RADIATION INDUCED BYSTANDER RESPONSES IN VERTEBRATES GIVEN LOW DOSES OF *IN VIVO* RADIATION

By

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A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfillment of the Requirements for the Degree of

Master of Science

BYSTANDER RESPONSES IN VERTEBRATES GIVEN *IN VIVO* RADIATION

Masters of Science (2009)
(Medical Physics)

McMaster University
Hamilton, Ontario

TITLE- RADIATION INDUCED BYSTANDER RESPONSES IN VERTEBRATES GIVEN LOW DOSES OF *IN VIVO* RADIATION

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Number of Pages (CXC) 190

Acknowledgments

I would like to thank, first and foremost, my supervisor as well as the other committee members. To my supervisor, Dr. Carmel Mothersill, I give thanks for the time and dedication she invested in me over the past two years. I am grateful her patience and her ongoing faith in my work, and always being there to offer support and advice. I would also like to thank Dr. Seymour Colin for all the helpful advice and support he has given me with over the past few years. Finally, I would like to give thanks to Dr. Gerry Moran for all his advice through the years.

A special thanks to all the people involved in treating the mice /frog samples at AECL. Without their hard work, this project would not have been possible.

Also, I would like to thanks my family for all their motivation and support during the past two years. . I am also grateful for this opportunity given to me by all members of the faculty and staff. This experience has allowed me to learn not only science but also, about myself

ABSTRACT

The bystander response phenomenon shows that radiation induced changes in cells that have not been directly targeted, but are neighbors to or receive medium from directly hit cells. Our group has performed a range of single and serial, low dose irradiations (*in vivo*) on two strains of mice that have been documented to show genetic differences in their response to radiation. This thesis also explores the impact of environmental radiation contamination on female and male Mink frogs (*Rana septentrionalis*) sampled from contaminated and background (control) radiation sites. Bladder explants established from these vertebrates are incubated in culture medium, which is then used to measure apoptotic response (cell survival and calcium flux) in the keratinocyte reporter system.

This study reveals that culture medium from acutely irradiated C57Bl6 mice, but not Balb/c mice, induces dose-dependant clonogenic death. The administration of a priming dose(s) to C57BL6, but not Balb/c mice, leads to stimulatory growth effects in reporters regardless of the time separation between the priming and challenge dose. Similarly, ITCM corresponding to male and female contaminated frogs results in a sex-dependent decrease in reporter survival, but no reduction is induced from ITCM sampled from explants from female and male frogs from uncontaminated sites. When the ITCM is measured for its calcium inducing ability, results show abnormal calcium levels in both strains of mice only after the administration of a priming dose.

However, chronic exposures to male and female frogs results in the production of ITCM that induces transient calcium flux in reporters. These results indicate that genetic predisposition in mice influences the type of bystander signal that is produced after exposure to low, acute doses of radiation. However, when mice are repeatedly exposed to radiation, the bystander signal is modified in a way that may be causing unregulated growth in reporter cells.

SUMMARY

This paper focuses on the effects of bystander signals that are produced *in vivo* from the irradiation of C57BL6 mice (show normal radio sensitivity) as well as Balb/c mice (show high radio-sensitivity). Bystander signals are produced by exposing these two genetically distinct mice to various acute as well as repeated doses of radiation, after which they are sacrificed for their bladder tissues. The bladder explants are cultured within medium, and this process is speculated to transfer bystander factor(s) that originate in the tissue at the time of irradiation, into the medium. This irradiated tissue culture medium or ITCM is exposed to completely unirradiated HPV-G transfected reporter cells, and various biological endpoints are monitored such as clonogenic survival, intracellular calcium signaling, growth rates, and onset of proliferation.

Results are showing that under acute dose exposures, C57BL6 mice are able to produce pro-death bystander signals that cause a decrease in clonogenic survival and a transient calcium flux in their corresponding reporters. This is indicative of a gnomically stable response to radiation damage, in that it rids the population of weak and damaged cells thus preventing the propagation of mutations. On the other hand, Balb/c mice fail to produce pro-death effects in their corresponding reporters; however subtle changes in growth rates are detected.

This indicates that either a) bystander production fails due to excessive damage of the tissue at the time of radiation or b) the bystander signal is modified from pro-death to pro-survival, due to the different genetic background of the mice. If the latter is true then these 'modified' signals are using calcium signaling to activate an alternative pathway that targets cell cycle progression rather then apoptosis.

Upon repeated exposures, both C57Bl6 and Balb/c mice induce a considerable increase in clonogenic survival and growth rates of the corresponding reporters. The increase in growth correlates with abnormal levels of calcium signaling which indicates that perhaps this excessive growth is associated with unregulated rather than adaptive growth. This allows one to conclude that the pro-death bystander signal that is associated with C57BL6 is switched into a pro-survival one, under repeated exposure conditions.

When female and male *Rana septentrionalis* frogs are sampled from radiation contaminated sites for their bladders, their corresponding ITCM produces a sex dependent (females are more radio-sensitive) decrease in percent colony survival and a transient calcium flux. This indicates that lifetime, chronic exposures to radiation contamination generate bystander signals *in vivo,* which shows pro-death effects in reporter cells *in vitro.*

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Golden Horseshoe

RRS Travel Award – For Savannah 2009 RRS conference
Radiation Research Society

1 INTRODUCTION

1.1 Radiation Damage

When radiation tracks deposit energy in a cell, there are several fates that can occur. If the cell suffers enough DNA damage after high doses of radiation, the result is loss of function of the cell, causing reproductive cell death (Hall, 2000) However, the most frequently encountered type of cell death after low doses of radiation is known as apoptosis, and it is characterized by a dense cytoplasm, membrane blebbing, cell shrinking (Kerr *et al.*, 1972 and Strasser *et al.*, 2000). On a molecular level, apoptosis is characterized by mitochondrial membrane depolarization and rupturing of the plasma membrane (Kroemer and Reed, 2000). Apoptosis functions to regulate cell proliferation as a part of a homeostatic process. One type of homeostatic process is referred to as the anti-tumorigenic mechanism, which selectively eliminates cells that demonstrate unregulated cell division (Pollycove *et al.*, 2003). Another type of cell death that can occur is termed senescence. In this scenario, the cell remains metabolically active but DNA fails to replicate (Gandarillas, 2000). Clonal growth can be limited through terminal differentiation as well. In this process, cells stop dividing without experiencing membrane blebbing or nuclear fragmentation (Alberts *et al.*, 2002 and Oleg *et al.*, 2006). Finally, radiation can alter or mutate DNA strand sequencing, resulting in unregulated, cancerous growth (Hall, 2000; Bertram, 2000). Sub-lethal doses of radiation can induce other types of biological responses, such as reduced proliferative capacity of progeny

(Dewey *et al.,* 1963 and Nias *et al.*, 1965), increased radio sensitivity (Sinclair *et al.*, 1964), and/or changes in cell cycle progression (Grote *et al.*, 1981 and Joshi *et al.*, 1982a)

1.1.1 Types of DNA Damage

These pathways to cell damage occur as a result of the direct or indirect action of radiation on cellular DNA. Direct DNA damage occurs when energy is deposited directly into DNA strands resulting in single strand breaks (SSB), double strand breaks (DSB), or base pair damage (Hall, 2000). High linear energy transfer (LET) radiation that results from neutron or alpha particle exposures cause direct DNA damage to occur more frequently (Hall, 2000). However, low LET radiation that results from gamma or X-ray radiation usually induces DNA damage indirectly through interaction with water molecules (Hall, 2000). When energy is deposited into water molecules, it causes water molecules to eject circulating electrons, thus becoming ionized. These ionized water molecules react with one another to produce hydroxyl radicals also referred to as reactive oxygen species, which have the potential to damage DNA (Hall, 2000)

1.1.2 Types of DNA Damage Repair

The most detrimental type of DNA damage are double strand breaks (DSB), which occur when two complimentary sites on a DNA molecule are broken at the same time. This can lead to genomic rearrangements that can prove to be very hazardous in nature (Hall, 2000). There are two primary mechanisms that serve to repair DSB, the non-homologous end joining (NHEJ) and homologous recombination (HR). In NHEJ, specialized DNA binding proteins are recruited to the site of DNA damage where they act as scaffolds that hold the broken DNA ends apart and remove any single stranded

overhangs that may be present. After DNA ends are processed, ligase IV and XRCC4 are recruited to the site to ligate or join the two ends together (DSB, 2007 and Hall, 2000). HR requires the utilization of a sister or homologous chromosome to repair a DSB. A strand of broken DNA is introduced into one of the two homologous chromosomes in a process called 'strand invasion'. The homologous strand is used as a template for synthesis of the missing DNA sequences. The newly synthesized strand is unwound and then ligated back the other end of the broken chromosome (DSB, 2007 and Hall, 2000).

1.2 Targeted Effects of Radiation

1.2.1 Origins of Radiobiology

The origins of radiobiology date back to the 1940's when scientists discussed the mechanistic action of radiation on the chemical constituents within a living system (Lea, 1946). For an extensive review refer to Mothersill and Seymour (2006a). Lea proposed that in dilute solutions, radiation initially reacts with water to create an intermediary body, which in turn catalyses a reaction with the solutes. Moreover, Lea stated that this indirect action of radiation was always proportional to dose. Simultaneously, Timofeeff-Reessovsky and Zimmer (1947) published a book which proposed that radiation damage results from the direct action of energy on a critical target. At this point, the structure of DNA was not known but extensive work on chromosomal damage and reproductive failure in response to radiation had been established (Laterjet, 1972 and Savage, 2002), making the nucleus or its content the primary candidate as the critical target for radiation damage. Studies such as the one done by Puck and Marcus (1956) supported the critical

target theory by showing that cells that are able to reach 5-6 population doublings are survivors of radiation damage because they must have not sustained any lethal damage. Any cells that fail to divide more than three times are considered 'hit' or inactivated, thus classified as 'non-survivors' of radiation damage. On the other hand, there was literature that proposed the presence of sub-lethal damage in the progeny of irradiated cells, such as cell cycle delay (Sinclair *et al.,* 1964 and Joshi *et al.*, 1982a), but this was largely dismissed.

1.2.2 Traditional Paradigms for Radiation Damage

Extending from the principals discussed by Timofeff-Reessovsky and Zimmer (1947), novel methods of quantifying radiation induced damage have been proposed. On of these models is the linear quadratic model, which is used for quantifying biological response to radiation damage.

$$SF = e^{-(\alpha D - \beta D2)} \qquad \text{Eqn 1.1}$$

This equation represents a survival curve defined by D (dose which produces 1 lethal event/target), α is the cell kill per Gy of the initial linear component, β the cell kill per Gy^2 of the quadratic component (Brenner *et al.*, 1998). These concepts gave way for another model for biological effect assessment called the linear no threshold model (LNT), which was initially proposed by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) in the 1950's. This paradigm for risk

estimation argues that radiation at any dose results in adverse effects. The foundation for this theory is based on the fact that the energy that is deposited by the transversal of radiation tracks in cellular DNA results in the formation of DNA double strand breaks (Hall, 2000). These DNA breaks are considered detrimental because they are hard to repair. If these lesions are left unfixed, they can cause incorrect DNA replication resulting in cell death (Hall, 2000). In addition, improper repair of these breaks can also result in the induction and propagation of various mutations that are non-fatal, but can lead to carcinogenesis if they occur in tumor suppresser genes or otherwise disrupt controls on the fidelity of DNA repair and replication (Ward, 1990). Consequently, this model implies that DNA strands serve as intracellular "target" sites for radiation tracks, so any increase in dose should lead to a proportional increase in number of cells hit, thus a corresponding increase in biological effect.

Currently, the validity of the LNT model at very low doses is under intense scrutiny as a result of various studies that reveal that DNA damage is not required to induce radiation related biological effects. Various trends have been thoroughly described in literature such as abscopal effects, hormeosis, adaptive responses, bystander effects, which suggest that the mechanisms underlying biological responses to low doses are different to those which operate after high dose exposures. Although the exact mechanistic actions of these effects are unknown, they all are predominant at very low doses.

1.3 Non- targeted Effects of Radiation

There has been a shift away from the traditional DNA centered paradigm for radiation damage with the observation that cells which survive irradiation may appear to be normal but can produce progeny with abnormalities. These abnormalities are non-clonal effects and are a manifestation of latent or continuing damage. Evidence for these effects came from studies in the 1980's when various groups demonstrated that survivors of irradiated cells exhibit chromosomal instability (Seymour *et al.*, 1986;Gorgojo *et al.*, 1989;Pampfer *et al.,* 1989;Kadhim *et al.*, 1992). Since then non-clonal effects have been extensively reviewed and consist of abnormal events such as lethal mutations, genomic instability, delayed reproductive death and chromosomal instability (Kronenberg, 1994; Mothersill *et al.*, 1997b). Several authors have proposed to explain how genomic instability persists through several generations. Clutton *et al.*, 1996 and Wright *et al.*, 1999 discuss that persistent production of ROS species in response to direct irradiation is the key to the propagation of non-clonal effects. This can be explained by the fact that low levels of radiation cause oxidative stress in cellular systems, which in turn forces cells to allocate its energy towards cellular repair. This results in the production of various proteins which stimulates the metabolic rate of the system. This leads to the further production of reactive oxygen species. However, the mechanism through which these ROS species are transferred to progeny remains to be explored.

Another challenge to the DNA centered dogma of radiation damage emerged as studies began to show how unirradiated cells that are near or receive signals from irradiated cells, express similar effects to those cells that have been directly irradiated

(Watson *et al.*, 2000; Lewis *et al.,* 2001; Lorimore *et al.*, 2003). These effects are different from non-clonal effects which are observed in the direct descendants of irradiated cells. This radiation-induced change observed in unirradiated (non progeny) cells is termed the 'bystander effect'. These findings are an extension of previous evidence dating back to the 1950's for abscopal and clastogenic effects (Parsons *et al.,* 1954). The nature of these bystander effects has been extensively reviewed (Mothersill and Seymour, 2006a;2006b;2006c). The fact that they are predominant in low regions of the survival curve (Seymour and Mothersill, 2000) emphasizes the importance of understanding these mechanisms in order to properly grasp the impact of low dose radiation on biology.

1.3.1 Bystander Effects

Bystander-effect associated changes in biological response are reported to consist of decreases in plating efficiency, increases in chromatid exchanges, oncogenic transformation, and changes in protein synthesis and/or gene expression (Morgan *et al.*, 2003; Nagasawa *et al.*, 1992; Kadhim *et al*, 1992).

One approach used to induce bystander signal production involves exposing a monolayer of cells to very low fluences of high LET radiation particles or through the use of a microbeam (Azzam *et al.,* 1998). This ensures that only a minute fraction of the total cell culture is being irradiated. One of the first studies to employ this method was by Kadhim *et al*'s (1992) group which shows that chromosomal damage appears in hematopoietic cell progeny present after numerous generations' post- irradiation. This is

a clear indication that the damage observed within these progeny cells is not a direct consequence of radiation track transversal on DNA. Similarly, Nagawasa *et al* (1992) used low fluences of alpha particles to expose less than 1% of Chinese hamster ovary cells. Interestingly, 30% of the cells showed a significantly (1.4x) higher proportion of SCE's at doses as low as .31mGy, indicating that genetic damage is induced in nuclei that have not been directly exposed to alpha particle tracks. These results are confirmed using normal human diploid lung fibroblasts which reveal an 8.6 fold increase in the percentage of cells showing excessive SCE induction at .4 cGy exposures (Deshpande *et al.*, 1996). The discrepancy between theoretically estimated frequencies of SCE's and the actual observed SCE's is seen to decrease with increasing dose of radiation, indicating a switch from majority bystander induced damage at lower doses to direct DNA damage at higher doses (Deshpande *et al.*, 1996). Moreover, Nagawasa and Little (1999) shows sensitivity to induction of mutations at very low doses of radiation and a saturation point for mutation induction at doses higher than 10 cGy. They propose that adjacent cells play a role in the enhancement of this biological response. Overall, these studies provide evidence that suggest that high LET radiation can produce signals in directly irradiated cells, which are transferred to nearby non-irradiated cells, where they induce a biological response.

Mothersill *et al.* (1997c) demonstrates a different approach of bystander signal production by exposing cell cultures to small doses of low LET gamma radiation. Upon exposure, these cells release factor(s) into the medium. When this medium is transferred onto unirradiated HPV-G or bystander cells, significant amount of cell death is observed.

However, no lethal effects are seen in cells after they are exposed to irradiated medium that did not contain cells. This medium transfer method can also incorporate the use of human tissue explants (irradiated *in vivo* or *in vitro*) as a source of bystander signals (Mothersill *et al.*, 2001 and 2002a, Seymour and Mothersill 2006). The use of tissue explants in medium transfer experiments has also been extended by O'Dowd *et al.'s* (2006) group that exposed various fish tissue explants to low levels of gamma radiation and then measured the effect that the explant medium (ITCM- irradiated tissue culture medium) has on unirradiated keratinocyte cultures. This method has been further modified by Mothersill et al. (2006d) and Mothersill *et al.* (2007), which show that fish irradiated *in vivo* can release factors into the water. These signals are received by unirradiated or bystander fish that were placed into the 'conditioned' water. The 'bystander' fish are sacrificed and their tissue explants are cultured to provide 'conditioned medium', which in turn causes tissue specific biological responses in the reporter cells. Mothersill *et al.*'s (2005) group irradiated distinct species of mice in order to asses the effect that the corresponding bystander signals has on an unirradiated reporter system.

1.3.2 Endpoints of the Bystander Effect

The most frequently reported endpoint of low dose and bystander signal exposure is apoptosis and/or other types of cell death. However, Mothersill and Seymour (2006a) discuss that at very low doses of radiation, cells work to allocate energy in an efficient manner that focuses on repair of some types of damage and the tolerance of other types of damage. This tolerance results in delayed effects that allow the cell to

'postpone' dealing with certain types of damage until it is better equipped. As a result, apoptosis is considered a direct or immediate response to a certain level of damage, which effectively eliminates damaged cells from the population. However, other types of damage such as mutations are dealt with through different avenues of damage control. For example, one group shows an increase in TP53 (p53) protein expression in rat lung epithelium cells after sustaining 0.1 Gy of direct alpha radiation (Hickman *et al*, 1994). It is known that an increase in p53 protein expression results in the induction of cycle cell arrest through activation of G1 arrest (Kastan *et al.*, 1991 and Kuerbitz *et al.*, 1992), suggesting that low dose radiation can also cell cycle progression. Similarly, Azzam *et al.'s* (1998) group shows that human fibroblasts that are exposed to low fluences of alpha particles have higher level of TP53 and (downstream substrate) cyclin dependant kinase inhibiter 1 (CDKIN1A) expression. More importantly, this increase is more then what is predicted based on the number of cells actually traversed by radiation tracks. This shows that induction of p53 and up-regulation of cell cycle checkpoint proteins can also induced in cells that have not been directly irradiated (bystander cells). In addition, Matsumoto *et al.'s* (2001) group shows an accumulation of stress response regulators such as P53 and HSP72 in wildtype P53 cells when they are co-cultured with irradiated mutant P53 cells. This provides further evidence that damaged observed in irradiated cells can up-regulate stress associated proteins in neighboring non-irradiated cells. An increase in P53 induced cell cycle alterations in response to bystander associated factors as well as direct radiation, permits the repair of damage before the cell reinitiates DNA replication (G1 arrest). This confirms that radiation induced increase in cell cycle regulation is a biological attempt to

repair the cell of low dose radiation damage. Failure to delay cell cycle checkpoints can result in the accumulation and propagation of mutagenic lesions which can result in genomic changes that may initiate transformation (Kuerbitz *et al.*, 1992).

1.3.3 Mechanism of transmission of the Bystander Effect (Gap Junctions and Medium Transfer)

The transmission of the bystander signal from irradiated cells to non-irradiated cells is thought to occur via multiple mechanisms. Some groups show the involvement of direct channels or gap junctions in mediation of the bystander response. Azzam *et al.* (2001) shows that when given low doses of alpha radiation (.3 cGy), there is an associated increase in $p21^{waf1}$ in GJIC (gap junctional intracellular communication) competent cell lines only. No such effect is detected in cell lines that show diminished gap junction communication. Furthermore, when cells were given .16 cGy (associated with the traversal of 1% of cells of the total population), there was an increase in $p21^{waf1}$ expression only in aggregates of neighboring cells (Azzam *et al.*, 2001). This confirms the involvement of gap junction communication in the transmission of damage signals to adjacent, unexposed cells. In addition, Bishayee *et al.*, (1998) reveals that Lindane (a gap junction inhibitor) causes an inhibition of the bystander response in unirradiated cells. Mechanistically, lindane activates the endocytosis of gap junction plaques from the plasma membrane, after which they are degraded by lyzosomes (Guan, 1998). This indicates that the role of cell to cell communication, more specifically the plasma membrane is important in transmitting bystander signals. Cell membranes contain signaling microdomains called GEMs (glycol-sphingo microdomains) that consist of sphingolipids, cholesterol and various proteins that serve as rafts for aggregation of

various factors (Grestner, 2009). Filipin administration results in the disruption of these lipid raft or GEMs, thus inhibiting signal transduction pathways. In bystander cells, damage manifests itself in the form of point mutations which can cause the formation SCE versus directly hit cells which experience partial or total gene deletions. The addition of Filipin can also suppress the induction of sister chromatid exchanges in bystander cells when compared to the controls (Nagawasa *et al.*, 2002). However, when exposure levels are increased to 10 cGy, filipin fails to suppress induction of SCE's in bystander cells. This shows that cell membrane signaling is vital for transmission of the bystander signals that result from very low doses of exposure (Nagasawa *et al*, 2002).

Lehnert *et al.* (1997) was the first to suggest that radiation damage could in part be due to transmissible factors. The authors show that alpha particle exposures at low doses result in the production of extracellular factors which can cause excessive SCE frequencies in unexposed normal human cells. Further exploration into these extracellular factors reveals that superoxide dismutase (SOD) administration can inhibit excessive SCE activity (Lehnert *et al.*, 1997). Azzam *et al*'s. (1998) experiment was repeated by taking the supernatant from alpha irradiated cells an exposing it to unirradiated cells. Results show a reduction in TP53 and CDK1A (negative regulators in cell cycle progression) protein expression in completely un-irradiated cells (Iyer *et al.*, 2000). Furthermore, when cell counts are performed 3 days after receiving supernatant from irradiated cultures, a 150% increase in growth is seen when compared to sham controls (Iyer *et al*, 2000). This suggests that medium soluble transmissible factors within medium can also contribute to radiation induced effects (cell death or cell proliferation)

in non-irradiated cells, thus direct cell to cell contact is not required. Mothersill and Seymour (1997c) group was the first to show that exposing cells with low LET gamma radiation causes them to release soluble factor(s) into the medium, which can cause increased cell death of unirradiated cells. The authors also show that not all cell lines are capable of producing bystander factors while some cell lines are not able to receive the signal (Mothersill and Seymour, 1997c). The natures of these soluble factors are a subject of intense debate, and will be further discussed in another section.

1.3.4 Mechanism of Action of the Bystander Signal(s)

Once the bystander signal reaches the bystander cell, it can cause a variety of cellular 'changes' that work on a higher homeostatic level to execute a 'bystander response'. Lyng *et al.*, (2000), shows rapid induction of intracellular calcium immediately upon exposure to conditioned medium (ICCM), followed by the loss of mitochondrial membrane potential and an increase in reactive species. In fact, the ICCM from initially irradiated (0.5 Gy) cells as well as the ICCM from their progeny (7^{th} generation) is able to induce calcium flux and loss in mitochondrial membrane potential (Lyng *et al.*,2002a and 2002b). (Kroemer *et al.*'s (1997) group shows that a reduction in mitochondrial membrane potential occurs before nuclear DNA fragmentation, after which reactive oxygen species are produced. These cellular changes are considered to be early stages of the apoptotic cascade. Similarly, Ojcius *et al*'s (1991) group demonstrates that when cells are exposed to various toxins, the DNA molecules become fragmented (associated with apoptosis). This is accomplished due to the release of calcium from

intracellular compartments into the cytoplasm. The cellular responses discussed above can cause various forms of damage such as delayed chromosomal instability, mutations, micronucleus formation, and delayed death. However, they all occur at very low doses and upon induction, they become permanent characteristics of the cell populations.

Others have explored additional pathways that could transmit the bystander response. For example, Zhou *et al.* (2005) shows that inhibition of COX-2 (Cyclooxygenase- 2) in bystander cells effectively reduced expression of bystander effects. COX-2 is a downstream substrate in the MAP-K (mitogen-activated protein kinase) pathways such as ERK, JNK, and P38. Activation of various MAPK pathways also occurs after direct radiation exposure (Dent *et al.*, 2003) as well as in bystander cells (Azzam *et al.*, 2002). Inhibition of some of the MAP-K proteins results in the inhibition of bystander responses (Lyng *et al.*, 2006).

1.4 Source/Nature of the Bystander Signal

1.4.1 Source of Bystander Signal(s): What is the critical target?

Most research papers show that radiation primarily targets DNA either directly or indirectly, and it is DNA damage that modulates cellular response. The recent understanding that radiation induced damage can occur in cells receiving medium from irradiated cells has challenged this conclusion. However, bystander effects do require that some cell receive energy from irradiation and the link between DNA damage and bystander effects is strong. Since bystander responses require an integrated effort from various constituents of the cell, research has started to explore the role of other cellular

organelles in mediating radiation response. As discussed previously, inhibition of cell membrane signaling can eliminate bystander responses in unirradiated cells (Guan, 1998). In fact, the role of cell membranes as a critical target for radiation has been previously reported. A study by Gulbins and Kolesnick (2003) states that ionizing radiation can alter phospholipid composition, and thus modulate transmembrane signaling. This observation is supported by Benderitter *et al.*'s (2003) work which shows changes in cell membrane content after exposure to irradiation. This suggests that perhaps the cell membrane and its constituents act as primary targets for radiation action and subsequent bystander signal production. It is also known that the lipid component of phospholipids can initiate peroxy-radical formations, which are known to cause DNA damage (Freeman and Crapo, 1982). So whether cell membranes or DNA strands serve as the primary target for radiation induced bystander effects after low dose exposure is still under investigation.

Mitochondria are also suspected as playing a role in expressing radiation damage. There is extensive research that deals with radiation induced mitochondrial responses such as initiation of apoptosis and the generation of ROS, both of which are involved in oxidative stress responses (Janssen *et al.*, 1993). However, mitochondria also contain their own DNA as well as an arsenal of genes that regulate various cellular functions (Koehler *et al.*, 2004) so naturally, studies have looked into effects of radiation damage on mitochondrial DNA. Gaziev and Podlutskii (2003) show that although mitochondria contain various regulatory genes, they lack an efficient DNA repair system. Their role in the production of ROS species and the initiation of the apoptotic cascade are well

accepted responses to radiation, however, they role as critical target for energy deposition has not been established.

The endoplasmic reticulum is another organelle that shows fluctuation in protein expression and protein assembly in response to radiation (Khizhniak *et al.*, 1990 and Shroder *et al.*, 2005). Extraceullar stimuli can result in the accumulation of misfolded proteins, which can result in programmed cell death. There is limited research that looks into the ER as the primary target of radiation (Rudner *et al.*, 2001), but their secondary role in mediating radiation induced stress due to protein misfolding (from point mutations in DNA) is undisputed.

Mothersill and Seymour (2006a) brings up an interesting concept, " *It could be argued that virtually all 'damage' is secondary, and that effects such as DNA strand breaks that we ascribe to energy deposition in a target are actually resulting from apoptosis, triggered by cellular response.*" This perspective gives a refreshing outlook on the issue of assigning primary and secondary sites of radiation damage. Is it possible that accumulation of stress induced cellular responses is modulating DNA damage? Consequently, does this imply that various components of the cell (membranes, ER, Mitochondria) are all the primary sites of radiation action?

1.4.2 Nature of the Bystander Signal

The nature of the bystander signal(s) is unknown, although various studies have shed light on its properties. Lehnert and Goodwin (1997) discuss that medium borne signals that are able to induce bystander responses, survive freeze-thaw cycles but are

susceptible to heat damage. This data suggests that bystander signals(s) are protein-like in nature but cell derived. It is also suggested that bystander responses can be communicated via soluble factors such as reactive oxygen species (ROS) (Lehnert *et al.*, 1997). The role of ROS in the induction of radiation damage is confirmed by showing that the addition of superoxide dismutase (SOD) immediately before medium transfer to un-irradiated cells prevents the excessive occurrence of SCE's (Lehnert *et al.*, 1997). In addition to this, Narayanan *et al.* (1997) shows intracellular generation of superoxide anions in cells receiving alpha radiation doses as low as .4 cGy, which peaks at 15 minutes after exposure. Similarly, hydrogen peroxide production peaks are seen at 15 minutes only in the lower dose ranges. This response is inhibited by NADPH oxidase, inferring that the primary source of radiation induced ROS signaling is the plasma membrane bound NADPH complex (Nayanan *et al*, 1997). In addition to ROS production, Narayanan et al (1999) shows up-regulation of Interleukin 8 protein and mRNA expression after 3.6 to 19 cGy of alpha radiation. These effects are completely suppressed by SOD administration at time of insult, suggesting that induction of IL8 occurs in parallel with increased ROS production. However, due to the short half life of ROS species (10^{-9} to 10^{-10}), it is doubtful that they act direct mediators of bystander response from irradiated to unirradiated cells. Therefore, logic dictates that a factor (s) that is manipulated or initiated by ROS could possibly be the primary bystander factor(s). In fact, ROS elicits a factor called transforming growth factor- beta 1 or TGF-beta-1 (Rhyu *et al.*, 2005), which is a cytokine known to initiate production of extracellular matrix, arrest cell cycle in G1 phase in epitheial and endothelial cells,

stimulate angiogenesis and participates in various other disease states (Globe *et al.*, 2000). This cytokine is also suggested to partake in the transmission of bystander effects in normal human fibroblasts, an effect which is eliminated by the addition of TGF-B1 inhibitors (Iyer *et al.*, 2000).

