-sided,  $P > 0.25$ ).

### Mutational Spectra

Each nucleotide substitution and inferred amino acid substitution is presented in Table I. All nucleotide substitutions observed in this study have been reported previously, and those from this study have been accessioned in the *lacI*,



**Fig. 1.** Somatic mutant frequencies observed in the *lacI* transgene of C57BL/6 hemizygous Big Blue<sup>®</sup> mice. Mice ( $n = 5$ ) belonging to the exposed group were chronically exposed (90 days) to the Chernobyl environment and absorbed a cumulative radiation dose of  $\sim 3.0$  Gy (300 rads). Mice ( $n = 3$ ) belonging to the reference group were maintained in an uncontaminated reference enclosure for a duration equivalent to the exposed mice. Bars represent standard errors. Mutant frequencies in the exposed and reference groups were not significantly different (one-sided,  $P > 0.25$ ).

Big Blue<sup>®</sup> mutation database (<http://eden.ceh.uvic.ca/bigblue.htm>). The mutation spectrum from each group did not differ significantly (LRT = 3.91,  $df = 3$ ,  $P > 0.27$ ). Furthermore, the mutation spectra from the two groups in this study did not differ significantly from the spontaneous spectrum observed in de Boer et al. [1997] (LRT = 11.39,  $df = 10$ ,  $P > 0.32$ ).

Eight G:C to A:T transitions, two A:T to G:C transitions, two G:C to T:A transversions, and one A:T to T:A transversion were observed in the mutation spectrum of the mice chronically exposed to the Chernobyl environment. Four G:C to A:T transitions, two A:T to G:C transitions, and five G:C to T:A transversions were observed in the mutation spectrum of the unexposed, reference mice. In both groups a single doublet mutation (tandem, two basepair substitution) was observed [Buettner et al., 2000]. Buettner et al. [2000] suggest that these two mutation events may not be independent; therefore, for analysis of mutant frequencies, tandem mutations were treated as a single mutational event. However, both nucleotide substitutions in each doublet are reported in Table I and used for mutation spectra comparisons.

Every nucleotide substitution observed in both groups resulted in an inferred amino acid substitution (Table I), with two exceptions. In both cases where tandem nucleotide substitutions occurred, one of the substitutions was silent (i.e., this substitution did not contribute to an inferred amino acid substitution). Two nucleotide substitutions in each group, four in total, resulted in an inferred termination signal. In the exposure group, a K and an R were altered to a termination codon. In the reference group, an E and a Q were altered to a termination codon. The remaining amino

TABLE I. Spectrum of Mutations Observed in Big Blue® Mice.

Plaque	Group	WT	Mut	Position(s)	WTAA	MutAA
TK84325-2	Exp	T/a G/c	C/g A/t	659-60	L	P
TK84325-3	Exp	C/g	T/a	236	A	V
TK84328-1	Exp	G/c	T/a	154	V	F
TK84328-2	Exp	G/c	T/a	178	V	L
TK84328-3*	Exp	G/c	A/t	218	R	H
TK84328-4	Exp	C/g	T/a	281	A	V
TK84328-5	Exp	C/g	T/a	26	A	V
TK84328-6	Exp	C/g	T/a	130	R	C
TK84328-7	Exp	C/g	T/a	367	R	TERM
TK84329-1a	Exp	C/g	T/a	80	T	M
TK84330-1	Exp	A/t	T/a	388	K	TERM
TK84330-2	Exp	T/a	C/g	200	L	P
TK84356-1	Ref	G/c	T/a	626	G	V
TK84356-3	Ref	C/g	T/a	217	R	C
TK84356-4	Ref	G/c	T/a	1043	R	I
TK84356-5	Ref	G/c G/c	T/a T/a	171-172	E	TERM
TK84356-6	Ref	C/g	T/a	118	Q	TERM
TK84356-7	Ref	C/g	T/a	95	A	V
TK84357-2*	Ref	G/c	A/t	218	R	H
TK84357-4	Ref	C/g	A/t	122	T	N
TK84359-1	Ref	T/a	C/g	278	L	P
TK84359-2	Ref	T/a	C/g	794	L	P

\*Mutation observed in two different individuals.

Each individual in this study was assigned a unique identification number prefixed by "TK." The number following each identification number corresponds to a mutant plaque identification number. Group indicates exposed ("Exp") and reference ("Ref") samples. "WT" denotes the wildtype nucleotide state and "Mut" denotes the mutant nucleotide state. Nucleotide position(s) correspond to those within the *lacI* mutation database maintained by Johann de Boer (<http://eden.ceh.uvic.ca/bigblue.htm>). "WTAA" denotes the inferred wildtype amino acid state and "MutAA" denotes the inferred mutant amino acid state. Single-letter abbreviations follow IUPAC guidelines.

acid substitutions inferred in the exposure group were three of A to V, two of L to P, R to C, R to H, T to M, V to F, and V to L. The remaining amino acid substitutions inferred in the reference group were A to V, G to V, two of L to P, R to C, R to H, R to I, and T to N.

No deletions or insertions were observed in either series.

## DISCUSSION

### Chronic, Low-Dose Rate Irradiation and Transgenic (*lacI*) Mutation Frequencies

Relatively few studies have examined the genetic effects of low doses (<10 cGy) of ionizing radiation and these studies have focused on acute exposures. One reason for the exposure bias is that chronic, continuous exposures of this magnitude are virtually impossible to accurately deliver in a laboratory situation. However, chronic, low-level exposures administered continuously or in fractions typifies those experienced in occupational and environmentally contaminated settings.