1.5 Types of Bystander Responses

Previously mentioned papers show that (Azzam *et al.*, 1998 and Iyer *et al.*, 2000) bystander cells can express different responses depending on whether the signal is transferred through gap junctions or through soluble media. This gives rise to the concept that perhaps the nature of the bystander signal and/or cellular response is subject to modulation from genetic as well as environmental conditions.

1.5.1 Genetic Background and Bystander Signals

Mothersill *et al.* (2001) show that tissue biopsy samples from over 100 patients and various mouse strains produce various types of effects in the recipient unirradiated cells. Mothersill *et al.*'s (2004) study shows a differential bystander response from repair deficient cell lines versus normal cell lines. Cells from various repair deficient cell lines show a severe reduction in clonogenic survival when compared to the repair proficient cells lines, which show very low or no bystander response. Furthermore, Mothersill *et al's.* (2005) group discusses how genetic predisposition can influence the type of bystander signal produced in murine bladder explants after receiving low doses of radiation. O'Dowd et al (2006) shows that a decrease in EPC (epithelioma papulosum cyprinid) reporter plating efficiency occurs in response to medium obtained from *ex vivo*

irradiated gill explants from the rainbow trout. However, there is an increase in reporter plating efficiency when they are exposed to medium from irradiated spleen explants. This reveals that signal production is modulated by tissue origin. O-Neil-Mehlenbacher *et al.*'s (2007) group compares the effect that ICCM obtained from various irradiated fish cell lines have on HPV-G reporters as well as autologous fish cell lines. Results indicate that half of the irradiated fish cells induce an increase in reporter cloning efficiency when their ICCM is placed onto unirradiated HPV-G and autologous cells, where as the other half show reduced reporter survival. This shows that not only is bystander signal production modulated by genetics, but so is cellular response. These studies reveal the importance of genetic background in determining the response to bystander signals in unirradiated cells.

1.5.2 Adaptive Responses

Most of the experimental evidence presented above shows adverse effects that are observed after exposure to a single dose of radiation. However, Olivieri *et al.*'s (1984) group was the first to show adaptive responses. The authors show significant reduction in chromosomal aberrations in lymphocytes only if they are initially exposed to tritiated thymidine before exposure to high doses of X-rays.

1.5.2.1 Adaptive Responses in Vitro

Since then, many studies have reported stimulatory rather then death inducing bystander effects as a result of repeated exposures to radiation. The time interval between a priming and challenge dose required to induce protective responses can be highly variable with some studies observing protective responses after only 4 hours

(Shadley *et al.*, 1987). Other groups show significant adaptive effects when the priming and challenge dose is separated by 24 hours (Maguire *et al.*, 2007) up to 40 days (Cai *et al.*, 1995). Maguire *et al.*'s (2007) group shows a 10 – 15 % increase in cell sparing in HPV-G reporter cells when they are initially exposed to ICCM and then challenged with a higher ICCM dose. This suggests that the presence of a priming dose can impose a protective effect against subsequent higher doses of radiation.

There is considerable interest in low dose radiation induced adaptive responses. Ryan *et al.* (2007) shows a 'protective' bystander response in three different fish cell lines that are given a .1 Gy priming dose eight hours before the challenge dose. The ICCM derived from the primed fish cells cause an increase in cloning efficiency in unirradiated reporters, when compared to the controls. A recent study by Seymour and Mothersill (2006) sampled blood samples from patients after their primary radiotherapy session, midway through the radiotherapy treatments and six weeks post radiotherapy. The serum extracted from these samples exhibit variability in the toxicity of the bystander effect it induces from non-irradiated HPV-G cells. The isolated serum samples that produce the greatest bystander effect (death) after the primary treatment also produces the greatest adaptive response (colony survival) six weeks post therapy (Seymour and Mothersill, 2006). This shows that the magnitude of initial damage can determine the magnitude of the adaptive response observed. The mechanism responsible for bystander induced adaptive effects is possibly the consequence of upregulation of an efficient repair system in cells that experience a priming insult low enough to cause damage but not significant enough to cause extensive cellular damage. Given that

enough time is provided after the priming dose, small amounts of DNA damage activate specific genes that consequently induce the transcription and activation of various proteins that allow for repair. In fact, the rate of rejoining DNA double strand breaks induced from challenge dose in adapted cells is higher than in non-adapted cells (Ikushima *et al.*, 1996). This effect can be completely suppressed by protein synthesis inhibition (Ikushmia *et al.*, 1996).

1.5.2.2 Adaptive Responses in Vivo

Adaptive responses are also reported within humans who are environmentally exposed to radiation (*in vivo*). For example, areas such as Ramsar (Iran) have background radiation levels that are considerably higher then those present in North America (5x higher). When lymphocytes from Ramsar residents are extracted and exposed to 1.5 Gy of radiation *in vitro*, results showed a significant reduction in the frequency of chromosomal aberrations when compared to the lymphocytes extracted from people in normal background radiation (Ghiassi-nejad *et al.*, 2002). Tao *et al.*'s (2000) group reports that residents from a high radiation areas in China (Yanjiang) show lower mortality rate when compared to people from control regions. Another *in vivo* study looks at micronucleus (MN) formation in lymphocytes extracted from medical radiation workers. The MN frequency within the lymphocytes is seen to decrease (relative to unexposed controls) after they are exposed to a 1 and 2 Gy challenge dose (Gourabi and Mozdarani, 1998). These studies indicate that repeated exposures to low doses of radiation may confer an adaptive response in *vitro*. Although the mechanism behind this

phenomenon is unknown, various studies propose the involvement of DNA repair related proteins such as poly (ADP-ribose) polymerase (PARP), DNA dependent protein kinase (DNA-PK), ataxia telangiectasia mutated (ATM) and p53 (Matsumoto *et al.*, 2007; Shadley *et al.*, 1987; Szumiel, 1998; Wiencke *et al.*, 1986).

These *in vivo* and *in vitro* studies suggest that low-dose radiation is protecting cells against subsequent doses of radiation. Further investigation is needed to understand the mechanism behind adaptive responses.

1.6 Imaging

Biological response to radiation is traditionally characterized by the ability of cells which survive a dose to form colonies that are clearly viable and have undergone 5 to 6 doublings – i.e. they contain at least 50 cells and visible to the naked eye. Small colonies are then examined under the microscope, using the 50 cell threshold (Puck and Marcus, 1956) criterion as an indicator that these cells have the potential to 'survive'. As a result, inhibition of cell growth as a response to radiation damage is assumed to correlate with the inhibition of single cells to form colonies. However, Dewey *et al.*, (1963) shows that cells that do survive a radiation dose and produce progeny do not necessarily have the same doubling time as cells obtained from control treatments. In fact, measuring growth rate inhibition shows a greater radiation effect than using colony formation as an index, because the former accounts for the reduced proliferative capacity of survivors (Dewey *et al.,* 1963). Consequently, using colony formation as a criterion

for radiation effect ignores the fact that proliferative cells may have suffered some sort of non fatal damage which has manifest itself in the form of a reduced growth rate.

1.6.1 Heterogeneity in Colony Size

Moreover, Nias *et al.* (1965) reveals another parameter that provides additional information about sub-lethal effects of radiation damage. This group shows a broadening of colony size distributions as a result of radiation dose, when compared to the controls. This implies that the presence of slower growing colonies after irradiation do maintain some sort of proliferative capacity, thus suggesting that a minimum colony size of 50 cells is highly arbitrary index for reproductive capacity (Nias *et al.*, 1965). Furthermore, Sinclair *et al.*'s (1964) group shows that slower growing Chinese hamster cells that result from high doses of X -rays show indefinite proliferation when re-plated, although they maintain a significant slower generation times as well as higher radio sensitivity. This confirms that colony size and growth rate may be indicative of inherent damage (Sinclair *et al.*, 1964). So, information about size distributions in cell populations can provide information about levels of sub-lethal damage, which would be impossible to obtain from simple colony formation counts. Fluctuations in mean colony size have also been reported to occur in a manner that is highly dose-dependant (Spadinger *et al.*, 1994). For example, when exposures are relatively low (between 0 - .5 Gy), the mean colony area is seen to decrease, which corresponds to the hypersensitivity of cells reported in literature (Lambin *et al.*, 1992). A sudden increase in colony size is experienced when dosage is raised to about 1.3 Gy, which may be attributed to the induction of repair (Spadinger *et*

al., 1994). By using various endpoints such as mitotic lag, growth inhibition, colony size and colony formation, a complete description of radiation damage may be understood.

1.6.2 *Causation of Cell Size Heterogeneity*

The presence of heterogeneity in colony size was recognized as early as Puck and Marcus (1956), who stated the necessity in "distinguishing between abortive and slow growing colonies". The cause for diversification of colony size was discussed extensively by various groups. Thompson *et al.*'s (1984) group has documented that various human tumor stem cell lines show a hierarchy of proliferative capacities that give rise to a whole range of cellular sizes. It is speculated that this may be the result of variable delay in the onset of proliferation or as a result of inherent differences in the rate of cell division (Thompson *et al.*, 1984). Considering that clonogenic cells are defined by their capacity to produce a specific colony within an assay, the presence of genetic heterogeneity within a cell population alters the way results are understood (Seymour *et al.,* 1986). Further investigation by Joshi *et al* (1982a) shows that when Syrian hamster cells are irradiated during the G1 phase, they show no significant changes in their G1 progression to M1. However, when colony formation is measured after M1 was completed, the proportion of slow growers increases in a dose dependant manner and these cells tend to show higher levels of micronucleus formation (Joshi *et al.*, 1982b). Similarly, Grote *et al.*'s (1981) group shows that fast growing colonies show no post- mitotic (after IR) chromosomal damage where as the stop and slow growth colonies expressed abundant fragment loss. This confirms that colony size differences arise from radiation induced differences in cell growth rate after mitosis is reached rather then from delay in the onset of proliferation

caused by a defect in G1 progression. Overall, differences in colony size are a result of inherent damage that may be non-fetal, but they still represent a biological response to radiation that must be explored.

1.6.3 Subjectivity in Clonogenic Assays

In order to investigate sub lethal effects that may manifest in cell populations, clonogenic assays are employed to assess colony –forming ability. Usually, this process requires a large sample size so as to maintain a level of statistical accuracy. However, the traditional method of manual colony counting creates major discrepancies in colony identification. Lumley *et al.'s* (1997) group shows that significant differences exist between the counts of twelve different laboratories when scoring colony forming units (granulocytes from myeloid line) on the same set of slides. Thus, novel methods are needed to identify and measure colonies. Dobson *et al.* (1999) shows that by using an image analysis software program to pinpoint individual bone marrow cell (BMC) colonies on a coordinate system, the group can accurately quantify colony size and cell number. This technique allows for a detailed quantification and distribution of cellular response to BMC inhibiting drugs. However, imaging techniques can also produce fundamental problems such as failure to detect merging colonies and discrete fuzzy colonies. Attempts to solve these problems have led to the development of various processing algorithms or mathematical models. Thielmann *et al.* (1985) shows that automated counts produced consistently higher colony sizes (12% deviance) when compared to manual counts because unlike scientists, computers were not able to differentiate large clusters of colonies into smaller individual colonies. In order to deal

with this issue, Thielmann's group implemented novel mathematical models that determines an accurate colony count based on the probability value of weather the detected colony was in fact a conglomerate of 1, 2, 3, etc colonies. Mukherjee *et al.* (1995) implemented a different model for colony separation (aimed at merging colonies) termed the distance transform algorithm. This approach converts cell flask images into binary numbers of 0 (background) and 1 (colony), then extracts information about the local maxima which corresponds to the central most point of a colony. The colonies are consequently reduced to their central points, thus eliminating any overlapping of merging colonies, after which the central points are expanded into squares so as to estimate each individual colony size Mukherjee *et al.* (1995). Although this approach tend to distort the topology of the detected colony, it's appropriate structure can be recovered effectively. The fuzzy recognition algorithm implemented by Barber *et al.* (2001) consists of first locating threshold maxima's from each detected object and than calculating the maximum and minimum distance from this central point to the edge of the boundary. Furthermore, radial searches from the central point are carried out in thirty two different directions so as gain information about where the greatest gradient or change in grey level occurred. This information confirms the location of the boundary or edge, allowing for a clear construction of colonies. Marotz *et al.* (2001) discusses yet another algorithm termed the fuzzy recognition method which assigns each pixel in an image a unique coordinate system, after which it was assigned a measure of goodness as a potential object center depending on the distance to object boundaries. These parameters are then

compared to a set of shape, structure, and goodness threshold values that filter out unwanted objects (Marotz *et al.* , 2001 and Saha *et al.*, 2002).

1.7 Objectives

The risk to cells from low doses of radiation is receiving significant attention. However, further focus on the *in vivo* generation of bystander signals, as well as the role of genetics in the production of these signals, is vital for understanding how non-targeted effects relate to risk assessment.

- This thesis investigates whether a range of doses delivered *in vivo* to vertebrates can induce radiation like effects *in vitro* in a keratinocyte reporter system.

- Secondly, the role of genetic predisposition in the production of bystander signals is explored. This is accomplished by exposing C57BL6 (apoptosis-prone) and Balb3 (cancer prone) mice to single doses of gamma radiation, and then measuring the effect the medium harvested from the mouse bladder explants (ITCM) has on un-irradiated reporter cells *in vitro.* Differences in clonogenic survival in these reporters will indicate whether genetic microenvironment dictates the nature of signal produced, thus the type of response observed. Clonogenic survival as well as a marker for apoptosis is investigated so as to determine if these biological endpoints can be modulated by genetic predisposition.

- Further emphasis is placed on investigating whether bystander responses in reporter cells can be modified. This is performed by exposing both C57BL6 and Balb3 mice to repeated doses of radiation. The response is measured in reporter cells that receive ITCM from bladders removed from both sets of irradiated mice. These responses are compared to the responses observed in the reporters receiving medium from bladder explants of acutely exposed mice. This comparison allows our group to determine if a) repeated exposures given to mice *in vivo* can alter the nature of the bystander signals produced so as to modify the biological response seen in cells *in vitro* and b) if genetic predisposition can alter the nature of these signals in such a way so as to elicit different biological responses from the same keratinocyte reporter system.

- Finally, a secondary focus is placed on whether implementing imaging techniques can enhance the detection of bystander responses by allowing for measurement of subtle changes (growth rate and size distribution) in biological response.

2 MATERIALS AND METHODS

2.1 Cell Culturing

Experiments are conducted using a human foreskin kertinocyte cell line supplied as a gift by J. Di Paolo, NIH, Bethedsa. These cells are immortalized through transfection with plasmids containing complete Human Papilloma viral (16) genome so as to extend the proliferative capacity of these cells (Pirisi *et al.,* 1987). This virus is most often associated with malignant cervical cancer (Pirisi *et al.,* 1987). The HPV genes responsible for this immortalizing activity have been isolated as E6 and E7 proteins (Hawley-Nelson *et al.*, 1989). Repression of E6 protein expression has been documented to activate p53 expression leading to growth inhibition, apoptosis, and senescence (Horner *et al.*, 2004). Therefore, sustained degradation via polyubiquitination by E6 of p53 is required for maintenance of the proliferative capacity for immortalized kerintocytes (Ristriani *et al.,* 2008). HPV-G cells used here grow in culture to form monolayers of characteristic cobblestone-like patterns that exhibit contact inhibition and gap junction intercellular communication.

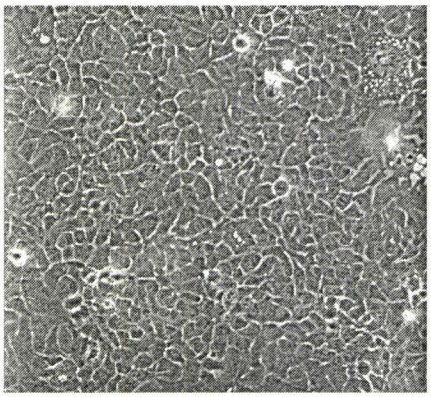


Figure 2.1: Phase contrast image of HPV-G cells in vitro (40x objective)

Moreover, these cells are used because when they are exposed to irradiated conditioned medium or (ICCM), they have been documented to give a reliable and stable bystander effect of about 40% reduction in colony survival over a wide range of exposure conditions (Mothersill *et al.*, 1998; Lyng *et al.*, 2006; Poon *et al.*, 2007; Mothersill *et al.*, 2006; Mothersill *et al.*, 2007). Under control conditions, this cell line showed a 15% plating capacity.

HPV G-transfected kertinocytes are maintained in RPMI 1640 (Gibco, Burlington,ON) that was first developed in Rosewell Park Memorial Institute. This is a basal medium consisting of vitamins, amino acids, salts, glucose and glutathione but lacks any proteins or growth factors (Moore *et al.*, 1967). Consequently, the medium must be further supplemented with 60ml of Fetal Bovine Serum (Invitrogen, Burlington, ON), 5 ml of Penicillian- Streptomycin (Gibco, Burlington, ON), 5 ml of L-Gluthamine (Gibco, Burlington, ON), 0.5 ug/ml hydrocortisone (Sigma-Aldrich, Oakville, ON), and 12.5 ml of 1M HEPES buffer solution (Gibco, Burlington, ON). All cells were

maintained in sterilized T75 cm^2 flasks (Falcon, Franklin Lanes, NJ) within 37°C, 95% humidity, and 5% carbon dioxide incubator.

2.2 Subculture

Cells that reach 80% -100% confluence are given a medium change 24 hours before they were sub-cultured. Cells are detached from the flask bottom by exposing the monolayer to 1:1 solution of 0.25 % trypsin/EDTA (10x) (Gibco, Burlington, ON) and Dulbecco's Phosphate Solution (1x) (Gibco, Burlington, ON) for 8 to 10 minutes in the incubator. After cells have detached, the trypsin/cell solution is suspended in 10 ml medium so as to neutralize the trypsin. Cell suspension is then pipetted gently so as to produce a single cell suspension, and 2 ml of this solution was added into a new T75cm^2 flask containing 20 ml of medium. This procedure was carried out under sterile conditions in a Class II biosafety unit.

2.3 Clonogenic Assay, Medium Change and Bystander Activity

1 ml of the stock cell suspension discussed in the above section is placed in 10ml isotonic buffer (VWR, Burlington, ON) and cell concentration of this aliquot is counted using a Coulter Counter model Z2 (Beckman Coulter, Fullerton, CA). A threshold is pre-set to gate the size corresponding to HPV-G cells. To correct for background materials of similar size present in the solution, 10 ml of isoton is placed in the counter and the resulting count is subtracted from the count obtained for the solution containing the cells. Once the corrected cell concentration is derived, the stock cell solution undergoes a series

of dilutions (1:10, 1:100, 1:1000) allowing for plating of appropriate cell numbers for survival (Puck and Marcus, 1956).

Flasks designated to receive transfer medium from bladder explants are plated with 500 cells per flask. The flasks are then incubated for six hours immediately after plating, after which the radiation conditioned medium is thawed and medium from replicate samples (explants) is pooled into a single sterile container. The pooled medium is then passed through a 0.2 um sterile filter (VWR, Mississauga, ON) so as to ensure that no cells or debris are transferred in the process. This is confirmed by examining aliquots of the filtered medium under the microscope. The medium from the recipient flasks is poured off, immediately followed by the addition of the 5 ml of filtered harvested medium. The non-treated controls do not require a medium change. All cultures were plated in 5 ml of medium in sterile T25cm^2 (Falcon, Franklin Lanes, NJ) total growth area, 50 ml total volume flaaqq1sks. Incubation occurred at 37^{o}C with 5% carbon dioxide in air.

After 7-14 days, cultures are stained with 2 ml carbol fuschin (VWR, Bridgeport, NJ) for maximum of 5 minutes. The dye is then rinsed off using water. Colonies are then counted using the 50 cell threshold described in Puck and Marcus (1956) in which cells reaching this limit are classified as true survivors. In order to compare treatments, percentage survival was calculated using the formula: PE = [# of colonies / # of cells plated] *100. Percent Survival is calculated using the formula = [PE of treatment/PE of non-treated controls] * 100. The colony counts are normalized to unirradiated controls

using the equation: MCF = Mean of [$\sum$ 100 / colonies counted in unirradiated controls]. % survival = [MCF * colonies counted].

2.4 Mouse Models and In Vivo Irradiations

All mice were bred and housed in AECL (Chalk River, ON). All the mice were non- pregnant females, approximately 4 months old, and all of them received whole body irradiation carried out with either a Co- 60 gamma beam 150C (for adaptive or priming doses), or a Co-60 Gamma_Cell 200 (for challenge doses). The dose rate for the gamma beam exposure is 0.5 Gy/min whereas the dose rate for the gamma cell 200 varied according to distance from the beam, between 162 and 168 mGy/min. The first types of bladders sent to McMaster University were C57BL/6J mice (about 4 months old). This strain is very common and refractory to various tumors, but still permissive towards mutation expression (Jackson Lab, 2009). The second strain of mice used was Balb3/CJ (4 months old) non pregnant female mice that were exposed to the same set of IR conditions as the C57BL6 mice. This strain of mice carries a mutation in a PK-DNA repair gene and shows radio-sensitivity. These mice were exposed to a range of doses under various exposure conditions listed in table 2.4.1.

Table 2.4.1 *Mice Bladders Sent to McMaster University*

	0 Gy	2 Gy	20 mGy	20 mGy followed by 2 Gy (4 hours later)	20 mGy followed by 2 Gy (24 hours later)	20 mGy followed by 20 mGy, followed by another 20 mGy which is followed by 2 Gy (48 hours later)
Arrived and Plated Sept, 2008 (C57Bl/6J)	2 mice	2 mice	2 mice	1 mouse	1 mouse	1 mouse
Arrived and plated Nov, 2008 (C57Bl/6J)	2 mice	0 mice	2 mice	3 mice	3 mice	3 mice
Arrived and plated Oct, 2008 (Balb3/cj)	3 mice	3 mice	3 mice	2 mice	2 mice	2 mice

The mice are sacrificed 24 hours after receiving the final dose. Bladders are surgically extracted from the mice under sterilized conditions, placed in sterilized transport medium, and couriered overnight to McMaster University.

Mink Frog (*Rana Septentrionalis)* samples were also collected from contaminated (tritium water and Carbon-14) and control sites. They were sacrificed without further radiation, placed in sterilized transport medium and shipped overnight to McMaster University.

Table 2.4.2 *Frog Bladders Sent to McMaster University*

Treatment				
Sex	Arrived and plated on July, 2008	Contaminated Site	Control Site	
	Male	2 frogs	1 frog	
	Female	3 frogs	3 frogs	

2.5 Bladder Explant Culture and Bystander Activity

Upon arrival to McMaster University, bladders are placed in sterilie petri dishes (VWR, Burlington, ON) and chopped into 3 pieces approximately $2mm^2$. The explants are then transferred into sterile T25cm2 50 ml flasks (Falcon, Franklin Lanes, NJ) with 2 ml of supplemented RPMI 1640 (Gibco, Burlington, ON) medium described above. The explants are placed in the 37˚C incubator, 5% carbon dioxide, and 95% humidity. After 48 hours, the medium from the explants is harvested and frozen in 2 ml aliquots. The explants are replaced with 2 ml of serum free medium (KGM, Clonetics Cooperation,) and incubated for 10-12 days. After this, the explants and surrounding cell growth are fixed in approximately 2 ml of 10% Formalin (Fischer Scientific, Kalamazoo, MI) and then stored in the dark.

In order to assess the effect of irradiated and control tissue conditioned medium (ITCM or CTCM) on unirradiated reporter HPV cells, medium that is harvested from the *in vivo* irradiated tissue explants is pooled with other samples of the identical condition. From the pool, 5 ml of the ITCM is syringed through a 0.2 micrometer filter (VWR,

Mississauga, ON) onto untreated HPV-G reporters. These reporters were plated at a density of 1000 or 500 cells/ flask. The remaining 1 ml of medium is reserved for further experimentation described later. After exposure to the control or bystander medium, the reporters were incubated for 7 – 10 days in 37°C, 5% CO2 and 95 % humidity. Once macroscopic colonies are observed containing at least 50 cells, the colonies are stained with 2 ml carbon fushin for 4 minutes and than rinsed off with water.

2.6 Ratiometric Measurement of Calcium

Intracellular levels of calcium relative to controls is a useful method in detecting cellular activity in response to an activating stimulus. This has proved to be difficult until recently with the synthesis of Fura -2 by Tsein *et al.* (1980 and 1984) that measures and displays the dynamics of cystosolic calcium. Fura indicators are derived from various chemical manipulations of p-hydroquinone into their final acetocymethyl ester (Tsein *et al.*, 1980) form. Fura -2 is then further manipulated through the addition of five acetoxymethyl groups linked to the five COO- groups on the parent molecule through ester bonds (Roe *et al.*, 1990).

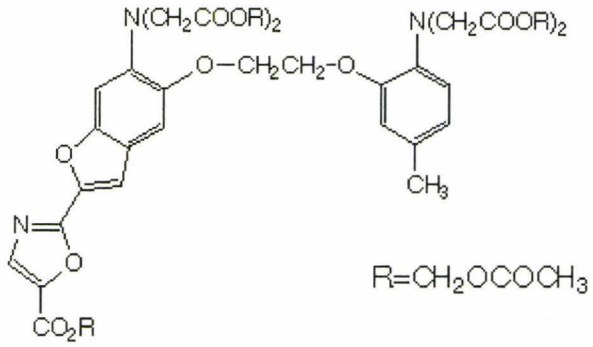


Figure 2: Molecular formula of Fura-2 derivative (Fura-2 AM)

This Fura-2 (AM) (Sigma –Aldrich, Oakville, ON) derivative is hydrophobic, thus able to penetrate the cell membrane effortlessly. Once inside the cell, cystosolic esterases cleave all the acetoxymethyl groups from the Fura-2 (AM) derivative recreating the highly charged and hydrophilic parent Fura-2 molecule that is unable to cross cellular or organelle membranes. This essentially traps the Fura within the cytosol, thus making it highly sensitive to changes in calcium concentration within the cystosol (Roe *et al.*, 1990). Calcium ion binding to Fura-2 has a unique ability to alter the wavelength of the fluorescence emission peaks upon excitation with UV light around 448nm. After excitation, any emission fluorescence seen at 380 (red) nm range represents free Fura-2 that is unbound to calcium; however, calcium bound Fura-2 shifts the emission spectra towards 345 nm (Roe *et al.*, 1990). The shift in the excitation spectra has been thoroughly described in literature as a result of the interaction between calcium and the lone pair of electrons on the amino nitrogen. This binding results in a disruption in the conjugation between the lone pair and the rest of the aromatic ring. Consequently, excitation spectra differ depending on whether calcium is bound or unbound to the Fura-2 molecule (Grynkiewicz *et al.*, 1985 and Tsien *et al.*, 1980). By taking the ratio of these two wavelengths (345:380), a meaningful measurement of cytosolic calcium concentration can be obtained. An increase in the ratio would represent an increase in free cytoplasmic calcium ions, whereas a decrease would show a decrease in cytoplasmic calcium concentrations. The detection of the fluorescence from the HPV-G cells is temporally measured with an automated Olympus 1X81 microscope using the 40x oil objective and a Fura filter cube with 510nm emission. Image acquisition is achieved by Photon

Technology International ImageMaster 5.0 software system. This system provides quantitative data of the spectral shifts of the Fura-2 emissions collected by the photomuliplier.

HPV-G cells are plated using the previously described method at 500,000 cells per plate (MatTek, Ashland, MA) and then left to incubate at 37°C and 5% carbon dioxide levels for 72 hours before measurements. After incubation, cells are washed in buffer solution that contained 130mM NaCl, 5mM KCL, 1mM Na2HPO4, 1mM MgCl2, and 25mM Hepes (pH7.4) three times. The Fura-2 (AM) is initially present in a 0.5 mg of yellow powder, which is then diluted with 1.2 ml of DMSO to yield 1200 uL of 420 uM Fura – 2 solutions. This solution is then aliquoted into 120 tubes, each containing 10 uL of Fura – 2 AM (420uM). Next, the cells are incubated with a 1 ml solution consisting of 990 uL of buffer to 10 uL of 420 uM concentration of Fura-2 (AM) for 30 minutes at 37°C. Next, the dye is discarded and the cells are rinsed with the same buffer three times next 300 uL of buffer is added to each plate for measurement purposes. With the camera binning set to 2*2 and the lens exposure time set for 977msec, twelve bit images are acquired. The field of view of the camera displays about 10 to 100 evenly distributed cells, out of which five are selected for the measurement. Once Fura- 2 is excited, measurements at 380nm and 345 nm are recorded every 4 seconds for 8-9 minutes. After 45 seconds to 1 minute of recording, 100uL of the harvested medium from treated and control explants samples is added to the cells. The pre-event time frame of 45sec – 1min is chosen so as to firmly establish a baseline reading. All measurements are made in the

dark at room temperature. The picture of the cells and ratio measurements are saved on a 500GB external hard drive.

Ratio of fluorescence at the two wavelengths versus time is then graphed using the Sigma Plus software system, thus illustrating the kinetics of free cytosolic calcium induction. The exact calcium concentration from fluorsence may also be quantified using the formula : $[Ca^{2+}]_i = K_d \times (R-R_{min})/(R_{max}-R) \times (S_{f2}/S_{b2})$ which has been thoroughly described by Grynkiewiez et al., 1985. R is the ratio of the dye's fluorescent intensities F1 and F2 at the two excitation wavelengths (345 and 380 nm). R min and R max represent the ratios for the unbound and bound of the Fura-2 and Ca2+ complex. The S factors are fluorescence intensities measured from calibration solutions with known amounts of low free calcium and calcium saturated dye. Thus, Sf2 is the limiting 380 nm fluorescence intensity without Ca2+, Sb2 is the limiting 380 nm fluorescence intensity in the presence of saturated amounts of calcium. Lastly, Kd is the dissociation constant for Fura-2 and calcium complex and has been determined previously by Dascalu *et al.* (2000) to be 244 nM.

2.7 Statistical Analysis

The data illustrated in this project are presented as a mean± standard deviation, where each treatment had N=3 for unless otherwise stated. Significance of differences are tested using two –tailed student's t test, with $P<0.05$ as the limit of significance. Differences in growth rates were analyzed with ANOVA, with sample sizes varying with each experiment set.

2.8 Image Analysis

This section will discuss modes of object (potential colony) detection and recognition as well as management of measurement data.

2.8.1 Experimental Setup, Calibration, and Filtering

After flasks are stained, they are photographed using a 8.1 mp digital Nikon camera. Once the image has been uploaded and imported into the Image –Pro Software page, it is vital to create/adjust the scale of the active image in terms of desired units of measurement. The software automatically makes all measurements in terms pixel positions, so this step will allow the user to define the number of pixels per unit of measurement that is under consideration (millimeters). This allows every measurement taken to be expressed in terms of a desired unit rather then standardized pixels. To make a standard calibration, a reference flask or image is placed under the CCD camera or it can be photographed and uploaded into the computer. In this case a photograph with a standard ruler aside a flask with a defined unit of measure (1 millimeter increment) was used.

[Select *Measure* from the Image Pro toolbar, than *Calibration*, and finally *Spatial …*]

- Proceed to click the *New* button. This allows all the calibration fields to become active. Change the text in the *Name* space to a desired title because this will specify the set of calibration about to be created (these values may be saved for future use).

- Alter the contents of the *Unit* space to the desired units you wish to work with (i.e. millimeters). This step allows the system to label all your measurements as millimeters (or any other preferred unit).

- In the *Pixel/Unit box*, select the *Image* button. Immediately, a *Scaling* box and a defining line will appear. First make sure that the reference represents 1 unit in the *Scaling* box. Then proceed to manipulate the defining line against a known standard- of – measure. In this paper, photographs from standardized distances are taken of T25 culture flasks and a plastic 12 inch ruler. The defining line was calibrated against the length of the ruler so as to represent 1 millimeter.

- Once calibration is completed, click *Ok* in the *Scaling* box and the *Spatial Calibration* box will reappear. The *Pixel/Unit* box should now read some pixel value for the given unit of measurement. This paper measures 22 pixels per unit of millimeter. Next, click *Ok* in the *Spatial Calibration Box.*

Once a unit of measure is calibrated for a certain pixel value, the user can recall this calibration from the system for any future flasks that may need analysis. To do this, select *Measure*, than *Calibration*, followed by *Spatial.* Once the *Spatial Calibration* window appears, select the desired name of the specific set of calibration values you want to use. Than click *Apply.* This will direct the system to employ the calibration value you want for the active image under analysis.

2.8.2 *Modification of Images : Filtering*

One of the problems with photographing flasks under laboratory conditions is the influence of external light sources. These conditions introduce uneven illumination patterns on the flasks which produce reflections and uneven background disturbances, especially near the edges of the flasks. Consequently, these 'disturbances' are detected as objects or they may hinder the detection of other objects. This may lead to error in object detection and inaccuracies in colony analysis. Nonetheless, an increase in precision can be obtained by increasing the resolution of the flasks, however, this also increases the duration of analysis. (Mukherjee *et al.*'s, 1995) group captures eight different views of the same image of each Petri dish and calculates the average pixel value of the eight views. Interestingly, the pixel values corresponding to the background remain constant in every image. However, since colonies display a non-planar surface, reflection of light off these surfaces always vary in a non-uniform way depending on the view. Knowing this, the authors subtract the averaged pixel value from pixel values in the individual slides, thus decreasing the pixel values of the background. This increases the contrast between colony intensities and the background intensity. Specialized algorithms have been employed by Dobson *et al.* (1999) and Dahle *et al.* (2004) that process and modify the image before it is analyzed. A filter called *Mean* calculates the arithmetic mean of each pixel and eight of its neighbors, thus suppressing noise and creating a smooth image. The filter *Laplace* strongly emphasizes the central pixel value, which sharpens the blurred image and drains out the edges. Finally, the *Median* filter places the median of nine adjacent pixels as the central pixel, thus suppressing noise and further smoothing out any

roughness in the image. The sequential applications of these filters compensate for the irregular illumination and provide a sharper image for analytical purposes. In this paper, similar image processing tools are used in order to dampen out illumination disturbances. These algorithms were implemented sequentially on a Pentium 4 processor in the Image – Pro Plus software system.

[Select *Process* from the Image –Pro toolbar, and than *Filters*]

- From the *Enhancement* tab, select the *Flatten* filter. This algorithm is commonly put in place because the background contains pixels of the same intensity as the objects of interest, and this evens out the intensity variations of the background pixels only. However, some flasks experience a green distortion in response to this filter. If this occurs, simply create an AOI (area of interest) surrounding the disturbance and apply the filter on a 10x feature width. Continue to apply the filter in various regions of interest until all disturbances are reduced. (Pay careful attention to the edges)

- Next, employ the *Laplace* filter on a 3 x 3 pixel grid from the *Edge* tab. This algorithm functions as an edge filter that accentuates intensity changes by modifying pixel values to exaggerate intensity differences from its neighbors. Basically, it amplifies intensity transitions, which allows slightly blurred and defocused object edges to sharpen.

- Next, utilize the *Median* (on a 3 x 3 pixel neighborhood) filter from the *Enhancement* tab. This algorithm modifies pixels that vary significantly from their

surroundings. This replaces the center pixel in a neighborhood with an average value of the neighborhood. This softens the image by removing random noise from the background, but at the same time preserves the edges.

- Finally, apply the *Open* filter from the *Morphological* tab on a large 7 x 7 pixel neighborhood. This algorithm performs erosions and dilations that function to smooth out breaks, removes all dark spots, and eliminate minor protrusions.

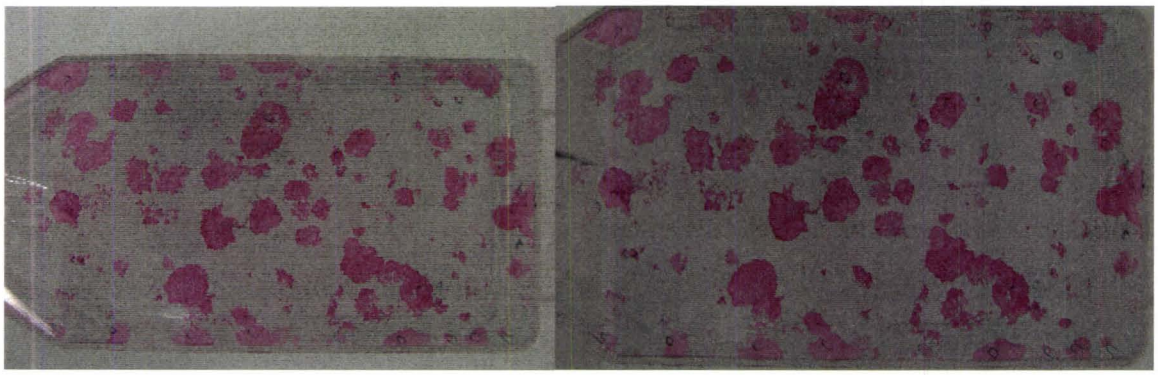


Figure: 2.8.2.1
Images taken before and after filter application: a) image on the left is before any type of image processing b) image on the right is after application of the four filters.

2.8.3 Object Segmentation and Colony Recognition

2.8.3.1 Segmentation

Once the image is processed, the next step is to detect potential colonies by performing grey level thresholding based on the color cube model. This allows for the separation of objects that are to be measured from the background. An object at this point is defined as a potential single colony or conglomerate of colonies with a grey level

intensity above a certain threshold. Initially, image processing counts used single grey level thresholding method that was able to detect colonies that possessed similar intensities (Corkidi *et al.*, 1998). In reality, colonies may have various grey – level values due to differences in illumination and size (Corkidi *et al.*, 1998). The first multi-level threshold algorithm incorporated the detection of a whole range of grey level intensities that allowed for automated detection of colonies of variant sizes (Corkidi *et al,* 1998).

[Select the *Count/Size* button from the Image Pro toolbar, then click on *Select Colors* from the *Intensity Range Selection* box followed by the *Color Cube Based* tab]

- Click on the *Eyedropper* button and move the cursor onto an area on the active image where you want to perform the segmentation. Left click on the area's or objects you want to extract, this will highlight all the other area's on the image which display the same intensity as the object you have selected. Repeat this step until all objects are highlighted. Be careful not to highlight any 'holes' within the objects, doing so will highlight the entire flask. If this occurs you may simply click the undo button which will remove the last action. Try to avoid selection of edges, but they can be manually removed later if necessary. Once the image is fully segmented, click Close. This will signal for the system to create an overlay which will define the range of objects you have just selected to be analyzed.
- Go to the *Measure tab* in the *Count/Size* box and click on *Select Measurements*. This box will display a range of possible parameters or measurement endpoints you wish to record for the objects previously selected. This paper looks at the area

which reports on all the pixels that have intensity values within the object perimeter recorded (minus any holes). The mean area will be calculated for each flask, and any colony with an area greater than the mean will be classified as 'large' whereas any colony with an area smaller than the mean will be classified as small. From this, a ratio of large to small colonies will be obtained from each flask, and then averaged across the entire treatment group (recipient, direct, and control). Also, the mean diameter which reports an averaged value at five degree intervals around the center of each object will be obtained for each flask, and then averaged across the entire treatment group.

- Return to the *Count/Size* window and select *Options*. The outline style should read "With Holes". This ensures that all the detected objects are surrounded by a line around its perimeter. The *Label Style* box should read *object number*. This tells the system to label all detected objects by number. After returning to the *Count/Size* window, press count. This will give you a count of all the objects detected. You may click on any object on the active image to arrive at the Attribute window which will define each object by its selected measurements.

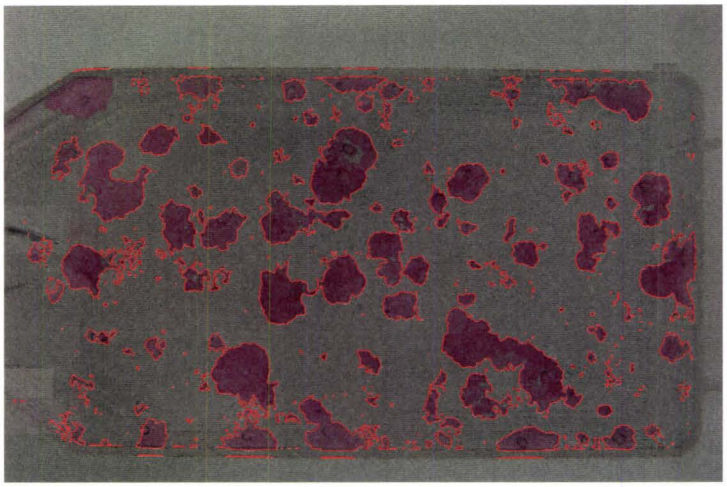


Figure 2.8.3.1
Image after grey level thresholding. Edges are erroneously detected as objects and merging colonies have been detected as a single colony.

2.8.3.2 Inclusion/ Exclusion of Ranges:

As shown in the above illustration, every object regardless of size has been detected. This again introduces error in colony count, because upon closer analysis, some selected objects are not viable colonies. Puck and Marcus (1956) found that abortive cell colonies rarely underwent more than four to five divisions after lethal damage, which corresponds to about 50 cells. Therefore, this threshold has a biological significance when distinguishing between a viable and non-viable colony. So implementing a similar threshold, in terms of millimeters, is a vital part of the procedure so as to ensure that analysis is only performed on viable or living cells colonies. In arriving at a threshold value in terms of pixels, timelapse photography of colonies is performed. This method requires that a live image of a cell flask is obtained using an inverted microscope and a CCD camera attached to a Dell desktop computer that is equipped with the Image Pro Plus system software. Once a colony is isolated, a photo snap is taken of the live image

under 10 x magnification, and each cell within the colony is manually tagged and counted. The colony which contained 50 cells is kept aside and it's associated area/ diameter is (same calibration scale used for all other flasks analysis) determined. The sample colony is displayed below.

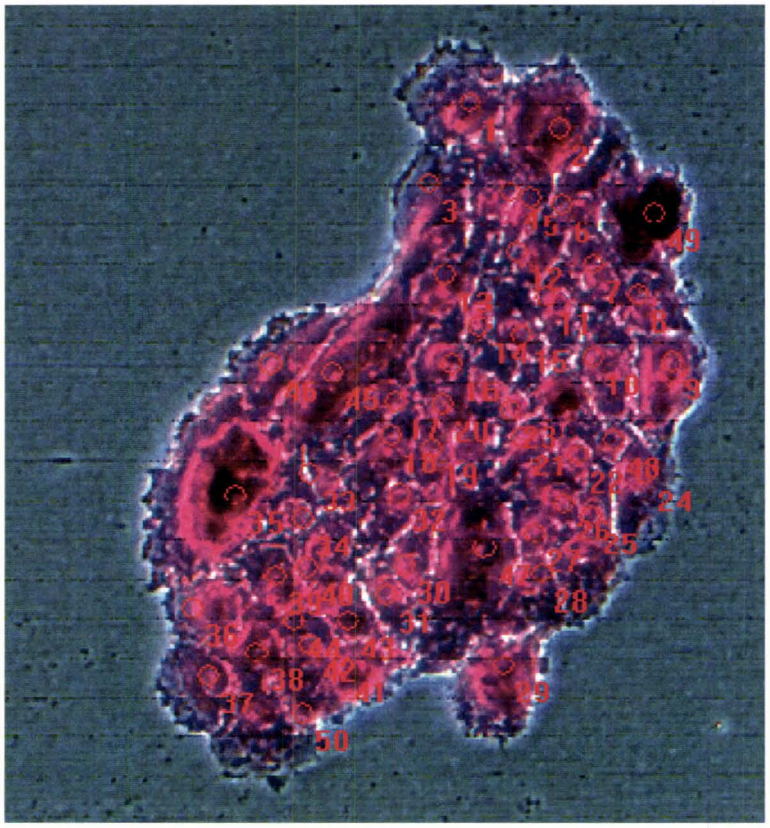


Figure 2.8.3.2 This colony contains 50 cells and its associated area was determined to be 0.2169 mm2. This area now represents the 50 cell threshold for cell survival.

Once the threshold values is determined, exclusion of all objects possessing a lower area and diameter value are removed. Previous literature stresses the importance of imposing thresholds so as achieve reproducible counts. Biston *et al.* (2003) enforces the exclusion of any object below brightness threshold value of 25 while Dobson *et al.* (1999) excludes

any objects below 1 mm in diameter or brightness threshold value of 20. To perform this type of object exclusion, go to the *Measure* tab in the *Count/Size* window and click on *Select Measurements*. Next, highlight Area, and place the threshold value in start range. Return to the Count/Size window and press Count. At this point a drastic reduction in colony count will be observed.

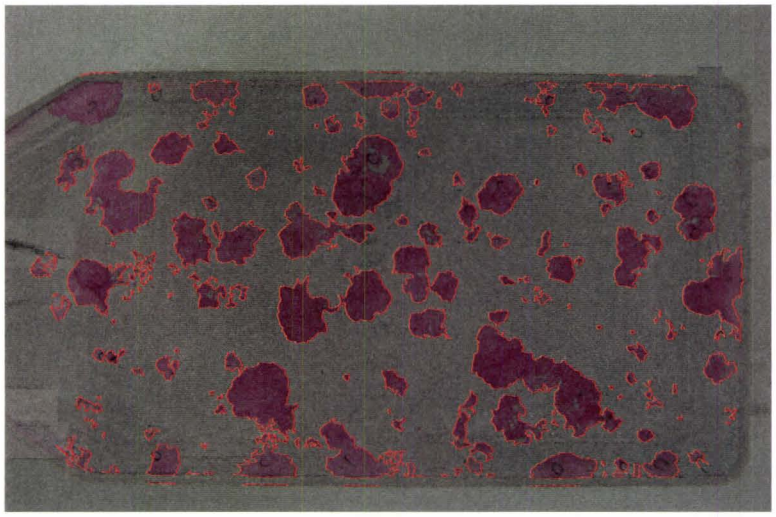


Figure 2.8.3.3 Image after the 50 cell area threshold is implemented. When compared to the last flask image, you can see a reduction in the number of objects now detected. The new count represents a true count of viable colonies only.

Due to the illumination techniques employed in this paper, the edges tend to display intensities similar to the objects located near the edges. As a result, when colonies are segmented by grey level thresholding, corners and unevenly shaded edges of the flasks are detected as colonies as well. Although filtering dampens out some of these uneven potions of the edges, the figure above shows that it can not completely remove them. This has proven to be a consistent problem when it comes to automated cell counting, and various approaches have been implemented to solve it. Initially, any colonies touching boundaries of the flasks were excluded from the counts (Thielmann *et al.*, 1985 and

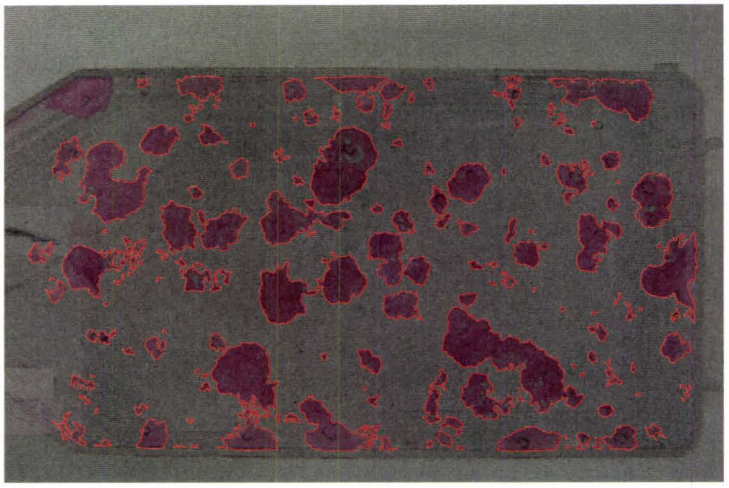


Figure 2.8.3.4 Image after false objects are toggled off around the edges. Reduction in erroneous colony count is

Another fundamental challenge with automated counting involves the proper segmentation of touching/merging colonies, which can prevent underestimation of colony count. Thielmann *et al.'s* (1985) group shows that automated counting yield significantly large deviations from manual counts due to the fact that counters recognize clusters of colonies as a single colony whereas experienced observers realize them as conglomerations of single colonies. This suggests that deviations from manual and automated counts are not random and that correctional programs must be implemented. Various statistical and technical approaches have been developed so as to reduce complex merging objects into their constituent single colonies. Thielmann *et al.* (1985) achieves this by applying a mathematical model that reflects the probability with which each counted colony would consist of one, two, or more colonies, as a function of total number of colonies per dish and the area they cover. Slocum *et al.* (1990) introduces a statistical approach that assigns a merged colony an area that is equal to the averaged sum of the areas of the individual colonies that make up the merger. Mukherjee *et al.* (1995)

discusses the application of the distance transform algorithm which identifies local maxima's of 3x3 pixel areas. These maxima's represents a core points (center of colony) which is expanded to a square using region-growing algorithms, and all the pixels within that square are assigned a specific integer values. However, during expansion, the growing region may encounter other core points (representing merging colonies) which will be subtracted from the pixel set under consideration. Eventually, all core points and their respective regions are considered separately and given specific pixel values. This way, conglomerate colonies are dissected into their component parts and recognized as separate colonies. Over time, gray-scale images are conceptually organized as topographic relief's in which the gray tone of a pixel was understood as an elevation at that point. This type of representation allows researchers to represent adjacent or overlapping colonies as catchment basins which are associated with the minimum of a set of pixels. The line that separates different catchment basins builds watersheds or dividing lines. In essence, catchment basins represent desired objects and if the counters of these basins can be extracted, than overlapping colonies may be properly segmented. Vincent *et al.* (1991) and Malpica *et al.* (1997) propose that binary images that have undergone distance transform algorithms display colonies as a central point that is surrounded by a region. Next contours (watershed lines) are identified by locating lines of pixels where the gray-tone varies quickly compared to the 'neighborhood', thus allowing for the separation of the overlapping components. Malpica *et al.* (1997) discusses that in order for histogram thresholding (grey level thresholding is a type of histogram thresholding) to correctly segment clusters of nuclei, there must be pixels with background intensity

between every two nuclei (in our case, between two colonies). Since this does not occur in most cases, separating clusters of cells become very challenging. The authors demonstrated that algorithms based on gradient watersheds (Vincent *et al.,* 1991) yield highly accurate divisions when compared to segmentation based on internuclei gradients or domains of influence. The latter method relies on simple geometry of the colonies whereas gradient watersheds rely on morphological information such as size, shape, and pixel intensity of the objects, thus producing more reliable segmentations. However, (Vincent *et al.*, 1991) implementation of watershed algorithms may still present erroneous counts since they tend to over segment merging colonies. Additional approaches (Barber *et al.,* 2001) use only edge information of an image and implement the Hough transform so as to highlight the centers of objects only. Since this algorithm does not take into account object contours or boundaries, chances of over segmentation decrease. However, edge enhancement leads to object detection of straight lines in addition to circular shapes. Correcting these errors require additional calculations that are time consuming. Dahle *et al.* (2004) describes a novel method that consists of analyzing the *inflection* (Nflex) and *shape* parameter of each conglomerate colony. Changes in colony curvature from being convex outward to convex inward is detected as an indentation in the colony perimeter, thus indicating a transition between colonies. Information of colony shape further supplements decisions on colony numbers of a spot. Nevertheless, some ambiguity remains as indentations may be blurred or unrecognizable as a result of smoothing during filter processing (Dahle *et al.*, 2004) Taking into consideration the level of ambiguity that remains despite implementation of various time

consuming algorithms, it is fair to suggest that manual splitting of merging colonies will produce a fairly accurate count of merging colonies. Consequently, this paper employs manual splitting of merging colonies based on the criterion that involves the consideration of degree of elongation and number of indentations described by Dahle *et al.* (2004).

[In the *Count/Size* window, select *the Edit* tab. Next select the *Split Objects* option]

- Place the cursor just outside the colony you want to separate and left click the mouse. This tells the system where to start the division line. Drag the cursor across the surface of the exact area you want to split and left click again to end the division line. Right click once to finalize the division. Scan the entire flask making sure to divide any merging colonies observed. Than click *Ok*, and your new count will automatically be updated on the *Count/Size* window.

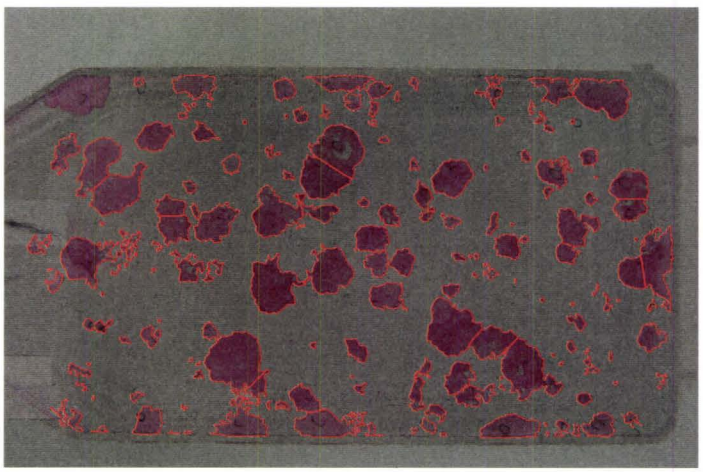


Figure 2.8.3.5 Image after merging colonies have been

2.8.4 Data Management and Analysis

After colony counts have been performed, the size distribution data and the counts are imported into an excel worksheet, after which they are plotted in the Sigma 9.0 software. Timelapse measurements are recorded manually and then transferred into excel and Sigma 9.0 for graphing purposes. Digitized images of the flasks are imported into excel as well.

2.8.5 *Time-lapse Photography and Growth Rates*

Serial photography of colonies chosen at random in each treatment flask are tracked over the course of the incubation period. Once an image is captured, the area of the colony is measured. At the end of the incubation trail, net growth of each colony is calculated using the formula: Net Growth = [Final Colony Size- Initial Colony Size]. The median of all the net growth values is used as the critical value from which fast growth are separated from slow growth colonies. After this point, slow and fast growth colonies are analyzed separately. All growth data is imported into Sigma Plus 9.0 software where growth rate graphs are constructed.

3 RADIATION INDUCED RESPONSES IN MICE GIVEN *IN VIVO* RADIATION

3.1 ABSTRACT

Until recently, the paradigm for radiation induced damage in living tissues has been modeled from observations based on the genetic changes in DNA from directly irradiated cells, tissues, and individuals. However, the bystander response phenomenon challenges these assumptions by showing radiation induced changes in cells that have not been directly targeted, but are neighbors to or receive medium from directly hit cells. Previous data show cellular responses to non-targeted radiation exposure *in vitro* but investigation of the *in vivo* production of bystander signals remains relatively unexplored. Our group has performed a range of single and serial, low dose irradiations on two strains of mice that have been documented to show genetic differences in their response to radiation. Bladder explants established from these mice are then incubated in culture medium, which is then used to measure apoptotic response (cell survival and calcium flux) in the keratinocyte reporter system. This study reveals that culture medium from acutely irradiated C57Bl6 mice, but not Balb3 mice, induces dose-dependant clonogenic death. The administration of a priming dose(s) to C57BL6, but not Balb mice, leads to stimulatory growth effects in reporters regardless of the time separation between the priming and challenge dose. When the transfer medium is measured for its calcium inducing ability, results show abnormal calcium levels in both strains of mice only after the administration of a priming dose. These results indicate that genetic predisposition influences the type of bystander signal that is produced after exposure to low, acute doses

of radiation. However, when mice are repeatedly exposed to radiation, the bystander signal is modified in a way that may be causing unregulated growth in reporter cells.

3.2 RESULTS

Medium samples are transferred from bladder explants established from *in vivo* irradiated C57BL6 and Balb3 mice to HPV- G transfected human keratinocyte reporter cells. Several endpoints of biological response such as clonogenic survival, intracellular calcium flux, growth rates and colony sizes, have been measured.

3.2.1 Clonogenic survival of reporters exposed to medium from bladder explants cultured from irradiated C57Bl6 mouse samples (Figures 3.1 and 3.2)

Manual colony counts show that medium taken from bladder explants obtained from C57BL6 mice given 2 Gy of whole body radiation, significantly reduces the percentage of survival of reporters from 100% (in reporters corresponding to unirradiated control mice) to 12% ± 52.835 (Figure 3.1). Medium from bladders established from 20 mGy irradiated mice significantly increases the percentage of surviving reporters when compared to the bladder medium harvested from 2 Gy irradiated, but causes a reduction (47%) in reporter survival when compared the reporters associated with the 0 Gy irradiated mice. The bladder medium from mice given a 20 mGy priming dose, twenty four hours before a 2 Gy challenge dose (24 hour group) causes an increase (84% increase in cell sparing when compared to 2 Gy reporters) in reporter percent survival to levels rivaling that of the unirradiated controls. An insignificant decrease in percent survival is observed in reporters given bladder medium from mice exposed to a 20 mGy priming dose four hours versus twenty four hours before the challenge dose. Similarly, when mice are repeatedly exposed to 20 mGy (3 times)

priming doses, forty eight hours before a 2 Gy challenge dose (48 hour group), no significant differences in colony survival can be seen when compared to the reporters exposed to medium from bladders extracted from mice in the 4 or 24 hour group.

After culture flasks are stained, they are photographed and uploaded into Image Pro where they are processed for colony counts. Figure 3.2 displays digitized images of stained reporter colonies that have been processed using the grey-level thresholding algorithm. The discrepancy between the manual and automated counts increases in reporters given bladder medium treatments from mice in the primed radiation groups. Colony counts of reporters given medium from bladders extracted from acute dose treatment groups (2 Gy and 20 mGy) show minimal discrepancy (fig 3.1) between the automated and manual counts. Looking at the photographed images in figure 3.2, the reporters that show the smallest discrepancies between the colony counts are also the ones that show the greatest cell death (C and D). The reporters that are given medium from bladders from treatments consisting of a priming and challenge dose, specifically the four hour treatment group, show drastic discrepancies between their manual and automated counts (fig 3.1). These discrepancies correlate with an increase in colony survival and greater automated detection of smaller colonies (Fig 3.2E, F, and G).

3.2.2 Clonogenic survival of reporters exposed to medium from bladder explants taken from irradiated Balb/c mice (Figure 3.3 and 3.4)

When Balb/c mice are exposed to various doses of radiation, the corresponding bladder medium induces different responses in reporters when compared to the reporters

associated with the irradiated C57B16 mice. The bladder medium from the 2 Gy exposed mice did not elicit a death response in the corresponding reporters (Fig 3.3). The bladder medium taken from 20 mGy irradiated mice induces a higher percentage of reporter survival when compared to the reporters given bladder medium from the 2 Gy irradiated mice. When the Balb/c mice are treated with a 20 mGy priming dose four (4 hour group), twenty four hours (24 hour group) before a challenge dose, the medium harvested from their corresponding bladder explants causes a significant reduction in the percentage of surviving reporters when compared to the reporters given bladder medium from the unirradiated controls and the acutely (2 Gy, 20 mGy) exposed mice. A similar decrease in reporter cell survival is induced by bladder medium extracted from mice exposed to a series of 20 mGy priming doses (3 priming doses delivered 48 hours apart) followed by a 2 Gy challenge dose given forty eight hours later (48 hour group). No statistical differences in survival exist between reporters given medium samples extracted from mice in the 4 hour, 24 hour, and 48 hours treatment groups.

Figure 3.3 illustrates that these reporters show relatively small amounts of discrepancies between automated and manual counts. However, the reporters given bladder medium from the mice exposed to a single dose of 20 mGy and 2 Gy, show drastically higher automated counts. This correlates with an increase in the automated detection of small sized colonies in these two sets of reporters (fig 3.4C and D). The majority of the reporters that receive bladder medium from mice exposed to a priming dose at various intervals (4, 24, and 48 hours) before a challenge dose, are larger, thus

causing them to merge with one another, indicating faster rate of growth (fig 3.4E,F and G).