Interestingly, standard markers (i.e., cytogenetic aberrations) of genetic damage used historically in detecting radiation exposure are insensitive to acute, low doses (<10 cGy). Previous studies examining the genetic effects of

acute, low-dose exposure using the widely accepted *hprt* gene as a mutation marker have documented an increase in point mutations and a corresponding lack of an increase in gross chromosomal aberrations [Schwartz et al., 2000; Huo et al., 2001]. Because these low-dose experiments have documented a significant increase in the frequency of point mutations, the Big Blue® system is an appropriate model for our experiment. Furthermore, the Big Blue® system has demonstrated a clear point mutation response to several mutagens, including ionizing radiation [Hoyes et al., 1998; Winegar et al., 1994]. Acutely administered  $\gamma$ -radiation doses of 1–3 Gy have significantly increased point mutation frequencies,  $\geq 4.5$ -fold, in the transgene of exposed animals when compared to unexposed controls [Hoyes et al., 1998; Winegar et al., 1994].

The issue with respect to radiation risk, especially that which results from environmentally relevant exposures, is whether or not equivalent increases in the transgene mutant frequency will occur following chronic, low-dose rate irradiation. Cumulative dose–response models indicate such an increase is mathematically expected, and a similar response will be observed if 1–3 Gy is administered either acutely or chronically. Rodgers et al. [2001a,b] and Chesser et al. [2001] documented that the external dose rate in the Red Forest region in Chernobyl, where these Big Blue® mice

were enclosed, is  $\sim 2.3 \times 10^{-3}$  cGy/min. Thus, the absorbed cumulative dose over the course of 90 days (chronic) exposure will be  $\sim 3$  Gy [Eaton and Klaasen, 1996; Wickliffe et al., 2001]. Therefore, if acute and chronic absorption of equivalent radiation doses yields equivalent effects (i.e., the current cumulative dose–response model is accurate), the Big Blue<sup>®</sup> mice chronically exposed to 3 Gy in the Chernobyl environment should exhibit a significant,  $\sim 4.5$ -fold increase in mutant frequency when compared to the unexposed reference mice [Hoyes et al., 1998; Winegar et al., 1994]. Our results do not support the equivalency of acute and cumulative radiation dose–response models. Not only did mice exposed to the Chernobyl environment not have a significant 4.5-fold increase in the mutant frequency of the transgene, both exposed and reference mice had mutant frequencies similar to the spontaneous mutant frequency,  $4.3 \times 10^{-5}$ , of the transgene in liver tissue [Young et al., 1995].

### Oxygen Radical Stress and Mutational Spectra

Oxygen radicals, such as those generated by ionizing radiation, are known to produce specific DNA base lesions. For example, one particularly mutagenic form of modified nucleotide generated when DNA is exposed to free radicals is 8-oxo-deoxyguanosine [De Souza-Pinto et al., 2001; Tani et al., 1998]. This lesion often results in a G:C to T:A transversion [Grollman and Moriya, 1993]. Thus, exposure to genotoxicants which induce oxidative stress may increase the number of mutants and alter the spectrum of mutations in comparison to the spontaneous or background mutant frequency and spectrum of mutations. Transcriptional responses may serve to modulate radical stress by either increasing the cellular capacity to scavenge free radicals and/or the ability to repair damaged DNA. This would essentially serve to maintain both the “spontaneous” frequency of mutants and spectrum of mutations. However, the spectrum of mutations may still be altered under such conditions, even in the absence of a significantly increased mutation frequency, because of the disproportionate number of radical interactions at specific DNA bases (e.g., guanine). This was not evident in the Big Blue<sup>®</sup> mice chronically exposed to the Chernobyl environment. In fact, there was no significant difference between the mutational spectra of the exposed and unexposed mice and neither of these groups differed significantly from the spontaneous mutational spectrum reported by de Boer et al. [1997]. However, the spontaneous mutational spectrum, considering transversion substitutions, observed in the *lacI* transgene of Big Blue<sup>®</sup> mice is biased towards G:C to T:A transversions and this was observed in this study. This “noise” property may serve to mask truly significant differences in the mutational spectra of unexposed and exposed individuals unless substantially more mutant plaques are characterized.

### Summary

The results of this study indicate that chronic, continuous exposure to 3 Gy of external  $\gamma$ -radiation in the Chernobyl environment does not induce mutations in the same manner as does acute exposure to 3 Gy of external  $\gamma$ -radiation in the laboratory. This finding is consistent with previous research investigating the cyto- and molecular genotoxicity of the Chernobyl environment [Baker et al., 1997, 1999a,b; DeWoody, 1999; Rodgers and Baker, 2000; Rodgers et al., 2001a,b; Wickliffe et al., 2002, 2003]. Therefore, these data do not support simple, cumulative dose–response models and downward extrapolations of genetic effects from acute, high-dose exposures [Doull and Rozman, 2000; Jaworoski, 1997, 1999; Neel, 1999; Sinclair, 1998; Vilenchik and Knudson, 2000]. In fact, a threshold may indeed exist, when dose rate is taken into account, at which mutations simply are not induced [Kondo, 1999; Kirsch-Volders et al., 2000]. Additional studies using genetically sensitive systems such as the Big Blue<sup>®</sup> Mutation Assay are necessary to further refine low-dose rate, chronic irradiation risk models. This study was designed to examine the paradigm in radiation risk assessment which equates deleterious effects with dose, irrespective of the rate at which the dose is administered. Our results indicate that dose rate is an essential variable that dictates the magnitude of response and simply equating biological responses to acute and chronic exposures, especially ultralow-dose rate, of equivalent magnitude is invalid. This study was not designed to determine a low dose, low-dose rate response curve. To achieve this goal, further research is required. In addition, recent technological advances (e.g., gene expression analysis) will need to be incorporated as transcriptional responses may modulate effects induced at low-dose rates. In the absence of induced mutations, this latter approach may prove uniquely sensitive to slight alterations in the homeostatic cellular environment.

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