3.2.3 Calcium flux in reporters given medium harvested from bladder explants from irradiated C57BL6 and Balb/c mice (Figure 3.5 and 3.6)

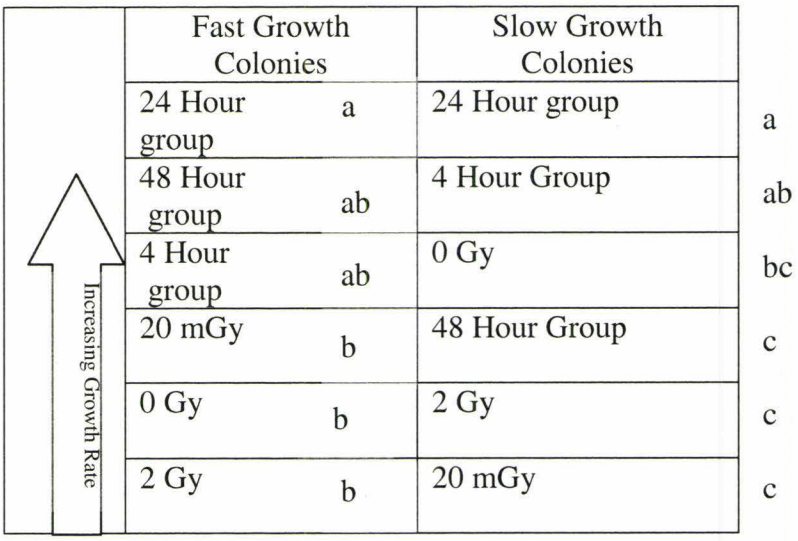
It is documented (Lyng *et al.,* 2002) that HPV-G reporter cells respond to bystander signals as a consequence of calcium signaling. This response is demonstrated by a transient induction of calcium within the cell cytosol after exposure to transfer medium harvested from bladder explants from irradiated C57BL6 or Balb3 mice. Medium that is removed from un-irradiated C57BL6 mice explants fails to induce a calcium flux in reporters (fig 3.5A). However, bladder medium from 2 Gy and 20 mGy irradiated mice (fig 3.5A) induces a transient increase in intracellular calcium levels that last for approximately 130 - 200 seconds. A transient calcium flux is also induced by medium that is taken from bladder explants from C57BL6 mice primed with 20 mGy, four hours before a subsequent 2 Gy challenge dose (fig 3.5B). However, medium from bladder explants from mice given 20 mGy and 2 Gy, twenty four hours apart, shows an initial decrease in intracellular calcium concentration at the time of addition of the transfer medium. This is followed by slight increase in the calcium levels which remain elevated for the remaining 250 seconds (fig 3.5B). Similarly, bladder medium taken from mice in the 24 hour group (20 mGy + 2 Gy exposures given 24 hours apart) also induces an initial decrease in intracellular calcium levels. This is followed by a rapid increase in cytosolic calcium concentration, which remains elevated for the remaining 200 seconds of measurement (fig 3.5B). Similarly, medium from bladders extracted from mice in the

48 hour group (20 +20 +20 mGy +2 Gy exposures given 48 hours apart) induces a spike in calcium levels at a faster rate and with a greater magnitude then the reporters exposed to the bladder medium from mice in he 24 hour group (20 + 2 Gy, 24 hours apart).

The bladder medium from irradiated Balb/c shows a slightly different pattern of calcium induction. Medium taken from bladders that originated in mice exposed to 2 Gy of radiation is seen to induce a transient calcium flux. However, the medium taken from bladder explants established from 20 mGy and 0 Gy irradiated mice, fail to do so (Fig 3.5A). On the other hand, medium harvested from bladder explants from Balb/c mice in the 24 hour group (20 mGy + 2 Gy exposures given 4 hours apart), shows a slight increase in the intracellular calcium concentration that remains elevated (Fig 3.6B). Bladder medium that corresponds to mice exposed to 20 mGy + 20 mGy + 20 mGy and another 2 Gy at forty eight hour time intervals, shows a similar calcium inducing response that is characterized by a transient decrease in calcium levels at the time of medium addition. This is followed by a rapid and persistent induction of intracellular calcium that lasts throughout the measurement period (approximately 300 seconds) (Fig 3.6B). In this case, bladder medium from mice in the 24 and the 48 hour group shows a similar magnitude of calcium induction (approximately 0.2 increase). However, the bladder medium taken from C57BL6 mice in the 24 hour and 48 hour group shows an increase in calcium levels around the magnitude of 0.02 and 0.3 respectively (fig 3.5B).

3.2.4 Growth kinetics and statistical analysis of reporters given medium from bladder explants from irradiated C57B16 mice (Figure 3.7 and Table 3.1)

Randomly selected colonies in each treatment group are photographed daily using a CCD camera that is mounted on a microscope, and then measured for area growth using Image Pro software. On day 9, the colony area measurements are divided into fast growers and slow growers within each treatment group, and their growth is plotted using Sigma Plus 9.0 software. This provides information about their growth kinetics in response to the medium harvested from bladders from C57BL6 mice given various radiation treatments. In considering the fast growth colonies only, the reporters given bladder medium from 2 Gy irradiated mice show the slowest growth rate (fig 3.7A) [0.0268]. Decreasing the dose to 20 mGy causes an increase in growth rate in the corresponding fast growers (fig 3.7A) [0.0375], followed by the fast growth reporters corresponding to the unirradaited mice [0.0413]. The fastest rate of growth is seen in the reporters given bladder medium from mice in the 24 hour group [0.1879] followed by the reporters corresponding to the mice in the 48 hour group [0.1215] and the 4 hour group [0.0854]. Conversely, the slow growth reporters associated with C57BL6 mice irradiated with 20 mGy show the slowest growth rate [0.0011] where as the slow growth reporters corresponding to the 2 Gy [0.0029] and 0 Gy [0.0141] irradiated mice show a higher rate of growth. The slow growth reporters associated with C57BL6 mice in the 48 hour group, 4 hour group, and the 24 hour group show a progressively faster rate of growth [0.0067, 0.028 and 0.0381 respectively]. The diagram below displays the growth rate of the fast and slow growers in order of increasing rate of growth.



Fast Growth Colonies		Slow Growth Colonies	
24 Hour group	a	24 Hour group	a
48 Hour group	ab	4 Hour Group	ab
4 Hour group	ab	0 Gy	bc
20 mGy	b	48 Hour Group	c
0 Gy	b	2 Gy	c
2 Gy	b	20 mGy	c

Diagram 3.1 : *Schematic comparing growth rates of fast and slow growth reporters associated with irradiated C57BL6 mice. Growth rate increases from bottom to top. ANOVA statistical test is used to measure similarities between the various treatments within fast and slow growth reporters. The same letter designation implies similar growth rate, where as different letters imply statistically distinct growth rates.*

Table 3.1 looks at how growth occurs within reporters in a given treatment group. This is done by measuring the statistical difference between area values on day 1 to day 9. By doing this, it is possible to see fluctuations in growth experienced by cells over the course of the incubation period. The fast growth reporters corresponding to the unirradiated control possess a slower rate of growth until day 7 of incubation, at which point the greatest amount of growth is achieved. Reporters given bladder medium from 2 Gy irradiated mice show a consistent rate of growth throughout their incubation period. However, the reporters corresponding to the 24, 4, and 48 hour group show the fastest amount of growth in their last 3 to 4 days of incubation.

When growth rates between slow and fast growth colonies are compared within each treatment group using student's t test (table 3.1A), results show that fast growth

reporters that receive medium from bladders extracted from unirradiated mice show an accelerated growth beyond that of the slow growers on day 3 of incubation. However, reporters that receive bladder medium from mice in the 20 mGy, the 4 hour and the 24 hour treatment groups, show divergence in the growth rates between the fast and slow growers starting from day 1 of incubation. Reporters exposed to medium from bladders of mice exposed to 2 Gy of radiation showed differences in growth rate between the fast and slow growth colonies only on days 1 through 4, after which the growth rates became similar.

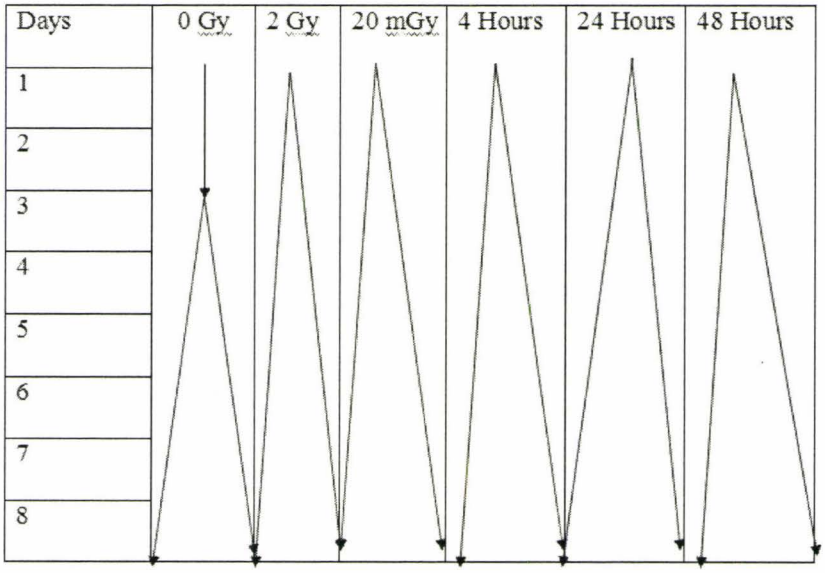


Diagram 3.2: *Onset of proliferation of fast growth reporters past the slow growth reporters across the various treatment groups. A single line represents statistically similar rate of growth between the fast and slow growers within a given treatment group. A fork in the line represents that the two types of growers are growing at statistically different rates. Significance determined by ANOVA (P>0.05).*

3.2.5 Growth kinetics and statistical analysis of reporters given medium from bladder explants from Balb/c mice (Figure 3.8 and Table 3.2)

Daily area measurements of reporters given bladder medium from irradiated Balb/c mice, provides information about the growth kinetics of the fast and slow growing colonies. Unlike the reporters corresponding to the irradiated C57BL6 mice, these fast and slow growth reporters show a homogenous stimulation (or inhibition) of growth under given treatment conditions. The reporters that are exposed bladder medium taken from 2 Gy and 20 mGy irradiated mice, show the slowest growth rate in both the fast [0.0313 and 0.0342 respectively] and slow growth colonies [0.0058 and 0.0064 respectively]. Reporters that are exposed to bladder medium from mice in the 24 hour (20 mGy followed by 2 Gy, 24 hours later) treatment group, show the fastest growth rate [Fast growth: 0.2355 and Slow growth: 0.514], followed by reporters exposed to bladder medium from mice in the 48 hour (20 mGy, 20 mGy, 20 mGy, followed by 2 Gy, 48 hours later) group [Fast growth: 0.1501 and Slow growth: 0.0347].

	Fast Growth Colonies		Slow Growth Colonies	
Increasing Growth Rate	24 Hour Group	a	24 Hour group	a
	48 Hour Group	a	48 Hour Group	ab
	4 Hour Group	a	4 Hour Group	ab
	0 Gy	a	0 Gy	ab
	20 mGy	a	20 mGy	ab
	2 Gy	a	2 Gy	b

Diagram 3.2 *Schematic comparing growth rates of fast and slow growth reporters associated with irradiated Balb/c mice. ANOVA statistical test ($P<0.05$) performed on these growth rates show that fast growth colonies growth at statically similar rates. The slow growth reporters corresponding to the 24 hour group show the fastest growth rate where as the reporters corresponding to the 2 Gy irradiated mice show the slowest growth rate. The remaining reporters all show statistically similar growth rates that are intermediate.*

Table 3.2 looks at the fluctuation of growth experienced by reporters within a treatment group over the course of incubation. Similar to the reporters from the C57Bl6 experiment, the reporters corresponding to unirradiated Balb3 mice show fastest rate of growth in their last three days of incubation. However, the reporters associated with 2 Gy and 20 mGy irradiated Balb3 mice show a similar distribution of their growth rates.

Growth rates are also compared between the fast and slow growth colonies, within each treatment group. Results show that fast growing and slow growing colonies within the control treatment group possess similar growth rates until day 5, after which fast growth colonies accelerate. Reporters given medium from bladders extracted from mice in the 20 mGy, the 4 hour group, and the 48 hour group show similar growth rates between their fast and slow growth colonies until day 4. However, reporters that are exposed to medium from bladders from mice in the 0 Gy, 2 Gy, and the 24 hour group showed divergence between their fast and slow growth rates immediately from day 1.

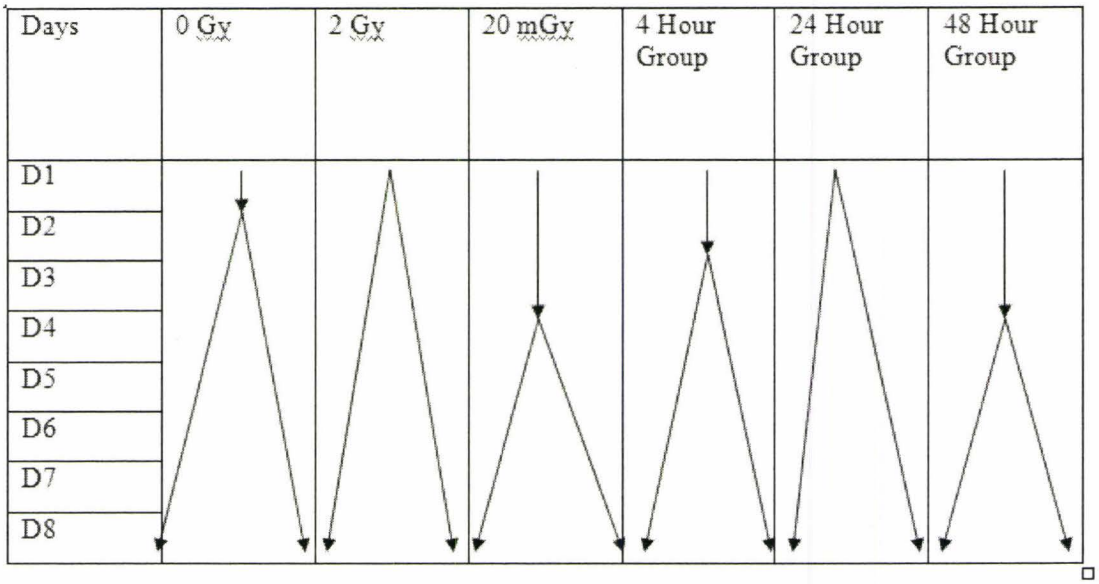


Diagram 3.4 : *Onset of proliferation of fast growth reporters past the slow growth reporters across the various treatment groups. A single line represents statistically similar rate of growth between the fast and slow growers within a given treatment group. A fork in the line represents that the two types of growers are growing at statistically different rates. Significance determined by ANOVA (P>0.05).*

3.2.6 Colony area distribution in reporters exposed to medium from bladder explants from irradiated C57BL6 and Balb/c mice (Figure 3.10)

After day 9 of incubation, colony area measurements of reporters in each treatment group are taken and their distribution pattern is graphed (fig 3.9 and 3.10). When C57BL6 mice are given a singe dose of 2 Gy (fig 3.9C), their corresponding bladder medium induces a considerable decrease in the variety of colony sizes observed in the reporters. Decreasing the dose to 20 mGy (fig 3.9D) results in an increase in the range of colony sizes observed in the corresponding reporters, however, not to the same degree observed in the controls. The presence of a priming dose(s) before a challenge dose in mice results in the emergence of a wide range of colony sizes. Although the

reporters exposed to medium from bladders from acutely irradiated Balb mice show high levels of cell survival, they do experience a reduction the variety of colony sizes observed (Fig 3.10).

3.3 DISCUSSION

The paper by Mothersill and Seymour (1997c) proves that the presence of medium soluble factor(s) that is/are capable of inducing a death response in unirradiated recipient cells. Moreover, Mothersill and Lyng (2005) show the production of bystander factor(s) after C57BL6 mice are irradiated *in vivo*, while O'Dowd *et al.* (2006) show the production of bystander factors after irradiation of fish tissue. More recently, Mothersill *et al.*'s (2006d and 2007) work shows that irradiation of fish results in the production of factor(s) that are released into the surrounding water. Unirradiated (bystander) fish are then placed within the water containing the bystander signals. The results presented in this thesis provide supporting evidence that bystander factors are produced *in vivo* within mice at the time of irradiation, and that medium harvested from their bladder explants transfer the bystander factors onto unirradiated repcipient cells. The fact that these signals are produced *in vivo* and can induce a biological response *in vitro* days after production, confirms that these medium soluble factor(s) are long lived in nature.

3.3.1 Effect of genetics and radiation exposures on clonogenic cell survival

Results clearly demonstrate tremendous variation in the growth response of reporter cells post- ICCM exposure from primary explant tissues. Medium from bladder explants established from acutely irradiated C57BL6, is able to induce cell death in the reporter cells, an effect that is more prominent with increasing dose. However, medium derived from bladder explants obtained from acutely irradiated Balb/c mice fails to

induce a cell death response in reporter cells. Previous work by Mothersill *et al.* (1999) characterizes two subtypes in biological response to radiation: C57BL6 mice bladder explant cells that show a pro-apoptotic response that is associated with 'chromosomal stability'. Consequently, these cells promote apoptosis and necrosis that function to effectively remove damaged cells from the population. The second response involves the survival of damaged CBA explant cells that is associated with chromosomal instability. Variation in the production of the bystander signal is also explored by Mothersill *et al.*'s (2001) group which shows that medium harvested from urothelial explant biopsies from non-smoking patients produces a greater reduction in plating efficiency where as the medium harvested from smoking patient urothelial explants induces increased reporter survival. This supports the current findings that a greater death response seen in reporters receiving ITCM from irradiated C57BL6 mice is a protective response. However, the failure to induce a death response from ITCM from Balb/c mice is a deregulated response, which allows for the propagation of mutations and genomic instability. Interestingly, Chen *et al.*, (2005) reveal that Balb/c mice show a higher proportion of immunosuppressive cells (CD4+CD25+) when compared to C57BL6 mice, resulting in the suppression of autoimmune responses against pathogens, thus inducing tolerance against antigens. This is speculated to cause an enhanced susceptibility to tumor genesis. Consequently, the ability of medium harvested from bladders from irradiated Balb/c mice to maintain reporter cell survival that rivals the unirradiated controls, is the result of an unchecked and unregulated growth.

Administering a priming dose to C57BL6 mice results in a significant moderation of the bystander effect as well as enhanced growth rates when compared to the unirradiated controls. This stimulatory effect is maximized when the priming and challenge dose is separated by twenty four hours. When a single priming dose or a series of priming doses are delivered to Balb/c mice at various time intervals before the challenge dose, the corresponding explants' ICCM causes a decrease in reporter survival, however, their growth rates surpass that of the unirradiated controls. The lower colony count can be attributed to large number of merging colonies that make an accurate manual count very difficult. It can be concluded that a single dose of radiation to C57BL6 mice produces a strong bystander effect in their corresponding reporters. However, repeated exposures to C57BL6 mice (prime + challenge dose), results in the modification of the bystander signal from pro-death to pro-survival. Whether this growth is adaptive or not is unclear at this point. It is postulated that a small amount of initial damage (priming dose) recruits repair proteins to the site of damage, thus allowing the cells to 'prepare' for any subsequent damage that may occur (Hall, 2000). In fact, Ikushima *et al.* (1996) shows an increase in the rate of double strand breaks rejoining in primed cells only. On the other hand, reporters receiving medium from acutely irradiated Balb/c mice show insignificant amount of cell death when compared reporters of the unirradiated controls. Regardless of this, the reporters given bladder medium from primed Balb/c mice show excessive growth, meaning that no bystander effect (cell death) is being induced. Since Balb/c mice are immunologically suppressed, the nature of the signal they produce after a single exposure is not the same as the signal produced by the C57BL6 mice. This fact

explains the difference in clonogenic survival observed after single exposures. However, serial exposures are inducing a similar stimulatory growth effect in both mice strains. This stimulatory phenomenon is also reported by Mothersill *et al.*'s (2006) group which show that blood serum (from patients undergoing radiotherapy) samples taken from the first round of treatment produces a large death inducing bystander effect. However, the serum samples that initially produce the greatest bystander effect also show the greatest adaptive response when blood serum is resampled six weeks post the last therapy treatment. This suggests that the adaptive response observed after a series of *in vivo* exposures is dependant on the amount of initial damage incurred after the first exposure. On the other hand, present data shows that the amount of initial damage does not dictate whether the signal is modified after subsequent exposures. It is important to note that the source of the signals in the Mothersill *et al.* (2006) are all human patients, and do not directly mirror the current experimental setup, which consists of two genetically distinct species of mice. It is possible that such a dependency may exist with in the C57BL6 mice but not the Balb/c mice.

3.3.2 Effect of genetic background and radiation on calcium signaling

The role of calcium as a signaling molecule is best described as a regulatory one. It is responsible for regulation of a wide range of cell functions such as secretion, enzyme activation, cell cycle regulation and apoptosis (Bygrave *et al.*, 1995). An increase in intracellular $[Ca^{2+}]_i$ is documented to cause production of mitochondria-derived reactive oxygen species (Rego *et al.*, 2003). Many studies demonstrate that oxidative stress and

calcium signaling is vital in the production of radiation-induced bystander effects (Lyng et al., 2000, 2002a and 2006). Current data show that calcium induction in response to *in vivo* generated bystander signals also exhibit genotypic differences. When a single dose of 20 mGy is administered to C57BL6 mice, the corresponding bladder medium induces a transient increase in cytosolic calcium levels in reporters, where as the same treatment in Balb/c mice fails to do so. This suggests that the genetic environment of Balb/c mice inhibits the production of death inducing signals. A recent study demonstrates that irradiated C57BL/6 mice but not CBA/Ca mice produce bystander signals that induce calcium fluxes, loss of mitochondrial potential, and apoptosis in reporter HPV-G keratinocytes (Mothersill *et al.*, 2005), indicating *in vivo* induction of bystander signals that are strongly influenced by genetic factors. It can be postulated that perhaps the lack of proficient immunological response abilities in Balb/c mice inhibits the detection of DNA damage after irradiation. If damage evades detection in the tissue post- IR, then the production of bystander signal(s) that serve to limit survival of neighboring cells as part of a homeostatic response will not be induced. Medium obtained from acutely irradiated C57 mice shows a transient calcium flux within the cytosol. However, when a priming dose is delivered twenty four hours before a challenge dose, the corresponding reporters show a modification in the pattern of calcium induction. The medium from these bladder tissues cause intercellular calcium levels within reporters to remain high within the cytosol rather than returning to basal levels as seen with the controls. This effect is also documented by Saroya *et al.*'s group (Honours Thesis McMaster University 2009) that shows that when bystander fish (placed in water that originally contained an irradiated

fish) receive an additional X ray, their corresponding ITCM is able to induce a persistent increase of calcium within the cytosol. This suggests that repeated (priming + challenge dose) exposures to radiation performed *in vivo* induce abnormal apoptotic signals in reporter cells, which may not necessarily signal for cell death. Interestingly, these abnormal calcium signals correspond to stimulatory growth effects in reporters. This suggests that the stimulation of cell growth observed in reporters as a result of repeated exposures may not be adaptive at all, instead, a result of unregulated growth. Moreover, medium from explants established from mice given a priming dose four hours before a challenge doses induces a transient (similar to acutely irradiated mice) calcium flux. Despite the presence of normal apoptotic signals, the medium from these bladder tissues fails to induce cell death when exposed to unirradiated reporter cells. This suggests that by manipulating the separation between the priming and challenge dose, one can also modulate how the bystander signal produced by the initial exposure dictates cellular response to the subsequent challenge.

The medium extracted from bladder explants taken from 20 mGy irradiated Balb/c mice, fails to induce calcium signaling, but the medium taken from bladder explants from 2 Gy exposed mice did show a transient increase in cytosolic calcium levels. This suggests that some sort of threshold dose is required to induce apoptotic signaling in Balb/c mice. Interestingly, the pro-apoptotic or cell death response induced in reporters that receive bladder medium from mice given 2 Gy is not reflected in the colony count; however, the growth rate for the reporters corresponding to this group is much slower than the reporters corresponding to the unrradiated controls. It is known that

calcium signaling is associated with other cellular responses such as loss of mitochondrial membrane potential and ROS production (Lyng et *al.,* 2000). An increase in ROS levels is also associated with DNA damage and an increase in p53 levels (Hall, 2000 and Azzam *et al.,* 2001), which functions to regulate cell cycle progression. Furthermore, the ability of a cell to actually undergo apoptosis as a result of calcium signaling depends on how downstream proteins such as p53interacts with the surrounding environment. Consequently, it is plausible that an increase in calcium levels observed in reporters given bladder medium from 2 Gy irradiated Balb/c mice, signals the activation of a different pathway that regulates cell progression rather than cell death and apoptosis. The fact that these cells are activating this alternative pathway may be in part due to the highly immunosuppressed microenvironment of the Balb/c mice. As discussed previously, Balb/c mice lack a proper immune response system. This type of immunosuppressed environment could prevent cells from recognizing severe damage. One can speculate that when damage of considerable magnitude can not be assessed, then regulatory proteins will fail to co-ordinate an appropriate response, which in this case would have been apoptosis.

Such variations in bystander responses, especially within *in vivo* systems are common, thus serving as a reminder of the complexity within and around a biological system. Clearly, more work is needed to fully understand the link between the nature of the bystander signal produced and its manifestation in terms of cellular response, and what that response means on a homeostatic level.

3.3.3 *Effect of genetic background and radiation exposure on growth rates*

Results show that discrepancies between the automated and manual are highly dose dependant (Fig 3.1 and 3.3). In both sets of experiments (C57BL6 and Balb/c mice), manual counts are mostly lower than the counts obtained through automated techniques. At a closer glance, reporters associated with primed C57BL6 show a considerable increase in the difference between automated and manual counts when compared to the reporters exposed to bladder medium from acutely irradiated mice. On the other hand, the reporters that are given bladder medium from Balb/c mice show very small amounts of discrepancies in colony counts except in those that are associated with acutely irradiated mice. Clearly, colony counting is a source of variation, a problem that has been discussed before (Wunder *et al.*, 1992 and Lumley *et al.*, 1997). Factors such as fatigue, merging colonies, obscure flask edges, and observer expertise can all contribute to such discrepancies. However, this thesis reports that bladder medium from primed C57BL6 mice causes an increase in the amount of smaller to intermediate colony sizes observed in the corresponding reporters (Figure 3.9). This also corresponds to a relative increase in the variation between the manual and automated colony counts because these colonies are better detected through automated techniques. The reporters associated with acutely irradiated mice experience a severe reduction in colony survival, thus giving rise to a few colonies that are easily detected through manual and automated techniques. Due to the tedious nature of cell counting and colony area measurements, there is limited work on low dose radiation induced bystander experiments. There are, however, quite a few papers on cell size and growth rates as endpoints of sub-lethal genetic damage after

exposure to direct radiation insults. Nias *et al.* (1965) report that a consistent percentage of cloned HeLa cells show a similar rate of division until about day 6 of incubation, after which a broad range of rates of divisions (thus colony size) is observed. This gives rise to a broad range of colony sizes that is characterized by majority intermediate to small sized clones and very few large sized clones. Under acute exposure conditions (6 Gy), clones show a drastic decrease in survival, but the ones that do survive show a broad range (heterogeneous) of clone size distribution. Nias *et al.*, (1965) further discusses that lowering radiation exposures will result in an increase in the amount of non-lethal damage that is experienced by surviving cells, thus producing more colonies that posses a delayed rate of growth. However with higher doses of radiation, greater proportion of cells will experience lethal damage, thus eliminating themselves from the population. This would essentially decrease the 'error' in manual counting that arises due to the presence of small, obscure colonies that are not in visible active growth. This supports the findings in this thesis in that an increase in the range of colony sizes occurs after primed conditions of radiation exposure(4, 24, and 48 hours- Figure 3.9) .

Colony heterogeneity is a consequence of inherent differences in growth rates. Results show that growth rates are also biological endpoints that are subject to modulation in response to radiation. Medium from bladders extracted from acutely irradiated (20 mGy and 2 Gy) C57BL6 mice, induces a dose –dependant decrease in the growth rates in only the fast growers while the slow growers show the opposite effect. Basically, a non-homogenous stimulation of fast and slow growers occurs in reporters associated with C57BL6 mice, thus suggesting that radiation induce damage does not

effect cells in a similar manner. On the other hand, reporters associated with Balb/c mice show a similar growth rate effects in both the fast and slow growth reporters, suggesting that both types of colonies are being affected in a similar way. In addition to this, statistical tests reveal all C57BL6 reporters show an immediate divergence in the rate of proliferation between the fast and slow growth reporters, except for the reporters associated with the unirradiated mice which show a one day delay. This reveals that a lag before proliferation of fast growth survivors represents genomic stability where as a failure to produce this lag or delay in proliferation is the result of either lethal damage (causing apoptosis) or sub-lethal damage (causing tumorgenic growth). On the other hand, fast and slow growers corresponding to Balb/c mice in the 2 Gy and 24 hour group show an immediate divergence response while the rest of the reporters show a delay in proliferation ranging from one (0 Gy reporters) to four days (20 mGy), 4 hour group and 24 hour group). This implies that perhaps this lag in proliferation also shows a 'damage' threshold. It can be that low enough damage can still elicit some sort of repair process where as more severe damage would cause cells to bypass any attempts at repair.

Colony distribution analysis of these reporters reveals a severe narrowing effect in colony sizes indicating the presence of mostly very small colonies (Fig 3.10). The presence of smaller colonies can go undetected by manual counting, thus resulting in large discrepancies against automated counts which are better equipped at consistently detecting them.

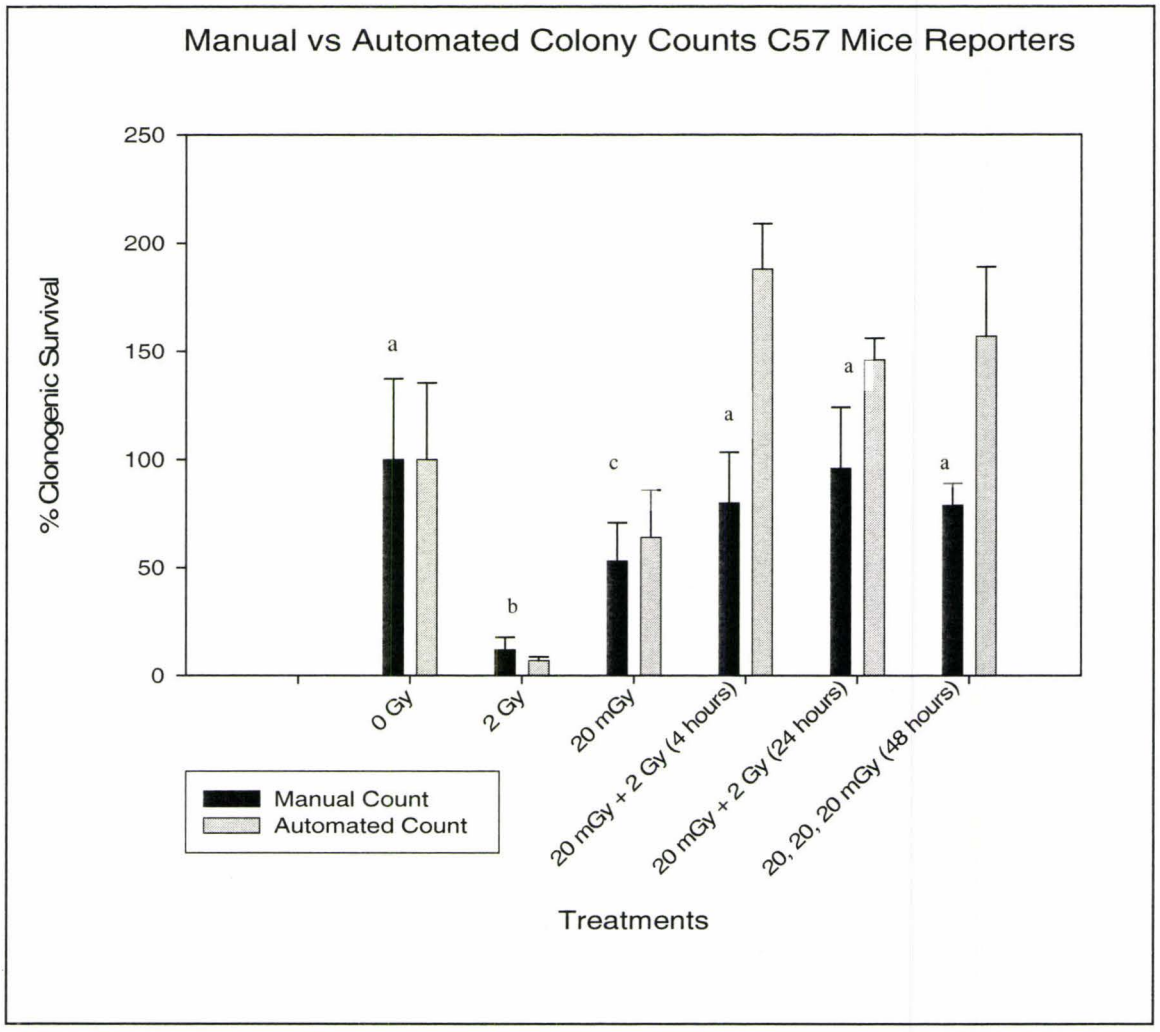


Figure 3.1: Manual versus automated colony counts in reporters associated with irradiated C57BL6 mice

Percent survival of reporters given transfer medium from bladders established from C57BL6 (apoptosis prone) mice that underwent the following treatments: mice exposed to 0 Gy of radiation, (2 Gy) Mice exposed to 2 Gy of radiation, (20 mGy) Mice exposed to 20 mGy of radiation, (4 hour group) Mice exposed to 20 mGy and then four hours later, exposed to 2 Gy, (24 hour group) Mice exposed to 20 mGy and then 24 hours later, exposed to 2 Gy of radiation, (48 hour group) Mice exposed to 20 mGy, then 48 hour hours later exposed to 20 mGy again, then 48 hours later exposed to another 20 mGy, and finally another 48 hours later exposed to 2 Gy challenge dose. N= 3 replicate flasks given bladder medium from 2 Gy and 20 mGy irradiated mice and N=4 replicate flasks for all other treatments. Significance is found using ANOVA ($p<0.05$). Treatments that display statistical similarity are designated with the same letter, where as different letters represent statistical difference.

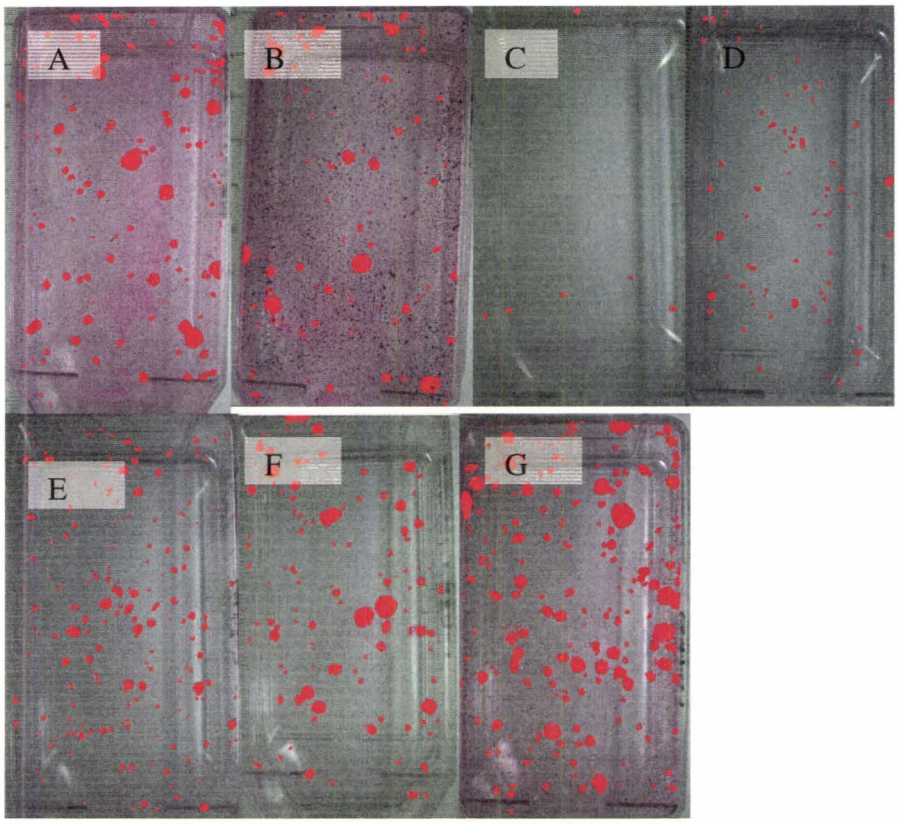


Figure 3.2: Grey level thresholding and colony detection of reporters associated with irradiated C57BL6 mice

HPV-G cells stained, photographed, filtered, and thresholded to allow for an automated colony count. (A) Controls (B) reporters receiving transfer medium from bladders of C57 mice exposed to 0 Gy of radiation, (C) reporters receiving transfer medium from bladders explants of mMice exposed to 2 Gy of radiation, (D)reporters relieving transfer medium from mice exposed to 20 mGy of radiation, (E) Mice exposed to 20 mGy and then four hours later, exposed to 2 Gy, (F)reporters receiving transfer medium from mMice exposed to 20 mGy and then 24 hours later, exposed to 2 Gy of radiation, (G) reporters receiving transfer medium from mice exposed to 20 mGy, then 48 hour hours later exposed to 20 mGy again, then 48 hours later exposed to another 20 mGy, and finally another 48 hours later exposed to 2 Gy challenge dose

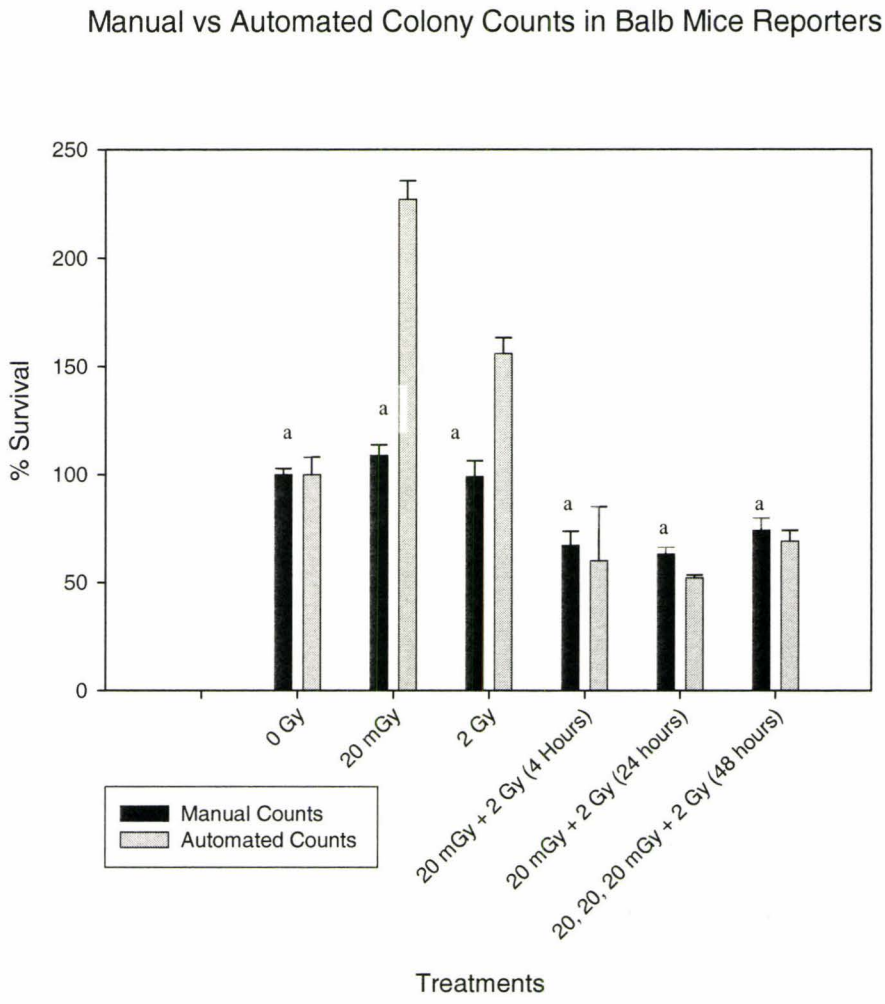


Figure 3.3: Manual versus automated colony counts in reporters associated with irradiated Balb/c mice.

Illustration of reporter percent survival after exposure to transfer medium from bladders established from Balb (sensitive) mice that underwent the following treatments: (0 Gy) Mice exposed to 0 Gy of radiation, (2 Gy) Mice exposed to 2 Gy of radiation, (20 mGy) Mice exposed to 20 mGy of radiation, (4 hour group) Mice exposed to 20 mGy and then four hours later, exposed to 2 Gy, (24 hour group) Mice exposed to 20 mGy and then 24 hours later, exposed to 2 Gy of radiation, (48 hour group) Mice exposed to 20 mGy, then 48 hour hours later exposed to 20 mGy again, then 48 hours later exposed to another 20 mGy, and finally another 48 hours later exposed to 2 Gy challenge dose. (N= 3 for all treatments)Significance is determined at P<0.05 by using ANOVA. Treatments that display statistical similarity are designated with the same letter, where as different letters represent statistical difference.

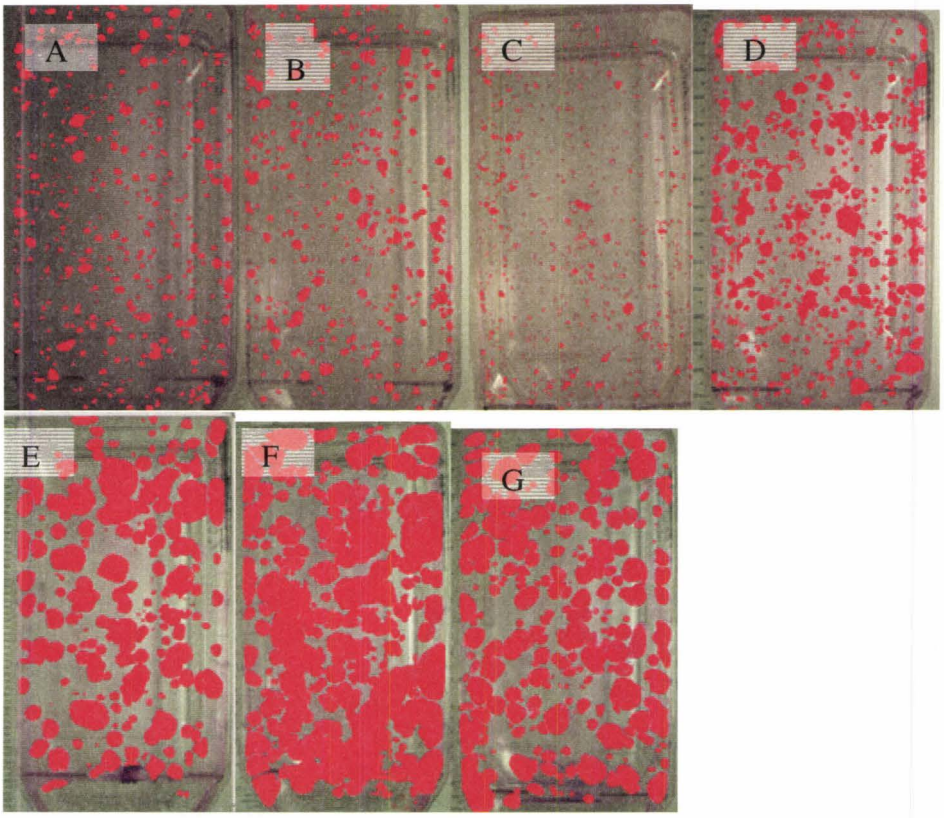


Figure 3.4: Grey level thresholding and colony detection of reporters associated with irradiated Balb/c

Digitized images of the reporter cell culture flasks have been processed with the grey level thresholding algorithm. The resulting automated counts are displayed.(A) untreated controls, reporters given bladder medium from mice exposed to (B) 0 Gy (C) 2 Gys (D) 20 mGy, (E) 20 mGy + 2 Gy (4 hours) (F) 20 mGy + 2 Gy (24 hours) and finally (G) 20 mGy, 20 mGy, 20 mGy + 2 Gy (48 hours)

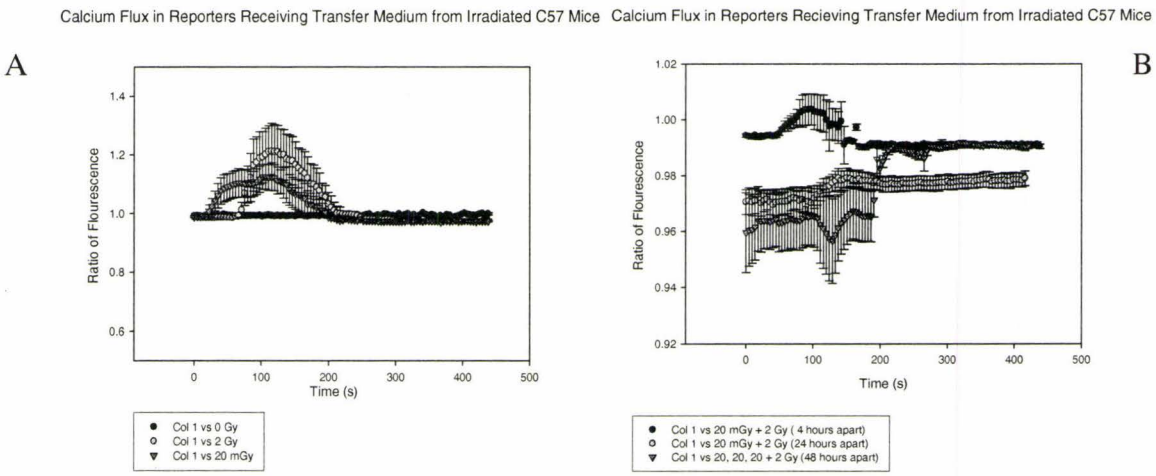
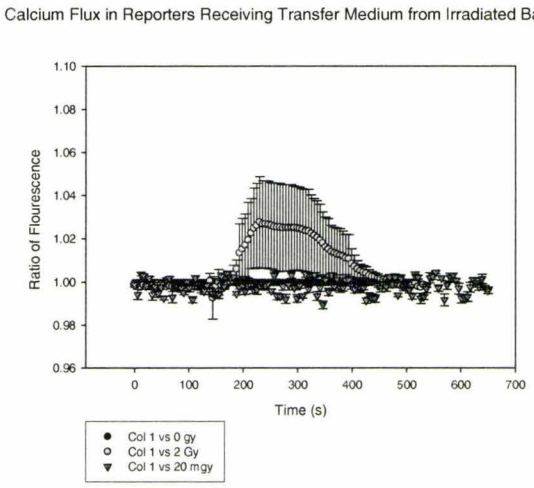
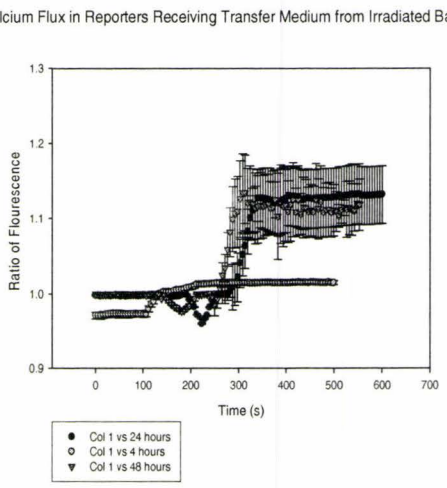


Figure 3.5: Calcium flux in reporters associated with irradiated C57BL6 mice

Cytosolic calcium is measured in HPV-G reporters that received transfer medium from bladder explants extracted from in vivo irradiated c5 7BL6 (normal apoptotic reponse) mice that were exposed to 0 gy, 2 Gy,20 mGy, 20 mGy followed by 2 Gy four hours later, 20 mGy followed by 2 Gy twenty four hours later, and 20 mGy, then 20 mGy, then 20 mGy followed by 2 Gy, at fourty eight hour intervals.(All measurements are taken from an avg of N=5 cells)

A



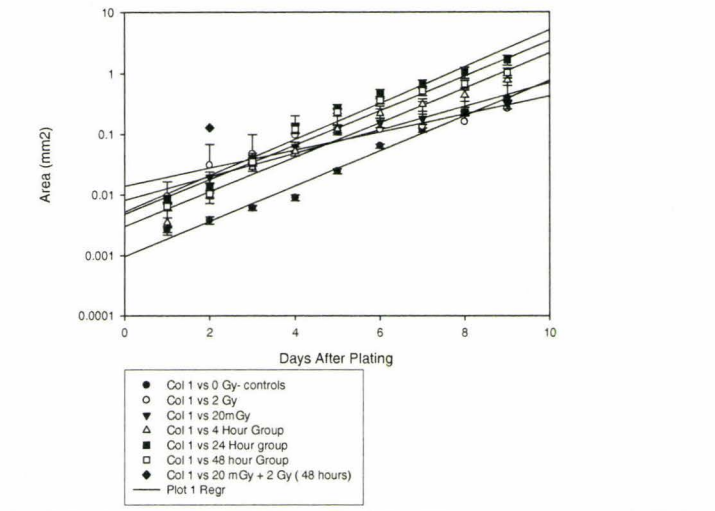


B

Figure 3.6: Calcium flux in reporters associated with irradiated Balb/c mice

Cytosolic calcium measurement in HPV-G cells that received transfer medium from bladder explants from in vivo irradiated Balb/c (radio-resistant) mice that are exposed to (A) 0 Gy, 20 mGy, 2 Gy (B) 20 mGy + 2 Gy (4 hours), 20 mGy + 2 Gy (24 hours apart), and 20, 20, 20 mGy followed by 2 Gy (48 hour intervals). (All measurements are taken from n=5 cells)

A



B

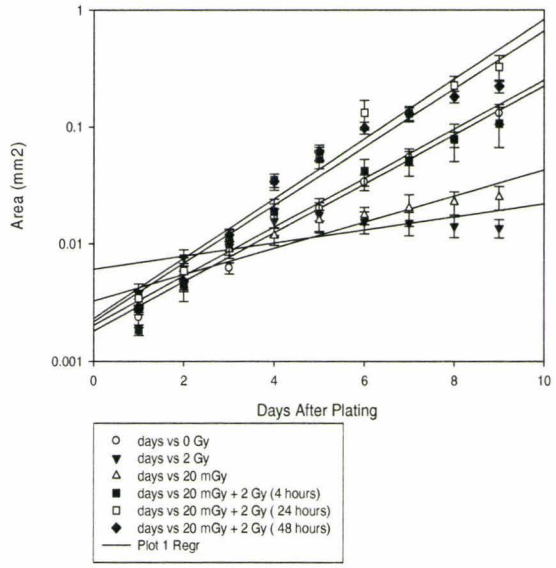


Figure 3.7:Growth kinetics of fast and slow growth reporters associated with irradiated C57BL6 mice

Serial photography colonies allowed for tacking of area growth over the incubation period. These colonies (N=25) were reporters that received transfer medium from C57 mice that were irradiated in vivo. Colonies are chosen at random and then divided into fast growth and slow growth colonies. Their growth is semi-logged. Cellular growth is defined with the relationship Y= mx-b where Y is area in mm^2 , m is the slope or growth rate and b is the y-intercept. For the fast growth colonies: (A) 0 Gy [m=.0413x, b= 0.01, R^2=0.7237, 2 Gy [m=0.0268, b=-0.02, R^2=.9065], 20 mGy [m=0.0375, b= -0.06, R^2= 0.9597],20 mGy followed by 2 Gy given four hours later [m=0.0854, b= -.208, R^2= 0.8196], 20 mGy followed by 2 Gy given twenty four hours later [m= .1879, b= 0.465, R^2= 0.8350], 20 mgy followed by 20 mGy followed by 20 mGy followed by 2 Gy given at forty eight hour later [m=0.1215, b= -0.2746, R^2= 0.8971. For slow growth colonies: (A) 0 Gy [m= 0.014, b= -0.036, R^2= 0.8154], 20 mGy [m= 0.0011, b= - 0.0069, R^2= 0.4847], 2 Gy [m= 0.020, b= 0.0005, R^2= 0.9902], 20 mGy followed by 2 Gy given four hours later [m= 0.0285, b= - 0.0594, R^2= 0.9351], 20 mGy followed by 2 Gy given twenty four hours later [m= 0.0381, b= -0.0872, R^2= 0.8644], and finally 20 mGy followed by 20 mGy followed by 20 mGy followed by 2 Gy given forty eight hours [m=.0067, b= - 0.0127, R^2= 0.8667].

Table 3.1: ANOVA and T test analysis of growth kinetics of reporters associated with irradiated C57BL6 mice

A) Fast Growth Colonies

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
0 Gy	e	e	e*	e*	d,e*	d*	c*	b*	a*
2 Gy	b*	b	a,b*	a,b*	a,b	a,b	A,b	A,b	a
20 mGy	e*	e*	e*	d,e*	c,d*	c*	B,c*	A,b*	a*
4 hour	e,*	e,*	e,*	de*	de*	cd*	bc*	b*	a*
24 hour	e*	e*	e*	de*	de*	cd*	c*	b*	a*
48 hour	f*	f*	ef*	ef*	de*	cd*	bc*	b*	a*

* shows that statistically significant difference exists between the fast growth and slow growth colonies

B) Slow Growth Colonies

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
0 Gy	e	De	de	de	de	cd	bc	b	a
2 Gy	d	Cd	bc	ab	a	ab	ab	ab	ab
20 mGy	d	D	cd	bcd	abc	abc	ab	a	a
4 hour	d	Cd	cd	cd	bcd	bcd	bc	ab	a
24 hour	d	D	d	d	cd	c	c	b	a
48 hour	f	F	f	ef	de	cd	c	b	a

ANOVA statistical analysis compares the average area values on each day of incubation, in each treatment group. The letter "a" implies fastest growth rate while the letter "f" implies the slowest growth rate. So, in the fast growers given bladder medium from unirradiated mice, the first four days of incubation is given the same letter designation "e". This means that these reporters experienced the statistically similar growth rate during this time frame, in addition to being the slowest growth rate. By day 6, the letter designation is changed to "d" which means that growth rate is slight higher at this point although still similar to the growth seen on day 5. Rate of growth progressively increases from this point meaning that the greatest increase in growth occurs in the last couple of days in incubation.

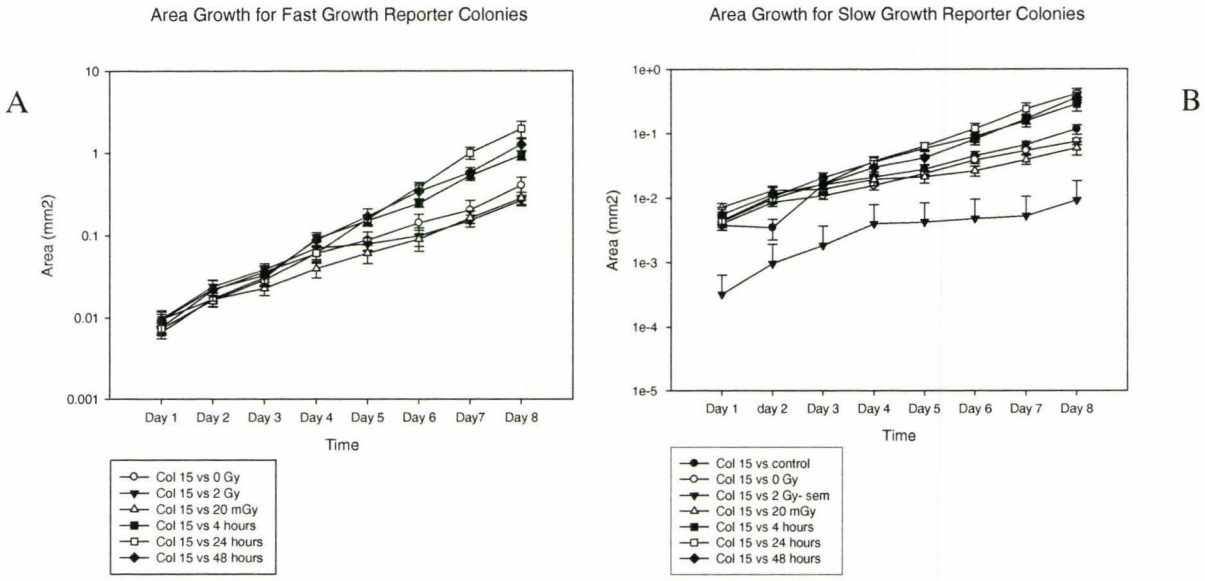


Figure 3.8: Growth Kinetics of fast and slow growth reporters associated with irradiated Balb/c mice

Serial photography of randomly picked colonies (N=25) allowed for daily measurements of area growth over the incubation period. These colonies received transfer medium from bladder explants there were established from in vivo irradiated Balb/c mice. Colonies are chosen at random and then divided into fast growth and slow growth colonies. Their growth is semi-logged. Cellular growth is defined with the relationship Y= mx-b where Y is area in mm^2 , m is the slope or growth rate and b is the y-intercept (A) for fast growth colonies: 0 Gy (m=0.0413, b= -0.1, R2=0.7237), 2 Gy (m= 0.0313, b= - 0.0486, R2=0.8353), 20 mGy (m= 0.0342, b= - 0.0684, R2=0.7844), 4 hour group (m=0.1169 b= -0.2742, R2= 0.7625), 24 hour group (m=0.2355, b= - 0.607, R2= 0.6872), 48 hour group (m= 0.1501, b= -0.3614, R2= 0.7243). (B) For the slow growth colonies: 0 Gy (m=0.0097, b= -0.0157, R2= 0.902), 2 Gy (m=0.0058, b=- 0.0053, R2= 0.9016), 20 mGy (m=0.0064, b=- 0.0037, R2= 0.8562), 4 hour group (m= 0.0347, b= - 0.0737, R2= 0.7998), 24 hour group (m=0.0514, b= -0.1189, R2= 0.7659), 48 hour group (m=0.0413, b= -0.0965, R2= 0.8966).

Table 3.2: ANOVA and T test statistical analysis of Growth Kinetics of Reporters associated with irradiated Balb/c mice

A) Fast Growth Colonies

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
0 Gy	d	cd*	cd*	cd*	bcd*	bc*	b*	a*
2 Gy	f*	ef*	def*	cde*	cd*	bc*	b*	a*
20 mGy	a	d	cd	cd*	cd*	c*	b*	a*
4 hour	e	de	de*	de*	cd*	c*	b*	a*
24 hour	c*	c*	c*	c*	c*	c*	b*	a*
48 hour	d	d	d	d*	cd*	c*	b*	a*

* shows that statistically significant difference exists between the fast growth and slow growth colonies

B) Slow Growth Colonies

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
0 Gy	e	e	e	De	d	c	b	a
2 Gy	f	ef	def	Cde	bcd	bc	b	a
20 mGy	d	cd	cd	Cd	cd	bc	b	a
4 hour	d	d	cd	Cd	cd	bc	b	a
24 hour	d	cd	cd	Cd	cd	c	b	A
48 hour	d	cd	cd	Cd	cd	c	b	a

ANOVA statistical analysis compares the average area values on each day of incubation, in each treatment group. The letter "a" implies fastest growth rate while the letter "f" implies the slowest growth rate.

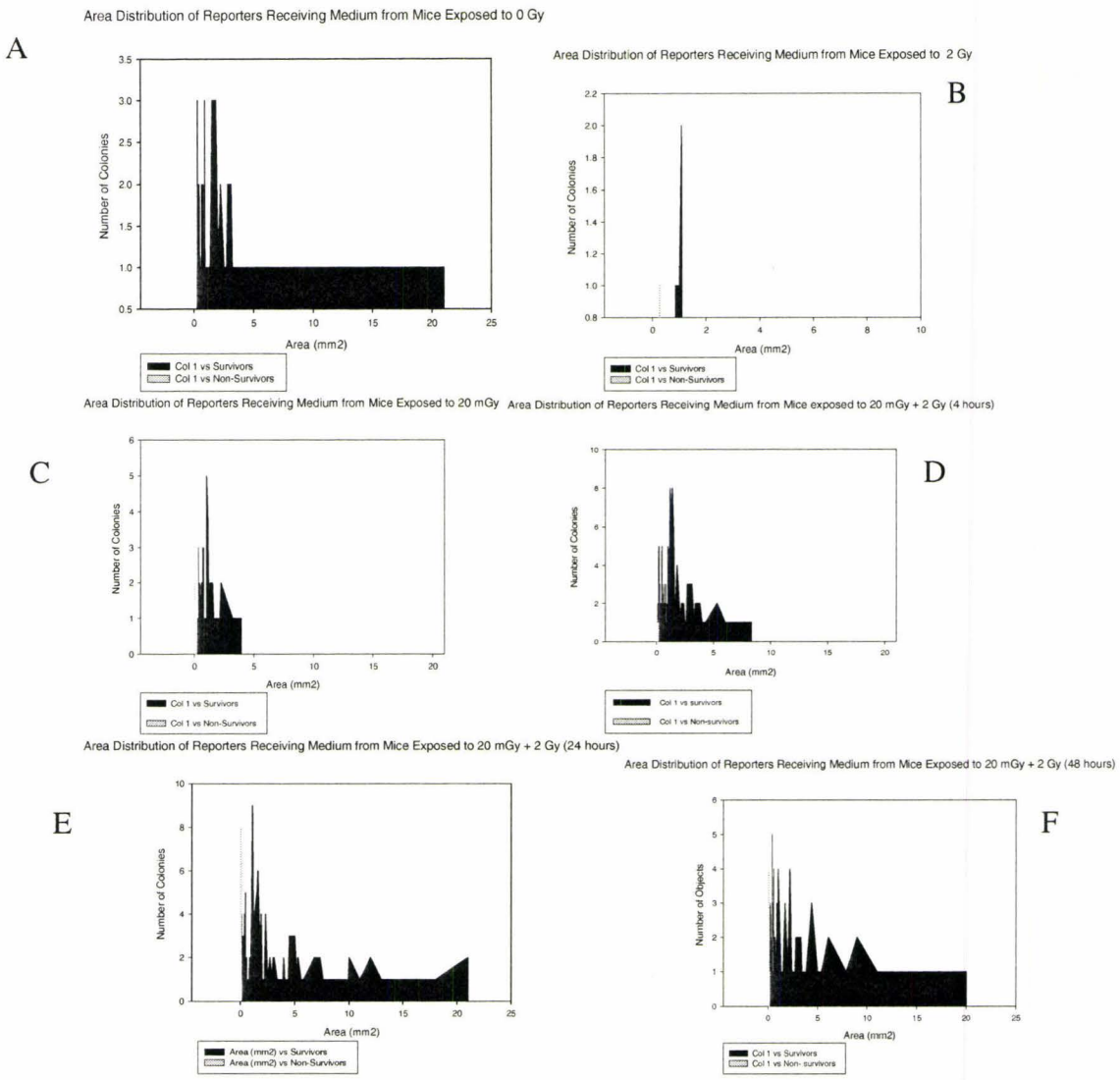


Figure 3.9 Area distribution of reporters associated with irradiated C57BL6 mice

Serial photography of randomly chosen colonies were processed for area measurement on a daily basis for nine consecutive days. These colonies were HPV-G reporters that received transfer medium from bladder explants extracted from in vivo irradiated c5 7BL6 (normal apoptotic reponse) mice that were exposed to a) 0 gy b) 2 Gy c) 20 mGy d) 20 mGy followed by 2 Gy four hours later e) 20 mGy followed by 2 Gy twenty four hours later f) 20 mGy, then 20 mGy, then 20 mGy followed by 2 Gy, at fourty eight hour intervals. For the number of cells, please refer to Appendix

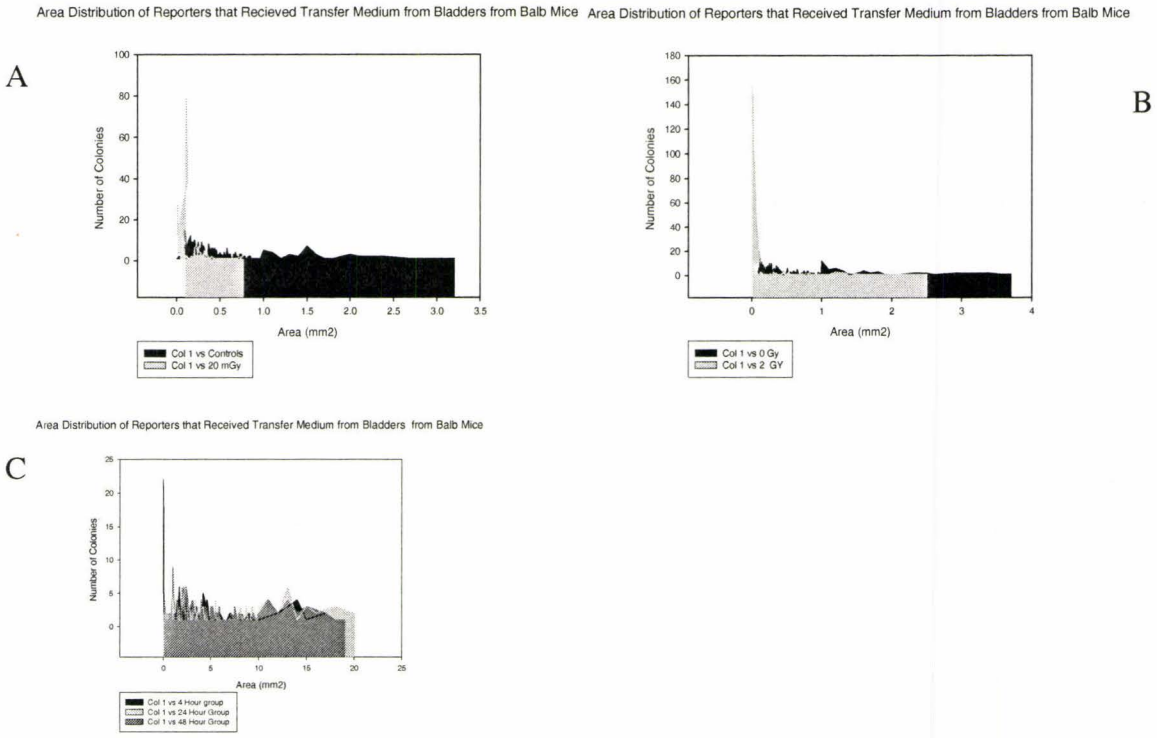


Figure 3.10: Area distribution of reporters associated with irradiated Balb/c mice

Serial photographs of randomly chosen colonies were processed for area measurement on a daily basis for nine consecutive days. These colonies were HPV-G reporters that received transfer medium from bladder explants extracted from in vivo irradiated Balb (radio-sensitive) mice that were exposed to A)incubator controls and 20 mGy B) 0 Gy and 2 Gy and C) 4 hour group (20 mGy followed by 2 Gy four hours later and 24 Hour group (20 mGy followed by 2 Gy twenty four hours later) and 48 hour group (20 mGy, then 20 mGy, then 20 mGy followed by 2 Gy, at fourty eight hour intervals).

4 RADIATION INDUCED EFFECTS IN FROGS FROM CONTAMINATED VS CONTROL AREAS

4.1 ABSTRACT

An accurate assessment of radiation induced effects comes from observing long-term damage in wildlife species. This ensures that various environmental and biological variables, some of which are unknown, are interacting in a natural way with radiation. Female and male Mink frogs (*Rana septentrionalis*) captured from contaminated and background (control) radiation sites, were sacrificed without further radiation treatments. The bladders were removed, shipped to McMaster and immediately plated in culture medium under sterile conditions. The culture medium is later harvested and used to treat unirradiated HPV-G transfected reporter cells. A pronounced reduction in the fraction of surviving reporters is seen after exposure to medium from bladders extracted from contaminated female and male frogs, but no reduction is seen when female and male frogs from uncontaminated sites are. Apoptosis is also measured in HPV-G cells that are given bladder medium samples from uncontaminated and contaminated frogs. A calcium flux is induced in cells that are exposed to medium from bladders harvested from contaminated female and male frogs but not in the cells exposed to bladder medium from background (uncontaminated) female and male frogs. Growth rates and colony size distribution are also measured in reporters as an index of biological response to radiation damage.

4.2 INTRODUCTION

The effects of radiation on amphibian species have been extensively reviewed by Brunst (1965). The author provides a thorough description of radiation – induced changes seen in various types of amphibian tissues and organs. The author states that the most radiosensitive tissue is that of the lymph nodes, followed by the small intestine, liver, spleen, kidney, muscle, and finally spinal cord. Nayar *et al.* (1975) reports that certain doses (120 R) of Co60 gamma irradiation results in a severe inhibition of sciatic nerve action potentials (95%) in *Rana tigrina* frogs. Hart *et al.* (1984) reports that when male frogs *(Xenopus laevis)* exposed to 1500 R of gamma radiation are bred with unexposed females, they experience severe reduction in the embryonic survival of their progeny. Ramirez *et al.* (1983) reports the destruction of bone marrow after exposure to 5000 R from a Co60 source in various species of Rana frogs. These authors have described detrimental effects that arise from relatively high levels of radiation exposure. There is limited literature available on low dose exposures to amphibians, and this proves to be a major setback when trying to understand the interaction between radiation and the environment. It must be taken into consideration that under natural conditions, animals receive radiation in chronic, low dose rates. A study by Miyachi *et al.* (2002) looks at how repeated exposures to radiation can affect biological response in *Rana porosa porosa* frogs. The authors show that when a small conditioning dose (0.1 Gy) is delivered before a high X ray dose, it induces radiation tolerance measured as suppression of emesis (vomiting). However, if the conditioning dose is increased to 0.5Gy, a higher occurrence of emesis occurs following a subsequently higher dose.

In the wild, individual sensitivities of species to radiation are often combined with other factors of the environment. As a result, radiation damage induced through short term and acute exposures that are delivered within a laboratory setting can prove to be misleading. In order to grasp a comprehensive understanding of radiation effects on non-human biota, it is vital that long term observations of radiation induced biological effects are considered as present in the natural setting. The "Environmental Protection from Ionizing Contaminants" or EPIC report authored by Sazykina and Kryshev (2006) consists of a collection of data on radiation induced effects in wildlife around the world, but with a specific focus on chronic/lifetime exposures at low, nonlethal doses and dose rates. Southern Urals, Russia has been contaminated with Sr-90 since 1957. EPIC report shows that brown frogs (*Rana arvalis*) that reside within the soil (56 MBq m^{-2} of activity) have smaller sized of eggs, show 10% reproductive success in young frogs and 17% of adults with morphological abnormalities (Sazykina and Kryshev, 2006). Radiation induced effects are also documented in frogs (*Rana arvalis*) sampled from contaminated sites in Chernobyl (Belarus, Khoiniki and Mogilev region) where residual activity from Cs-137 (11110kBq m^{-2}), Sr-90 (77.7 kBq m^{-2}) and Ru-106 persists in the soil. Some of the effects that have been reported are increased frequency of chromosomal aberrations in bone marrow cells (Cherdantsev *et al.*, 1993 and Sazykina and Kryshev, 2006), increase in the percentage of infertile eggs, as well as the presence of bone marrow tumors (Eliseeva *et al.*, 1994 and Sazykina and Kryshev, 2006).

This section of the thesis aims to show a sex-dependant response in various markers of apoptosis (clonogenic assay and calcium signaling) to bladder medium

extracted from contaminated and control frogs. Imaging techniques serve as methods for detecting subtle biological responses to sub lethal amounts of damage induced by contamination.

4.3 RESULTS

4.3.1. Clonogenic survival in reporters given medium from bladders extracted from Mink Frogs. (Figure 4.1 and 4.2)

HPV-G transfected clonogenic cell cultures treated with medium taken from bladder explants established from male and female frogs captured from contaminated areas, show a decrease in the percentage of surviving colonies to 69 % (males) and 26 % (females). Reporters given medium from bladder explants established from male or female frogs captured from control (uncontaminated) sites, show a high percentage of survivors of 146% and 149% respectively. Comparison between the manual and automated counts (average discrepancy of 12.8%) show that threshold based automated estimates are relatively lower than those obtained via manual scoring (Figure 4.1). The average threshold value is .085576 mm^2, which corresponds to approxiamtely 50 cells as described by Puck and Marcus (1956).

4.3.2 Growth rates of fast growth reporters after exposure to medium taken from bladder explants of Mink frogs. (Figure 4.3)

The reporter cells, selected for tracking, are mapped out directly on each treatment flask and their daily area measurements are taken over the course of ten days. Net area growth is calculated and used to separate the fast growing colonies from those colonies which show a reduced growth rate. The two types of colonies are analyzed separately across different treatment conditions. The growth kinetics displayed in Figure

4.3 show that the fast growing colonies that are given bladder medium from female frogs captured from contaminated (also referred to as contaminated females or CF) areas possess the slowest rate of growth (0.0029) followed by the reporters given bladder medium from male frogs captured contaminated sites (also referred to as contaminated males or CM) (0.0495). In addition, the reporters that are given bladder medium obtained from male and female frogs from control or background sites (referred to as background make or BM and background females or BF) show considerably higher growth rates (0.3434 and 0.1822 respectively) than the reporters exposed to bladder medium from the CM's. Refer the diagram below for a brief schematic of growth rates.

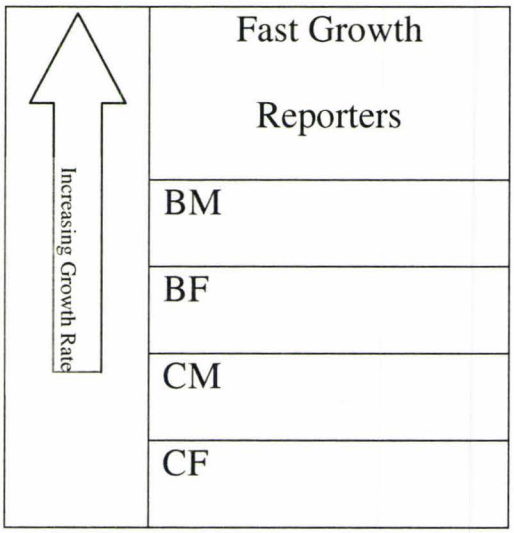


Diagram 4.1 – *Schematic comparing growth rates of fast growth reporters associated with radiation contaminated and control mink frogs.*

4.3.3 Growth rates of slow growth reporters after exposure to medium taken from bladder explants of Mink frogs. (Figure 4.4)

Similarly, slow growth HPV-G reporter cells are analyzed for their growth rates in Figure 4.4, and they reveal a similar trend as seen with the fast growth colonies. The reporters given medium from bladders extracted from contaminated female frogs (CF) show the slowest growth rate (0.0004), followed by the reporters given bladder medium from contaminated male frogs (CM) (0.004). The reporters given medium obtained from bladders from the background female frogs (BF- 0.0311) and background male frogs (BM- 0.0349) show drastically higher growth rates when compared to the reporters given bladder medium from the contaminated females (CF) and males (CM). However, in this case, the controls show the highest growth rate (0.0412).

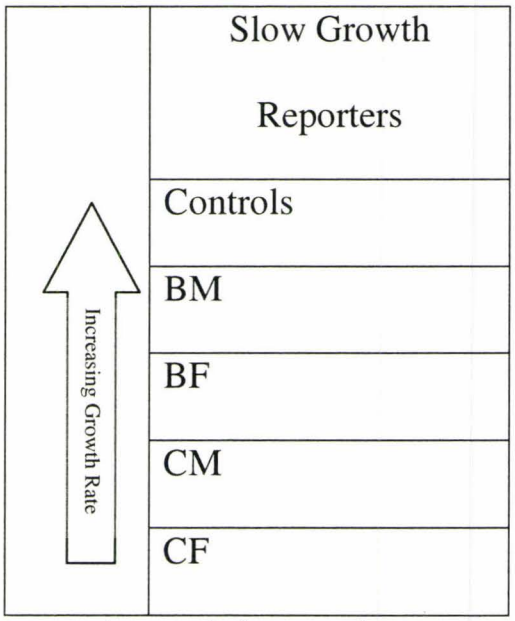


Diagram 4.2 – *Schematic comparing growth rates of slow growth reporters associated with radiation contaminated and control mink frogs*

4.3.4 Distribution of growth rates and delay in proliferation in reporters after exposure to bladder medium from Mink frogs (Table 4.1 and 4.2).

Statistical analysis of growth patterns between the fast growth reporters of different treatments groups reveal differences in daily growth patterns (Table 4.1). Reporters across all treatment groups show similar growth rates until day three of incubation, at which point the fast growth reporters given bladder medium from background males and females (BM and BF) show a distinct increase in their growth rates. Refer to diagram 4.3 below for a flow chart that describes growth patterns across the five treatment groups. However, the reporters given bladder medium from the contaminated males (CM) show a reduced growth rate, followed by the reporters given medium from the contaminated females. By day four of incubation, the reporters corresponding to the contaminated males have decreased their growth rate. By day seven and onwards, the reporters corresponding to the background females decrease their growth rate while the reporters corresponding to the background males show a consistently higher growth rate. This distribution pattern reveals that in fast growth colonies, there is a delay time of two days before divergence in reporters of different treatments is initiated.

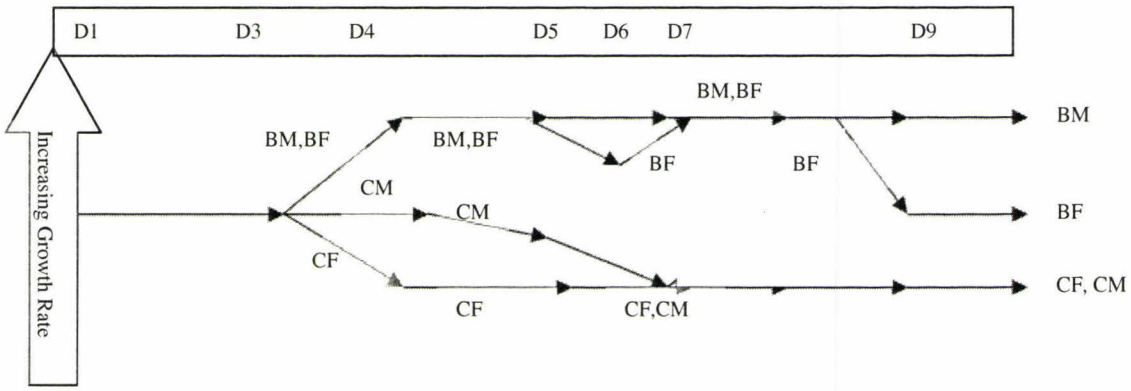


Diagram 4.3: Growth rate comparisons between fast growth reporters associated with radiation contaminated and control mink frogs

Every arrow represents a single or a group of reporters that possess similar growth rates. A merging of arrows means that growth rates that were once different, are now similar. An arrow that forks means that growth rates that were once similar are now diverging. An arrow pointed upward means that the reporters are increasing their rate of growth, where as a downward pointed arrow indicates the corresponding reporters are decreasing their rate of growth.

Similarly, statistical analysis of growth rates in slow growth colonies across the different treatments show unique growth patterns when compared to the fast growth colonies. In this case, all reporters show similar rates of growth until day four of incubation, after which reporters given bladder medium from background females and male (BF's and BM's) possess the slowest growth rates whereas the reporters corresponding to the contaminated female and males show the highest growth rates. By day five, the reporters given bladder medium from contaminated male and female frogs switch into the slowest growers while the reporters in the background male and female groups become the fastest growers.

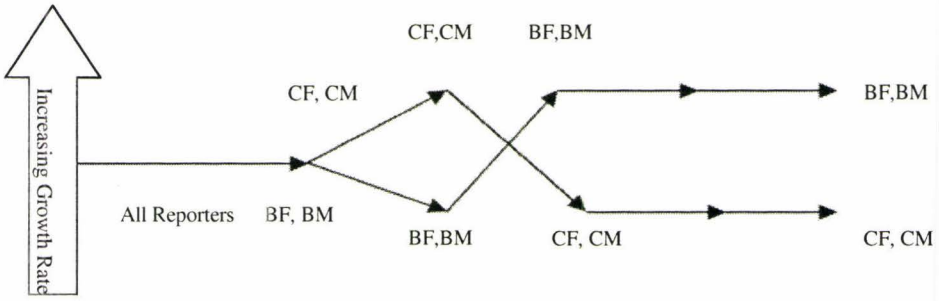


Diagram 4.4: Growth rate comparisons between slow growth reporters associated with radiation contaminated and control mink frogs

Every arrow represents a single or a group of reporters that possess similar growth rates. A merging of arrows means that growth rates that were once different, are now similar. An arrow that forks means that growth rates that were once similar are now diverging. An arrow pointed upward means that the reporters are increasing their rate of growth, where as a downward pointed arrow indicates the corresponding reporters are decreasing their rate of growth.

Daily observations of HPV-G cells *in vitro* show that divergence in cell proliferation between fast and slow growers often occurs after a variable delay, depending on the conditioned medium to which they are exposed. A t-test statistical analysis (Table 4.1) of mean area values between fast and slow growth colonies of each treatment reveals variations in proliferation times. Refer to diagram 4.3 for a schematic of relative proliferation times. The reporters corresponding to the contaminated male frogs show similar growth rates between their respective slow and fast colonies until day four of incubation. In contrast, reporters given bladder medium from female and male frogs from background sites, (Table 4.1) show an immediate proliferation of their fast growth colonies. The fast and slow growth reporters given medium from female frogs from contaminated sites show similar growth unrates until day 2 (Table 4.1).

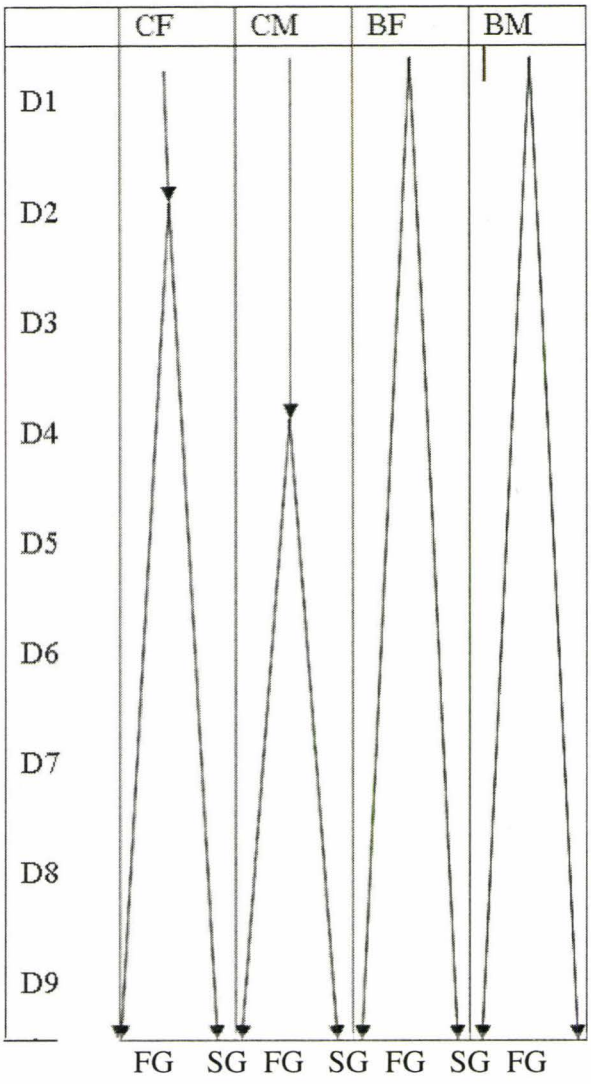


Diagram 4.5 – Delay in divergence between fast and slow growth reporters associated with radiation contamination and control mink frogs

A single arrow represents similar growth rate between the fast and slow growth colonies within that treatment group. A fort in the arrow shows that the growth rate between the fast and slow growth colonies are no longer similar. Divergence of Growth Rates is located on the x axis and number of days is on the vertical axis.

4.3.5 Calcium Signaling after addition of medium harvested from bladder explants established from Mink frogs (Figure 4.5).

HPV-G transfected reporter cell response to bystander signals is reported to occur via calcium signaling (Lyng *et al.,* 2002a and 2002b). This can be demonstrated (figure 4.5) by a rapid but transient increase in intracellular calcium levels after addition of conditioned tissue medium. A calcium flux is observed in reporters given tissue medium from female and male frogs from contaminated sites. This effect persists for approximately 150 seconds, before ratio measurements drop down to baseline levels. The magnitude of the calcium flux is slightly greater after addition of bladder medium harvested from contaminated female frogs. Medium from bladder explants obtained from male and female frogs from control or background sites fail to initiate a calcium flux.

4.3.6 Colony Area Distribution of Reporters exposed to medium from bladders extracted from Mink Frogs (Figure 4.6)

Bladder medium from the non-contaminated (males and females) controls cause an increase in range of colony sizes are seen. When reporters are given bladder medium from contaminated female frogs, all of the colonies that survive are within a narrow 0 to 1 mm^2 range. When reporters are exposed to bladder medium from contaminated males, they do not show a reduction in the range of resulting colony sizes

4.4 DISCUSSION

These data are unique in that they reveal biological effects in HPV-G reporters (*in vitro)* from damage that is initially produced in Mink (*Rana septentrionalis*) frogs (*in vivo)* through lifetime, environmental contamination of tritium and traces of carbon-14. The unirradiated reporter system lacks the dynamics and complexity of living organisms, so the biological effects observed in response to transfer medium from the frog bladder explants must be considered cautiously. Results indicate that lifetime exposure to radiation can elicit production of signals that can alter biological response in completely unirradiated HPV-G cells. Also, these biological responses to radiation are sex-dependant.

4.4.1 Clonogenic survival

Due to the limited amount of frogs collected, there are only enough bladder samples to yield one reporter flask for each treatment, thus no statistical analysis could be performed on the clonogenic assays. Consequently, sufficient data does not exist at this time point to give more then a suggestion of trends. Reporters that are given bladder medium from contaminated female frogs (CF group) show greater radio sensitivity when compared to the reporters given bladder medium from contaminated male (CM-group) frogs. Interestingly, human and mouse male fetal germ cells show severe radiosensitivity at doses as low as .1 Gy (Lombert *et al.,* 2007 and Guerquin *et al.,* 2009) where as female human and mouse oogonia sex cells show resistance until 1.5 Gy of radiation (Guerquin *et al.,* 2009). Even though both males and females show similar amount of DNA damage (γH2AX), the female sex cells show faster rate of DNA repair than the males (Guerquin *et al.*, 2009). In addition, when low doses of radiation (.05 Gy for 10 days) are delivered

repeatedly to C57BL6 mice, it significantly decreases pro-survival signaling (AKT in the hippocampus) in male mice only. No such effect is seen to occur in either males or females when they are subjected to acute exposures (.5 Gy) (Silasi et al., 2004). Similarly, Korturbush *et al.*, 2008 shows that cranial and whole body, *in vivo* X-radiation (1 Gy) leads to global hypomethylation that is significantly higher in males than females. A decrease in methyl constituents on DNA residues is a well known epigenetic mechanism that is associated with alteration of gene expression, gene silencing, and chromosomal rearrangements (carcinogenic) (Nagar *et al.,* 2003 and Klose *et al.*, 2006). When reporters receive medium associated with background male and female frog bladder explants, they experience high levels of survival. However, the reporters given bladder medium from unirradiated control C57BL6 mice show a lower percentage of survival. It can be speculated that perhaps laboratory animals experienced elevated stress from human handling where as the Mink frogs were taken from within their natural habitat without any human-associated stress. Since the frogs sampled from the controls sites are able to interact with the various components of environment (different temperature, sunlight, diet, etc), it is possible that this equips them with 'healthier' organs, thus allowing them to release stimulatory factors *in vitro.* In fact, growth inhibition, DNA damage, and behavioral changes are induced in various species of plants and animals in response to UV-B, CO2, and temperature changes in an environment. Whatever the mechanism, the sheer complexity in the interaction between the environment and an animal allows room for the possibility that perhaps species in the wild possess

mechanisms that are entirely different then animals that have been bred in captivity for numerous generations.

4.4.2 Calcium Signaling

Medium taken from bladder explants originating in contaminated female and male frogs show a transient calcium flux that is indicative of normal pro-apoptotic signaling. The medium taken from bladders explant from frogs captured from control sites fail to do so. It is interesting to compare calcium flux patterns in these contaminated frogs that experienced low dose rates of exposure with those of the C57BL6 mice which were irradiated serially over the span of 4 hour, 24 hours, and six days (larger dose rate). In contaminated sites, dose exposures are spread out over long periods of time, which means that these samples experienced very low dose rates (LDR) of radiation. The medium extracted from bladders obtained from LDR frogs is able to produce normal calcium signals *in vitro*, as well as an associated increase in clonogenic cell death in the reporters (fig 4.1). However, when mice were given radiation exposures over a small span of time, their ITCM produces abnormal patterns of calcium signaling which also correlates with excessive cell growth in the reporters. Similarly, Gow *et al.* (2008) shows that increasing the dose rate to 10 Gy/min from 20 MeV electrons results in significantly higher levels of bystander cell survival, where as the directly irradiated cells are severely affected. This shows that higher levels of damage sustained as a result of a high dose rate by the irradiated species (HPV-G cells or C57 mice) can create bystander signals that can induce growth in bystander reporters. Chen *et al.* (2008) also shows induction of DNA damage (γH2AX foci, MN frequency, and apoptosis) in bystander cells after low dose rate

exposures using ^{125}I seeds. It is plausible that excessive growth that is observed in reporters corresponding to primed C57BL6 mice, could be the result of the modification of the signals produced *in vivo*. It could also be that repeated exposures to directly irradiated mice results in excessive damage within their tissue, thus abolishing bystander signal production altogether.

4.4.3 Growth Rates

Reporters given bladder medium from female and male contaminated frogs show a highly reduced growth rate. In addition, medium taken from bladders from contaminated females (CF) induces a much shorter delay in the onset of proliferation, as well as a decrease in the overall range of reporter colony sizes. Both of these responses are consistent with excessive damage. The reporters given bladder medium from the contaminated males show a 3 day delay in growth rate proliferation, as well as a broader range of colonies. It is possible that this delay occurs because DNA repair mechanisms are induced so as to prevent the propagation of damage. This is contradictory to the reporters given bladder medium from repeatedly exposed C57 mice which fail to show reproductive delay in their growth rates. This provides further evidence that the method and rate of dose delivery can also impact biological response elicited from cells. Similarly, reporters given bladder medium from contaminated males show the highest growth rate, but also fail to show a delay in reproductive proliferation that is expected from gnomically stable cell populations.

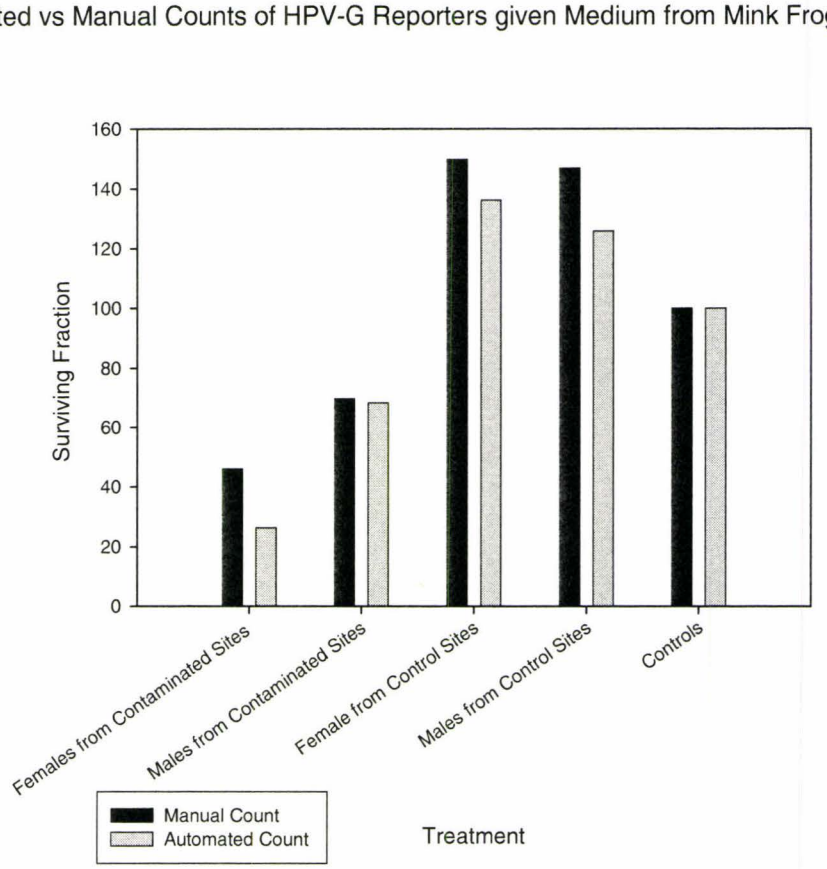


Figure 4.1: Manual versus automated colony counts in reporters associated with radiation contaminated and control mink frogs.

Surviving fraction of HPV- G transfected reporter cells exposed to culture medium harvested from male and female frog bladder explants established from non-irradiated frogs from control and contaminated areas. Percent survival is measured in reporter cells after receiving medium harvested from bladder explants taken from female frogs captured from contaminated areas, male frogs captured from a contaminated area, female frogs captured from background areas, and male frogs captured from background areas, and controls (N=1 flask/treatment).

Figure 4.2: Grey level thresholding and colony counts of reporters associated with radiation contaminated and control mink frogs

Multi-level threshold algorithm allows for the detection and separation of bright objects or colonies from background. This allowed for an automated count of: (A) HPV-G reporters receiving medium from female frogs captured from contaminated sites, (B) he reporters that received medium from male frogs from contaminated sites, (C) the reporters that received medium from female mice from control site, (D) the reporters that received medium from the male frogs from control sites. (E) no-treatment control.

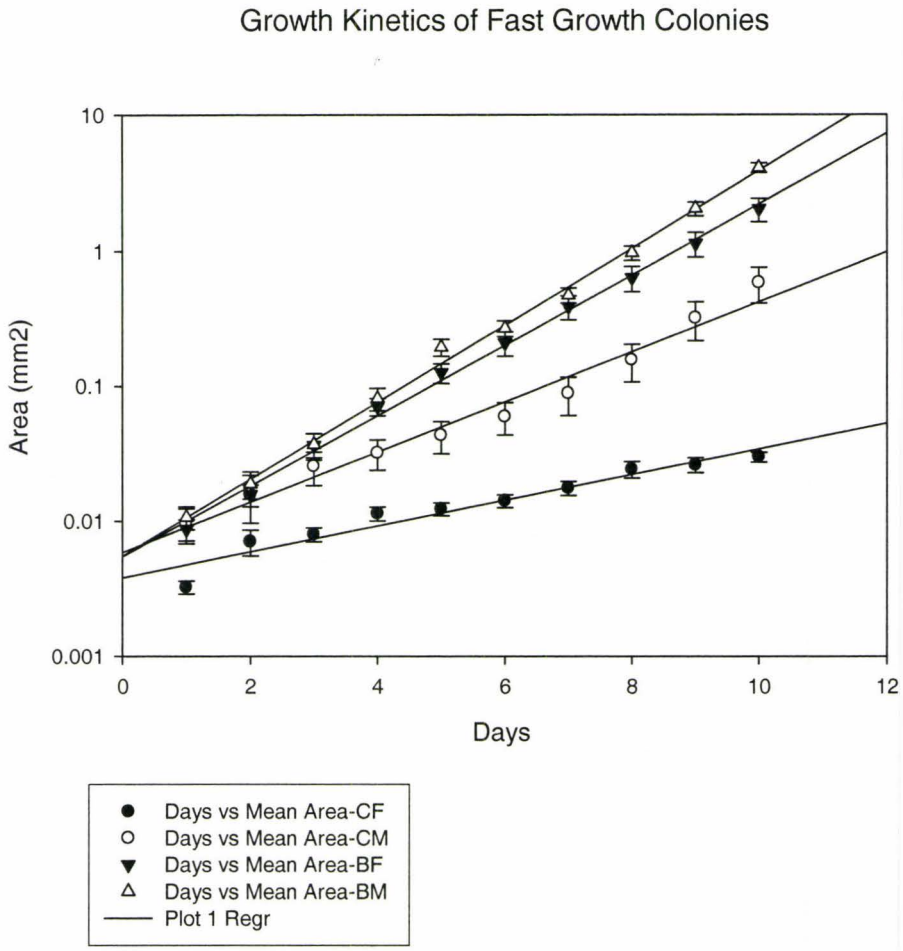


Figure 4.3: Area Growth kinetics for fast growth reporters associated with radiation contaminated and control mink frogs

Growth rates of four sets of fast growth HPV-G reporter colonies after exposure to medium harvested from bladder explants taken from : female frogs taken from contaminated sites (CF- or contaminated females), male frogs from contaminated sires (CM- contaminated males), female frogs from control sites (BF- background females), and male frogs from background sites (BM- background males). Linear Regression was performed on the growth curves, so cellular growth follows the relationship Y = mx + b where Y is colony area in mm^2, X is culture time in days, b is the y intercept and m is the slope or growth rate R value is the correlation coefficient. CF: b=-.0004, m=.0029 (r=.9829). CM: b=-.1384, m=.0495 r=(.8098). BF: b= -.5362, m=.1822 (r=.8429). BM:b=-1.0714, m= .3434 (r=.7956). Controls: b= - .6552, mb=.2155 (r=.8280). N= number of colonies tracked in each treatment various from (20-34). For details refer to the appendix 5 (A.5.2).

Table 4.1: ANOVA and T-test Analysis of growth kinetics of reporters associated with radiation contaminated and control mink frogs

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
CF	c	a*	b*	b*	c*	b*	b*	c*	c*	c*
CM	a,b	a	a,b	b,c*	c*	b*	b*	c*	c*	c*
BF	a,b,c*	a*	a*	a*	b*	a*	a*	b*	b*	b*
BM	a*	a*	a*	a*	a*	a*	a*	a*	a*	a*

*Table 4.1 Growth rates of five sets of fast growth HPV-G reporter colonies after exposure to medium harvested from bladder explants taken from: female frogs taken from contaminated sites (CF- or contaminated females), male frogs from contaminated sires (CM- contaminated males), female frogs from control sites (BF- background females), and male frogs from background sites (BM- background males. The data set shows ANOVA statistical analysis followed by Least Square Difference on each mean area value for fast growth colonies, on day one through ten for each treatment group. Similar growth is labeled with the same letter while different growth is labeled with different letters, the highest growth value corresponds to "a" while the lowest growth value corresponds to the letter "c". P<.0.05 is considered significant. The (*) indicates a comparison made between the fast and slow growth colonies of each treatment group on each day using the student's t-test (p< .05). Presence of an * indicates statistically significant difference in growth between the slow and fast growth colonies of that particular treatment.*

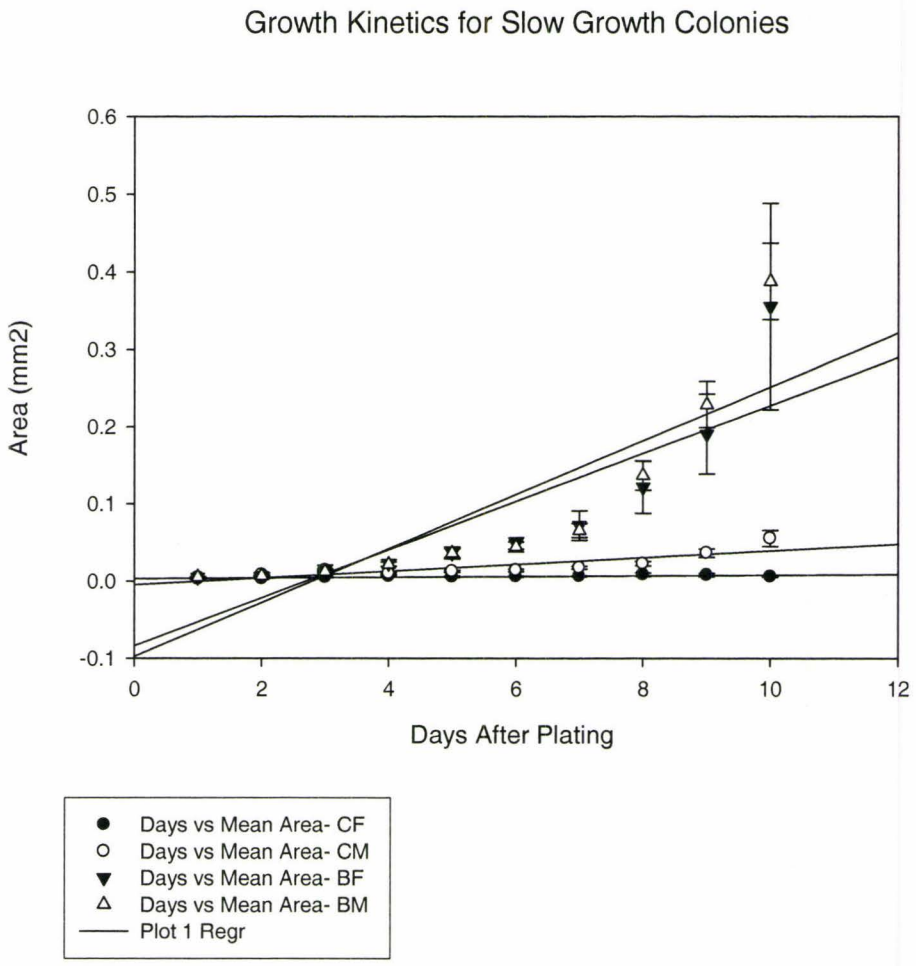


Figure 4.4: Growth kinetics of slow growth reporters associated with radiation contaminated and control mink frogs

. Growth rates of four sets of slow growth HPV-G reporter colonies after exposure to medium harvested from bladder explants from: female frogs taken from contaminated sites (CF- or contaminated females), male frogs from contaminated sires (CM- contaminated males), female frogs from control sites (BF- background females), and male frogs from background sites (BM- background males), and non-treated controls. Linear Regression was performed on the growth curves, so cellular growth follows the relationship Y = mx + b where Y is colony area in mm^2, X is culture time in days, b is the y intercept and m is the slope or growth rate R value is the correlation coefficient. CF: b=.0034, m=.0004 (r=.7905). CM: b= -.0045, m= .004 (r=.8582). BF: b= -.0840, m=.0311 (r=.8498). BM: b= -.0974, m= .0349 (r=.8465). Controls: b= -.1089, m= .0412 (r= .8904). N= number of colonies tracked range from 21 – 31, for details refer to Appendix 5 (A.5.2).

Table4.2: ANOVA analysis of slow growth reporters associated with radiation contaminated and control mink frogs

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
CF	b	B	b	a	b	b	c	b	c	b
CM	a	A	a,b	a	b	b	c	b	c	b
BF	a	a,b	a,b	b	a	a	a,b	a	b	a
BM	a	a,b	a,b	b	a	a	b	a	a,b	a

Table 4.2 This table shows distribution of growth rates in reporters across all treatment groups: female frogs taken from contaminated sites (CF- or contaminated females), male frogs from contaminated sires (CM- contaminated males), female frogs from control sites (BF- background females), and male frogs from background sites (BM- background males), and non-treated controls. The data set shows ANOVA statistical analysis on each mean area value for slow growth colonies, for each treatment group. Similar growth is labeled with the same letter while different growth is labeled with different letters; the highest growth value corresponds to "a" while the lowest growth value corresponds to the letter "c". P<.05 was considered significant.

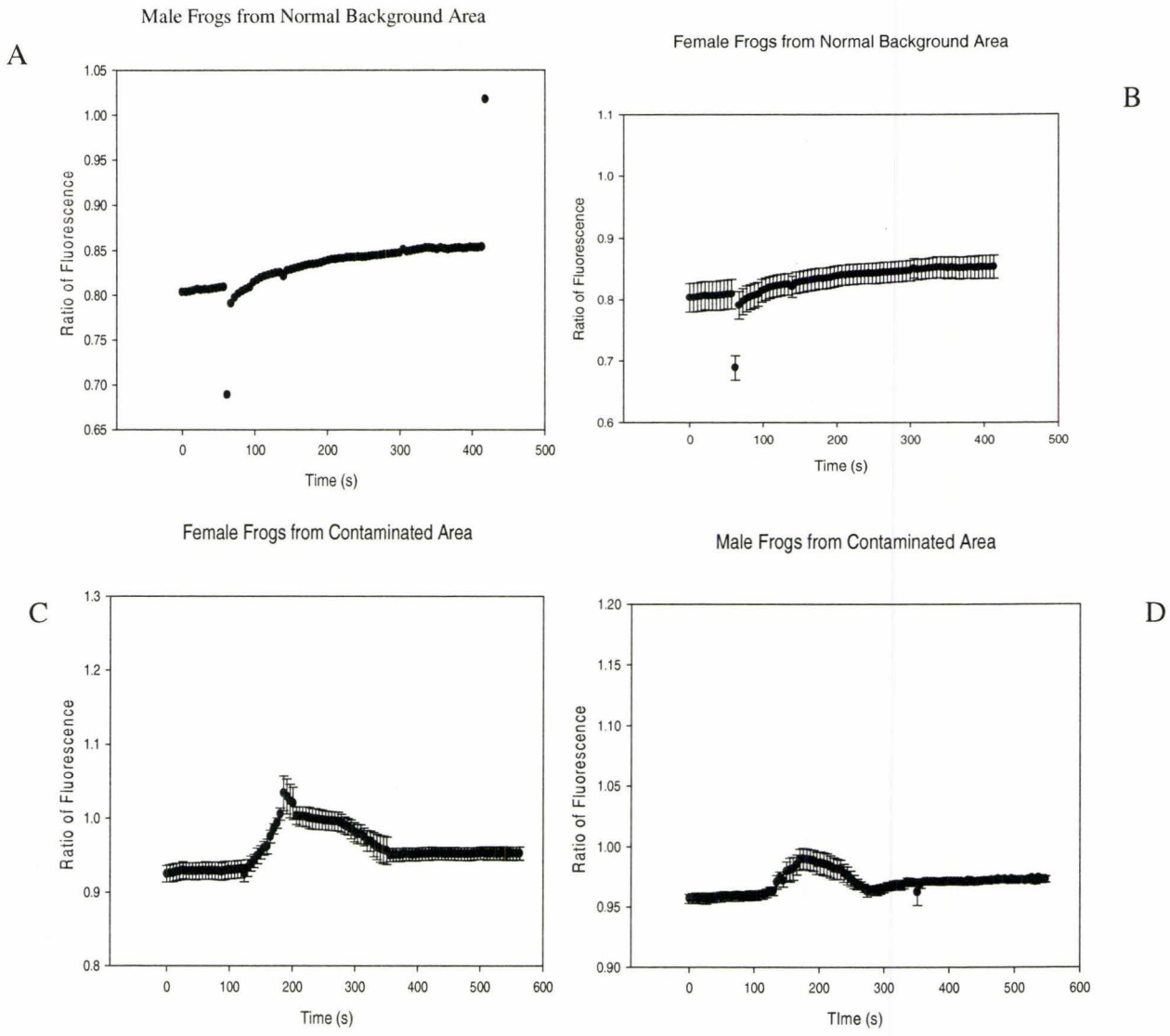
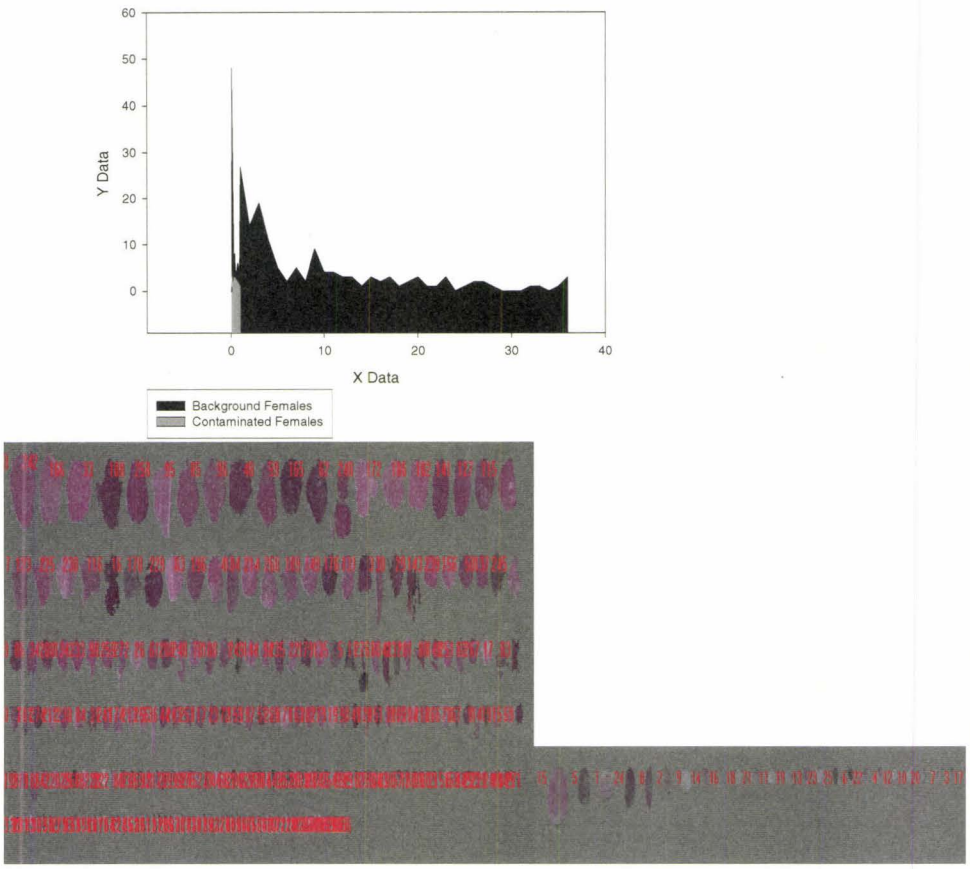


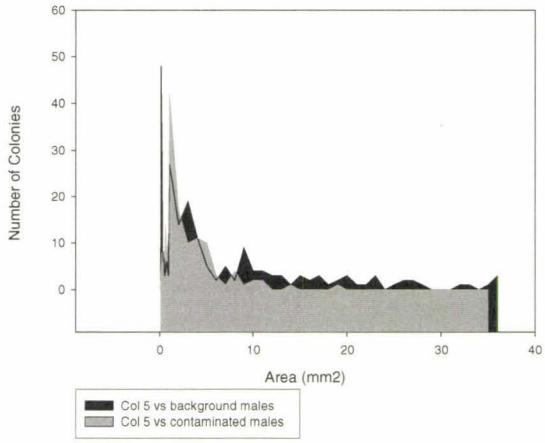
Figure 4.5: Calcium flux in reporters associated with radiation contaminated and control mink frogs

Intracellular Calcium signaling in un-irradiated HPV-G transfected reporters is measured in response to exposure to medium derived from bladder explants from female and male frogs from control sites (A and B), in addition to medium derived from explants from female and male frogs from contaminated sites (C and D). . (N= 5 cells measured/treatment) All values shown are Mean ± SEM.

A


Area Distribution of Reporters Recieving Medium from Background vs Contaminated Females
Y Data
X Data
Background Females
Contaminated Females

B


Area Distribution of Reporters Receiving Medium from Background vs Contaminated Males
Number of Colonies
Area (mm2)
Col 5 vs background males
Col 5 vs contaminated males

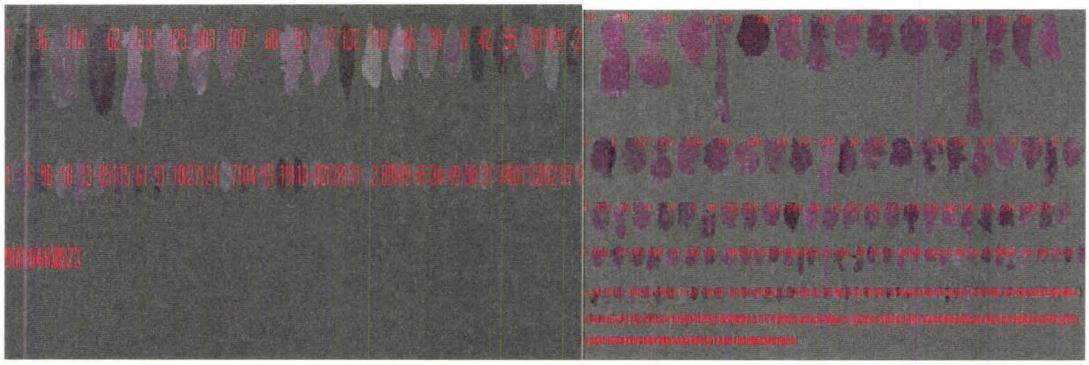


Figure 4.6: Area distribution of reporters associated with radiation contaminated and control mink frogs

Colony area size distribution of cells on day 10 after incubation in (A) in reporters receiving medium from bladder explants from female frogs taken from contaminated versus control or background radiation sites (B) and in reporters receiving medium from explants from male frogs taken from contaminated versus background or control sites. Number of cells in each treatment varies, refer to Appendix 5 (A.5.4). Below each graph are the actually colonies segmented by the Image pro software system.

5 CONCLUSIONS AND FUTURE WORK

The thesis provides further evidence that bystander signals are produced *in vivo* in C57Bl6 (gnomically stable) and Balb/c (gnomically unstable) mice after various doses of radiation under various exposure conditions. Similarly, chronic exposures of radiation through lifetime exposure in contaminated areas to Mink frogs also show the production of bystander signals. In all cases, the signals that are produced are in the form of long lived entities that survive freeze-thaw conditions. In lieu of previous work (Mothersill *et al.*, 2001 and 2005) which shows that the signal production process is separate from signal response process, both of which may be modulated by genetic background. This concept is consistent with current findings, with the exception being the repeated exposure conditions.

As expected, the C57Bl6 mice show a dose –dependent decrease in percent reporter survival as well as their growth rates after exposure to acute doses, where as the Balb/c mice fail to show a decrease in colony survival but they do show a dose-dependant decrease in reporter growth rates. After repeated exposures, C57Bl6 and Balb/c mice induced an increase in percent reporter survival in addition to a considerable increase in their corresponding growth rates. Interestingly, both mouse strains also induced abnormal calcium induction patterns characterized by rapid and sustained increase in calcium levels. When this information is coupled with the fact that these reporters also show stimulated growth rates, it allows for the conclusion that perhaps repeated exposures are promoting tumorgenic rather then adaptive growth. C57Bl6 mice also showed immediate divergence in growth rates between

fast and slow growth reporters after acute or repeated exposures when compared to the unirradiated controls which showed a three day delay before fast growers began to proliferate. On the other hand, acutely or repeatedly exposed Balb/c mice show a two to three day delay between fast and slow growth reporters where as the fast growth reporters corresponding to the unirradiated controls show an immediate divergence of proliferation patterns. It is well understood that slow growing cells in a population possess radiation induced damage (chromosomal damage) (Grote *et al.*, 1981). A delay in proliferation implies that all cells are growing at the same rate, until some cells within a population begin to manifest forms of damage that results in a decrease in their rate of proliferation. Results in this thesis show that reporters associated with gnomically stable mice show a delay in proliferation, but the reporters corresponding to exposed C57Bl6 show immediate divergence between the fast and slow growth reporters indicating induction of biological effect within 24 hours after exposure to ITCM. Conversely, reporters corresponding to uniiradiated Balb/c mice show immediate divergence in slow and fast growth reporters where as some of the reporters exposed to ITCM from irradiated Balb/c mice show the induction of this delay in proliferation. The reason for these differences in delay patterns is not clear from the present research, but it is feasible that the nature of the signal produced may also determine the rate at which biological effect is apparent in recipients.

Reporters given ITCM from frogs sampled from contaminated areas versus control sites show a sex-dependant modulation of bystander effects, with females showing great radio-sensitivity than males. Interestingly, delay in proliferation is

observed in reporters associated with radiation exposed samples (similar to Balb/c reporters). Furthermore, the radiosensitive females show a decrease in the lag time between the corresponding slow and fast growers. This supports the conclusion that genetic background can dictate the nature of bystander signals to such an extreme extent that even onset of biological effect is modulated.

Clearly, such tremendous variation in responses to bystander signals alludes to the complexity between radiation and the biological system. More specifically, repeated exposures show interesting responses, and it would be interestingly to explore these effects in more detail *in vitro*. It would be interesting to monitor growth rates as a biological endpoint after the priming dose, and watch how they fluctuate after given the challenge dose. Moreover, further exploration into the inherent differences between fast and slow growth colonies would prove to be very interesting. Growing these reporters to confluence, and re-plating them would show how well they maintain their plating efficiency and proliferation delays. In addition to this, more studies involving chronic, low dose exposures to radiation on non-human biota must be performed in order to gain a more comprehensive understanding of realistic exposure conditions.

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A Appendix 1

A.1 Raw Data- Clonogenic Survival of reporters exposed to bladder medium from irradiated C57BL6 and Balb/c mice

A.1.1 reporters associated with irradiated C57B1/6J Mice

Treatment	Cells Plated	Colony Count		PE (%)		%Survival		SD	T value	Sig
		Man	Auto	M	A	M	A			
0 Gy n=4	500	198 70 67 27 Avg= 90	92 33 116 203 Avg= 111	18	22	100	100	M-37.1494 A- 35.277		
2 Gy n=3	500	17 7 10 Avg= 11	6 7 10 Avg= 7.66	2	4.42	12	6.9	M-2.835 A-1.083		
20 mGy n=3	500	22 20 75 78 Avg= 48	76 93 43 Avg= 70.70	9.7	14	53.86	63.66	M- 17.772 A- 13.23		
4 Hour Group n=4	500	49 58 49 136 Avg= 73	196 154 265 224 Avg= 209.8	14.6	41	80.66	188.96	M- 23.322 A- 21.002		
24 Hour Group n=4	500	61 64 60 163 Avg = 87	182 141 145 180 Avg= 162	17	32	96.13	145.94	M- 28.008 A- 9.99167		
48 Hour Group n=4	500	56 61 72 97 Avg= 72	117 265 201 115 Avg= 175	14	35	79.00	157.20	M- 10.092 A- 32.62		

Controls n=8	500	130 136 129 224 241 220 248 250 Avg= 197	195 122 233 239 212 Avg= 200	39	40	n/a	n/a	M- 34.875 A- 28.797	

A.1.2 reporters exposed to irradiated Balb/c Mice

Treatment	Cells Plated	Colony Count		PE (%)		SF		SD	T value	Sig
		Man	Auto	M	A	M	A			
0 Gy n=3	1000	355 321 301 Avg= 326	380 360 400 Avg= 380	33	38	100	100	M- 15.621 A- 11.547	0	
2 Gy n=3	1000	350 319 302 Avg=323	926 855 815 Avg= 865	32	87	99.84	227	M- 1.732 A- 8.5415		
20 mGy n=3	1000	372 352 341 Avg= 355	644 591 550 Avg= 596	36	60	109.5092	156.8689	M- 4.825 A- 4.1329		
4 Hour Group n=3	1000	241 200 213 Avg =	25 21 26 Avg=	22	25	66.941	60.414	M-6.434 A- 14.38		

		218	243						
24 Hour Group n=3	1000	208 215 195 Avg= 206	193 200 210 Avg = 201	21	20	63.54619	52.99269	M- 3.1165 A- 1.2981	
48 Hour Group n=3	1000	250 221 255 Avg= 242	238 256 301 Avg= 266	24	27	74.65135	69.86598	M- 5.636 A- 4.9393	
Controls N=3	1000	361 340 370 Avg= 357	427 414 395 Avg = 412	35	41	N/a	N/a	M- 8.88819 A-9.2915	

A Appendix 2

A.2 Calcium Signaling in reporters exposed to transfer medium from irradiated C57Bl6 and Balb/c mice

A.2.1 Calcium Signaling induced by bladder medium from irradiated C57BL6 mice

Time (s)	*0 gy*	*S.E.M*	*2gy*	*Sem*	*20mgy*	*S.E.M*	*4 hour group*	*S.E.M*	*24 hour group*	*S.*
0.0000	0.9917	2.0888e	0.9862	1.6589e-	0.9868	1.0360e-	0.9942	6.2439e-	0.9705	4.

				3		3		4		3
4.4530	0.9922	2.7614e	0.9860	1.4311e-3	0.9882	3.5251e-3	0.9938	4.9924e-4	0.9709	4.3
8.9060	0.9908	5.1795e	0.9860	2.6326e-3	0.9884	1.8064e-3	0.9938	7.4974e-4	0.9704	4.3
13.2970	0.9885	1.7494e	0.9855	1.1503e-3	0.9903	2.4756e-3	0.9943	6.4273e-4	0.9710	4.3
17.8430	0.9937	1.2297e	0.9854	1.8315e-3	0.9890	7.8660e-3	0.9938	9.5031e-4	0.9732	3.3
22.2810	0.9921	2.1697e	0.9855	1.6365e-3	1.0085	0.0207	0.9939	4.6150e-4	0.9701	4.3
26.6870	0.9932	1.6277e	0.9857	1.8406e-3	1.0243	0.0275	0.9938	4.2409e-4	0.9711	3.3
31.0930	0.9907	1.7763e	0.9857	2.3705e-3	1.0429	0.0338	0.9942	6.5328e-4	0.9714	3.3
35.5000	0.9935	2.1508e	0.9858	2.2967e-3	1.0602	0.0419	0.9943	4.7926e-4	0.9704	4.3
40.0470	0.9919	2.4019e	0.9858	1.1631e-3	1.0771	0.0449	0.9939	7.4195e-4	0.9695	5.3
44.4530	0.9966	3.8903e	0.9857	1.3578e-3	1.0855	0.0461	0.9943	8.6747e-4	0.9704	4.3
48.8430	0.9936	3.6560e	0.9857	9.7673e-4	1.0899	0.0466	0.9950	6.8874e-4	0.9705	4.3
53.2180	0.9925	1.0383e	0.9857	1.9540e-3	1.0936	0.0443	0.9964	7.6567e-4	0.9707	4.3
57.6090	0.9924	3.5511e	0.9856	7.0931e-4	1.0984	0.0458	0.9976	1.8687e-3	0.9706	4.3
61.9840	0.9912	9.8946e	0.9908	1.4006e-3	1.1016	0.0457	0.9982	2.3682e-3	0.9709	4.3
66.4220	0.9901	3.1735e	0.9921	1.4338e-3	1.1038	0.0498	0.9990	2.3600e-3	0.9716	4.3
70.8590	0.9921	1.3717e	1.0121	0.0262	1.1037	0.0471	0.9996	2.5414e-3	0.9720	3.3
75.2810	0.9925	3.8063e	1.0689	0.0418	1.1018	0.0470	1.0008	2.7791e-3	0.9717	3.3
79.7500	0.9921	1.6994e	1.0991	0.0630	1.1051	0.0460	1.0023	4.8278e-3	0.9709	3.3
84.1870	0.9922	3.5086e	1.1225	0.0823	1.1001	0.0439	1.0030	5.7369e-3	0.9713	4.3
88.5930	0.9928	2.3400e	1.1651	0.0884	1.1016	0.0429	1.0035	5.6564e-3	0.9678	3.3
93.0310	0.9917	7.5193e	1.1746	0.0870	1.1086	0.0444	1.0035	5.5521e-3	0.9706	3.3

97.5930	0.9934	1.9877e	1.1886	0.0894	1.1166	0.0444	1.0037	5.5995e-3	0.9701	3.3
102.0310	0.9901	3.9276e	1.1921	0.0873	1.1250	0.0433	1.0028	6.3405e-3	0.9718	3.3
106.4680	0.9918	1.6803e	1.2012	0.0883	1.1247	0.0418	1.0026	6.2910e-3	0.9730	3.3
111.0470	0.9945	1.0539e	1.2100	0.0890	1.1233	0.0449	1.0024	6.5167e-3	0.9742	3.3
115.4530	0.9919	1.1463e	1.2112	0.0958	1.1259	0.0475	1.0021	6.6918e-3	0.9756	3.3
119.8900	0.9906	1.9045e	1.2100	0.0930	1.1219	0.0446	0.9998	7.2979e-3	0.9759	3.3
124.3280	0.9931	2.4786e	1.2100	0.0895	1.1128	0.0461	0.9975	8.8413e-3	0.9763	3.3
128.8120	0.9913	1.1493e	1.2001	0.0877	1.1092	0.0403	0.9983	5.6300e-3	0.9773	3.3
133.2650	0.9934	4.7411e	1.1998	0.0858	1.1049	0.0523	0.9981	5.7763e-3	0.9781	3.3
137.6560	0.9904	2.1811e	1.1855	0.0843	1.0986	0.0617	0.9971	5.7508e-3	0.9792	3.3
142.0310	0.9921	3.5266e	1.1820	0.0849	1.0809	0.0524	0.9993	6.9982e-3	0.9782	3.3
146.4680	0.9928	3.9686e	1.1796	0.0817	1.0721	0.0509	0.9909	6.7475e-3	0.9786	3.3
150.8900	0.9908	8.8623e	1.1662	0.0782	1.0657	0.0510	0.9922	7.2354e-4	0.9792	3.3
155.4370	0.9939	1.2191e	1.1552	0.0680	1.0632	0.0539	0.9924	6.9374e-4	0.9789	3.3
159.8590	0.9931	3.1110e	1.1445	0.0740	1.0550	0.0566	0.9920	6.2243e-4	0.9784	3.3
164.3120	0.9911	1.3735e	1.1366	0.0740	1.0522	0.0503	0.9972	1.1544e-3	0.9783	3.3
168.7180	0.9894	1.9650e	1.1211	0.0713	1.0319	0.0460	0.9902	7.7414e-4	0.9788	3.3
173.2810	0.9919	1.2847e	1.1001	0.0696	1.0314	0.0373	0.9899	8.2026e-4	0.9781	2.3
177.6870	0.9926	1.6267e	1.0992	0.0662	1.0267	0.0310	0.9900	8.2570e-4	0.9782	3.3
182.0620	0.9921	2.0210e	1.0900	0.0644	1.0209	0.0262	0.9904	7.6757e-4	0.9781	2.3
186.4220	0.9911	1.5010e	1.0775	0.0609	1.0199	0.0305	0.9911	7.1846e-4	0.9778	2.3
190.7970	0.9937	2.3822e	1.0612	0.0423	1.0106	0.0240	0.9908	6.4289e-	0.9773	2.

								4		3
195.1870	0.9918	1.5630e	1.0550	0.0414	1.0031	5.1588e-3	0.9907	9.0772e-4	0.9779	2.3
199.5620	0.9925	8.4713e	1.0331	0.0389	0.9873	1.5982e-3	0.9902	6.7727e-4	0.9773	2.3
203.9370	0.9925	2.5812e	1.0210	0.0386	0.9840	1.5636e-3	0.9908	9.0329e-4	0.9770	2.3
208.3430	0.9965	1.8110e	1.0101	0.0276	0.9836	2.0627e-3	0.9909	7.0337e-4	0.9768	2.3
212.7180	0.9938	2.1552e	1.0010	0.0239	0.9788	2.5309e-3	0.9906	5.4095e-4	0.9768	2.3
217.1090	0.9918	4.3002e	0.9988	0.0234	0.9757	4.0183e-3	0.9911	8.4829e-4	0.9771	2.3
221.4530	0.9915	1.0706e	0.9982	9.4269e-4	0.9771	3.9254e-3	0.9904	5.8895e-4	0.9768	2.3
225.8900	0.9903	5.1446e	0.9971	5.8476e-4	0.9825	4.4885e-3	0.9904	1.1200e-3	0.9768	2.3
230.2810	0.9974	3.6400e	0.9951	6.7473e-4	0.9797	4.9235e-3	0.9906	7.4523e-4	0.9768	2.3
234.7180	0.9935	2.7930e	0.9962	1.1750e-3	0.9777	1.7037e-3	0.9907	7.4711e-4	0.9772	2.3
239.2030	0.9884	2.1094e	0.9950	1.9825e-3	0.9770	2.5786e-3	0.9909	7.6389e-4	0.9770	2.3
243.6560	0.9949	7.1406e	0.9923	1.5912e-3	0.9762	1.7237e-3	0.9906	4.6312e-4	0.9771	2.3
248.0470	0.9909	3.9558e	0.9856	8.5497e-4	0.9811	1.0409e-3	0.9902	8.4605e-4	0.9770	2.3
252.4370	0.9927	3.0755e	0.9810	6.0080e-4	0.9749	1.8636e-3	0.9906	7.8353e-4	0.9772	2.3
256.8120	0.9954	4.2668e	0.9800	1.6400e-3	0.9751	2.5189e-3	0.9906	8.8944e-4	0.9774	2.3
261.1560	0.9973	1.6015e	0.9790	2.0126e-3	0.9750	4.4706e-3	0.9904	1.0029e-3	0.9775	2.3
265.5620	0.9902	1.6152e	0.9782	1.2144e-3	0.9741	3.2818e-3	0.9912	8.9218e-4	0.9771	2.3
269.9370	0.9895	3.2264e	0.9781	9.8083e-4	0.9740	2.4316e-3	0.9907	1.1265e-3	0.9771	2.3
274.3120	0.9945	1.7896e	0.9771	1.2488e-3	0.9734	3.1169e-3	0.9907	1.0208e-3	0.9773	2.3
278.6560	0.9868	5.1950e	0.9776	1.2220e-3	0.9721	5.7902e-4	0.9902	8.2747e-4	0.9771	2.3
283.0780	0.9979	5.1214e	0.9775	1.2276e-3	0.9720	3.3806e-3	0.9903	1.2569e-3	0.9773	2.3

287.5310	0.9966	2.8032e	0.9774	1.0790e-3	0.9735	4.3052e-3	0.9906	1.3385e-3	0.9774	2.3
291.9220	0.9925	2.9508e	0.9770	6.4413e-4	0.9722	4.7052e-3	0.9910	1.6604e-3	0.9775	2.3
296.3280	0.9920	5.2521e	0.9750	6.5549e-4	0.9721	2.7199e-3	0.9905	1.4170e-3	0.9775	2.3
300.7180	0.9906	2.2386e	0.9721	1.4157e-3	0.9720	3.2589e-3	0.9901	8.8804e-4	0.9779	2.3
305.1400	0.9984	3.0189e			0.9724	3.0414e-3	0.9909	1.0325e-3	0.9774	2.3
309.5470	0.9870	5.1449e			0.9722	2.5682e-3	0.9902	9.3630e-4	0.9775	2.3
314.1090	0.9955	3.0764e			0.9736	3.3714e-3	0.9908	8.8266e-4	0.9778	2.3
318.4840	0.9988	2.0911e			0.9769	4.2192e-3	0.9906	9.1073e-4	0.9778	2.3
322.8900	0.9881	2.0131e			0.9718	3.7697e-3	0.9908	5.7983e-4	0.9780	2.3
327.3280	0.9949	3.9427e			0.9725	3.0898e-3	0.9906	3.8703e-4	0.9778	2.3
331.8590	0.9874	3.1822e			0.9745	6.3626e-3	0.9906	5.8701e-4	0.9779	2.3
336.2970	0.9934	3.2522e			0.9738	3.9824e-3	0.9906	5.1915e-4	0.9781	2.3
340.7030	1.0021	3.4111e			0.9726	3.5712e-3	0.9910	7.6819e-4	0.9778	2.3
345.2810	0.9958	2.3896e			0.9749	1.3086e-3	0.9908	5.6917e-4	0.9782	2.3
349.7340	0.9925	4.6865e			0.9744	1.6927e-3	0.9905	4.1212e-4	0.9783	2.3
354.1250	0.9953	5.9028e			0.9739	4.5256e-3	0.9904	7.3429e-4	0.9786	2.3
358.4840	0.9918	3.5594e			0.9744	3.5446e-3	0.9908	5.7245e-4	0.9787	2.3
362.8750	0.9861	5.5344e			0.9731	2.9262e-3	0.9909	6.8753e-4	0.9785	2.3
367.2970	0.9921	1.0189e			0.9694	1.5932e-3	0.9908	5.5528e-4	0.9778	2.3
371.7030	0.9865	2.3706e			0.9743	3.9626e-3	0.9910	5.8226e-4	0.9780	2.3
376.2810	0.9949	1.6551e			0.9743	2.7798e-3	0.9906	7.0892e-4	0.9781	2.3
380.7030	0.9933	2.3768e			0.9735	4.1040e-	0.9908	8.5528e-	0.9782	2.

						3		4		3
385.0930	0.9851	4.4104e			0.9745	5.4574e-3	0.9907	6.3320e-4	0.9783	2.3
389.4680	0.9887	1.9699e			0.9759	2.8752e-3	0.9911	4.6809e-4	0.9779	2.3
393.9060	0.9917	1.4144e			0.9718	4.5633e-3	0.9910	5.5886e-4	0.9780	2.3
398.4680	0.9962	3.2149e			0.9736	3.6329e-3	0.9906	6.3583e-4	0.9785	2.3
402.8430	1.0003	3.2501e			0.9747	4.3839e-3	0.9908	7.7459e-4	0.9785	2.3
407.2650	0.9963	2.9946e			0.9747	2.5690e-3	0.9906	7.3529e-4	0.9784	2.3
411.6400	0.9942	1.6672e			0.9731	3.0550e-3	0.9910	5.5652e-4	0.9783	2.3
416.0780	0.9937	1.3495e			0.9734	5.0831e-3	0.9907	5.8804e-4	0.9790	2.3
420.5150	0.9937	2.2536e			0.9714	4.9327e-3	0.9909	7.1370e-4		
425.0470	0.9981	4.1805e			0.9743	3.4806e-3	0.9909	7.3770e-4		
429.4220	0.9998	2.5765e			0.9731	4.2279e-3	0.9906	7.5683e-4		
433.8280	0.9826	2.3282e			0.9732	1.8306e-3	0.9910	8.7413e-4		

A.2.2 Calcium signaling induced by bladder medium from irradiated Balb/c Mice

Time (s)	*0 gy*	*S.E.M*	*2gy*	*Sem*	*20mgy*	*S.E.M*	*4 hour group*	*S.E.M*	*24 hour group*	*S*
0.0000	0.9996	2.8174e-5	0.9983	4.8834e-4	0.9987	8.5795e-4	0.9976	1.9246e-4	0.9711	4 3
5.4850	0.9997	1.2075e-5	0.9985	7.3028e-4	0.9943	2.3445e-3	0.9979	4.5632e-4	0.9712	4 3
11.2970	0.9997	4.0249e-6	0.9976	3.7109e-4	1.0036	8.5186e-4	0.9968	3.8019e-4	0.9710	4 3
16.5780	0.9997	1.3864e-5	0.9986	2.4107e-4	1.0029	8.0983e-4	0.9975	4.9448e-4	0.9720	4 3

21.8910	0.9997	5.3666e-6	0.9976	5.7293e-4	0.9984	2.4327e-4	0.9977	1.5992e-4	0.9726	3 3
27.2030	0.9997	2.1019e-5	0.9979	6.1909e-4	1.0017	7.4527e-4	0.9976	3.8058e-4	0.9730	4 3
32.5160	0.9997	1.3864e-5	0.9981	7.2577e-4	0.9941	2.4185e-3	0.9971	4.0454e-5	0.9737	3 3
37.8600	0.9997	0.0000	0.9975	4.0302e-4	0.9979	1.9826e-3	0.9977	2.0917e-4	0.9739	3 3
43.1100	0.9997	2.2361e-6	0.9993	6.9526e-4	0.9947	1.7643e-3	0.9982	4.7350e-4	0.9732	4 3
48.4220	0.9997	0.0000	0.9989	9.8560e-4	0.9976	8.5841e-4	0.9978	3.5901e-4	0.9731	5 3
53.7190	0.9997	0.0000	0.9983	7.4500e-4	0.9927	8.3442e-4	0.9968	5.6254e-4	0.9730	4 3
59.0310	0.9997	2.2360e-6	0.9978	6.1283e-4	1.0011	8.7010e-4	0.9975	5.3961e-4	0.9725	4 3
64.3600	0.9997	0.0000	0.9982	4.1415e-4	1.0017	8.0470e-4	0.9964	3.8229e-4	0.9726	4 3
69.6720	0.9997	0.0000	0.9979	8.2094e-4	0.9931	2.6039e-3	0.9971	3.5204e-4	0.9727	4 3
74.9220	0.9997	1.2075e-5	0.9972	7.8047e-4	0.9979	1.7553e-3	0.9973	1.9169e-4	0.9730	4 3
80.2190	0.9997	1.3416e-5	0.9980	6.6537e-4	0.9978	4.4177e-4	0.9980	3.3571e-4	0.9732	4 3
85.5470	0.9997	1.0286e-5	0.9979	7.7894e-4	0.9970	1.6380e-3	0.9978	3.9138e-4	0.9735	3 3
90.8910	0.9997	3.1305e-6	0.9981	4.9499e-4	0.9952	9.0257e-4	0.9971	4.8637e-4	0.9730	3 3
96.1880	0.9997	1.2522e-5	0.9984	6.8486e-4	0.9998	7.8945e-4	0.9975	4.3266e-4	0.9733	3 3
101.4690	0.9997	3.2199e-5	0.9981	6.9782e-4	0.9973	1.7788e-3	0.9984	2.6136e-4	0.9735	4 3
106.8130	0.9997	7.1554e-6	0.9979	2.1311e-4	0.9919	1.4650e-3	0.9977	4.2343e-4	0.9732	3 3
112.1410	0.9997	0.0000	0.9976	3.2677e-4	1.0017	1.1495e-3	0.9974	2.4963e-4	0.9800	3 3
117.4690	0.9997	0.0000	0.9976	4.8110e-4	0.9986	1.6599e-3	0.9974	2.8301e-4	0.9862	3 3
122.7810	0.9997	0.0000	0.9984	7.7559e-4	0.9970	1.8948e-3	0.9970	2.8382e-4	0.9900	3 3
128.0780	0.9997	0.0000	0.9980	4.8488e-4	0.9958	1.4971e-3	0.9976	3.7338e-4	0.9957	3 3
133.3910	0.9997	0.0000	0.9980	5.8561e-	0.9986	1.1178e-	0.9978	4.5003e-	1.0001	3

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138.7500	0.9997	0.0000	1.0004	3.6833e-3	0.9959	1.6358e-3	0.9971	3.0696e-4	1.0010	3 3
144.1100	0.9997	0.0000	0.9926	9.8728e-3	0.9946	1.0878e-3	0.9966	2.6385e-4	1.0027	3 3
149.4220	0.9997	0.0000	0.9980	3.5788e-3	0.9946	1.3272e-3	0.9964	2.4798e-4	1.0026	3 3
154.7190	0.9997	0.0000	0.9999	5.9210e-3	0.9941	1.6515e-3	0.9964	2.9657e-4	1.0024	3 3
159.9850	0.9995	3.0187e-4	0.9986	1.0445e-3	1.0024	1.6762e-3	0.9965	3.1855e-4	1.0023	3 3
165.3130	0.9996	5.3666e-5	0.9983	9.9071e-4	0.9978	1.0067e-3	0.9966	2.6426e-4	1.0037	3 3
170.9060	0.9996	6.9318e-5	0.9989	4.0163e-4	1.0035	1.4906e-3	0.9967	2.4592e-4	1.0051	3 3
176.5000	0.9997	1.5652e-5	0.9996	7.7001e-4	1.0017	1.3217e-3	0.9972	4.1209e-4	1.0052	3 3
182.0470	0.9996	2.5491e-5	1.0035	4.2734e-3	1.0023	4.4651e-4	0.9983	4.7247e-4	1.0057	3 3
187.5780	0.9997	7.6026e-6	1.0059	4.2113e-3	0.9948	7.3132e-4	0.9997	3.9153e-4	1.0060	3 3
192.8280	0.9997	3.8460e-5	1.0133	0.0136	0.9974	5.9463e-4	0.9988	9.3086e-4	1.0078	3 3
198.0940	0.9996	3.0858e-5	1.0151	0.0142	0.9981	1.3940e-3	0.9921	8.5417e-4	1.0081	3 3
203.3750	0.9996	2.4150e-5	1.0170	0.0144	0.9958	1.4816e-3	0.9893	2.9687e-4	1.0091	3 3
208.7030	0.9996	1.8336e-5	1.0193	0.0175	0.9979	1.4527e-3	0.9825	3.5890e-4	1.0100	2 3
214.0160	0.9997	8.9444e-7	1.0224	0.0161	0.9983	1.6718e-3	0.9721	4.6607e-4	1.0123	3 3
219.3440	0.9996	4.1144e-5	1.0247	0.0186	0.9964	1.6225e-3	0.9693	5.9344e-4	1.0124	2 3
224.6560	0.9996	2.0125e-5	1.0261	0.0196	0.9963	1.9451e-3	0.9601	7.9268e-4	1.0125	2 3
229.9690	0.9996	4.9193e-5	1.0275	0.0212	0.9984	3.6294e-4	0.9662	5.9986e-4	1.0126	2 3
235.2970	0.9997	4.0249e-6	1.0265	0.0203	0.9989	2.3132e-3	0.9712	6.0095e-4	1.0128	2 3
240.6250	0.9997	1.4758e-5	1.0266	0.0203	0.9983	2.0230e-3	0.9821	5.7345e-4	1.0129	2 3
245.9380	0.9996	2.6833e-5	1.0264	0.0203	0.9989	2.2056e-3	0.9912	7.3952e-4	1.0132	2 3

251.2810	0.9997	1.3417e-6	1.0259	0.0200	0.9959	8.4747e-4	0.9963	1.0686e-3	1.0133	2 3
256.5310	0.9996	5.1430e-5	1.0255	0.0203	1.0028	1.4759e-3	0.9979	2.4345e-3	1.0141	2 3
261.8440	0.9996	1.8783e-5	1.0252	0.0205	0.9934	1.5286e-3	0.9982	2.6015e-3	1.0142	2 3
267.1100	0.9997	1.4311e-5	1.0252	0.0205	0.9934	1.1135e-3	0.9989	0.0119	1.0143	2 3
272.3600	0.9997	5.8138e-6	1.0249	0.0200	0.9981	1.7956e-3	0.9990	0.0119	1.0143	2 3
277.6100	0.9997	7.6026e-6	1.0250	0.0201	0.9922	2.0491e-3	1.0001	8.8724e-3	1.0146	2 3
282.8600	0.9996	3.1752e-5	1.0250	0.0201	0.9987	2.2965e-3	1.0025	7.3456e-3	1.0145	2 3
288.1880	0.9996	3.8908e-5	1.0249	0.0200	1.0032	1.4725e-3	1.0086	0.0300	1.0145	2 3
293.4380	0.9996	4.0249e-5	1.0249	0.0195	0.9972	7.9955e-4	1.0126	0.0280	1.0144	2 3
298.7810	0.9997	4.9193e-6	1.0247	0.0193	0.9924	1.7572e-3	1.0214	0.0323	1.0143	2 3
304.0470	0.9996	1.8783e-5	1.0245	0.0193	0.9983	1.2403e-3	1.0400	0.0319	1.0142	2 3
309.3910	0.9997	3.1305e-6	1.0241	0.0190	0.9936	9.9907e-4	1.0511	0.0280	1.0141	2 3
314.7190	0.9997	1.6100e-5	1.0236	0.0193	1.0035	1.2529e-3	1.0629	0.0297	1.0142	2 3
320.0780	0.9997	1.7889e-6	1.0233	0.0194	0.9968	1.5910e-3	1.0822	0.0298	1.0141	2 3
325.3130	0.9996	2.7727e-5	1.0222	0.0179	1.0031	1.1409e-3	1.1000	0.0297	1.0139	2 3
330.5940	0.9997	4.9193e-6	1.0214	0.0171	1.0031	1.0433e-3	1.1099	0.0300	1.0137	2 3
335.9220	0.9996	3.1305e-5	1.0203	0.0171	0.9935	1.9884e-3	1.1123	0.0301	1.0139	2 3
341.2190	0.9997	3.5777e-6	1.0192	0.0166	0.9974	1.7374e-3	1.1263	0.0306	1.0140	2 3
346.4690	0.9997	8.0498e-6	1.0177	0.0145	0.9894	1.7655e-3	1.1266	0.0395	1.0141	2 3
351.7500	0.9997	4.4703e-7	1.0161	0.0139	1.0009	1.0366e-3	1.1260	0.0409	1.0144	2 3
357.0000	0.9997	1.9677e-5	1.0150	0.0141	0.9954	1.7123e-3	1.1256	0.0415	1.0145	2 3
362.2500	0.9997	3.1305e-	1.0138	0.0122	1.0031	8.4995e-	1.1247	0.0425	1.0144	2

		6				4				3
367.6100	0.9997	0.0000	1.0133	0.0120	0.9978	1.6811e-3	1.1237	0.0430	1.0143	2 3
372.8600	0.9997	0.0000	1.0127	0.0122	1.0010	2.1425e-3	1.1201	0.0429	1.0142	2 3
378.1100	0.9997	0.0000	1.0122	0.0124	0.9973	2.1149e-3	1.1211	0.0434	1.0141	2 3
383.4220	0.9997	0.0000	1.0118	0.0116	0.9972	1.4224e-3	1.1200	0.0445	1.0142	2 3
388.6720	0.9997	0.0000	1.0109	0.0110	1.0008	2.6293e-3	1.1223	0.0444	1.0141	2 3
394.0000	0.9997	0.0000	1.0106	0.0110	1.0009	1.5508e-3	1.1242	0.0443	1.0141	2 3
399.3130	0.9997	0.0000	1.0083	6.9218e-3	0.9981	1.4359e-3	1.1251	0.0443	1.0140	2 3
404.6560	0.9997	0.0000	1.0071	5.2003e-3	0.9965	1.5068e-3	1.1299	0.0399	1.0142	2 3
409.9060	0.9997	0.0000	1.0059	3.8792e-3	0.9981	2.9899e-3	1.1293	0.0399	1.0141	2 3
415.1720	0.9997	0.0000	1.0050	3.0912e-3	0.9969	1.7417e-3	1.1292	0.0399	1.0143	2 3
420.4220	0.9997	0.0000	1.0044	3.1568e-3	0.9974	9.7340e-4	1.1282	0.0399	1.0146	2 3
425.7030	0.9997	0.0000	1.0039	3.0892e-3	0.9915	1.2750e-3	1.1270	0.0398	1.0149	2 3
430.9690	0.9997	0.0000	1.0029	2.7258e-3	0.9941	8.5331e-4	1.1252	0.0397	1.0144	2 3
436.2190	0.9997	0.0000	1.0024	2.3597e-3	0.9929	1.5893e-3	1.1242	0.0398	1.0144	2 3
441.5630	0.9997	0.0000	1.0024	1.7445e-3	0.9924	1.7270e-3	1.1252	0.0398	1.0146	2 3
446.8130	0.9997	0.0000	1.0018	1.8666e-3	0.9983	8.4622e-4	1.1267	0.0394	1.0142	2 3
452.0780	0.9997	0.0000	1.0014	1.4541e-3	0.9973	1.9812e-3	1.1255	0.0394	1.0149	2 3
457.3280	0.9997	0.0000	1.0009	1.2292e-3	0.9987	1.6044e-3	1.1254	0.0394	1.0150	2 3
462.6100	0.9997	0.0000	1.0006	8.5157e-4	0.9977	1.9931e-3	1.1260	0.0393	1.0145	2 3
467.8910	0.9997	0.0000	1.0004	4.6717e-4	1.0022	1.2162e-3	1.1266	0.0393	1.0144	2 3
473.1880	0.9997	0.0000	0.9999	8.5792e-4	0.9990	1.6403e-3	1.1267	0.0393	1.0142	2 3

478.4380	0.9997	0.0000	0.9996	4.8718e-4	1.0020	1.6508e-3	1.1268	0.0393	1.0140	2 3
483.7660	0.9997	0.0000	0.9989	7.3731e-4	0.9940	8.3128e-4	1.1270	0.0393	1.0142	2 3
489.0160	0.9997	0.0000	0.9981	3.3789e-4	1.0014	1.7772e-3	1.1270	0.0393	1.0149	2 3
494.2660	0.9997	0.0000	0.9978	5.3985e-4	0.9978	1.8512e-3	1.1270	0.0392	1.0141	2 3
499.5160	0.9997	0.0000	0.9978	6.1136e-4	0.9969	6.4190e-4	1.1278	0.0392	1.0142	2 3
504.8130	0.9997	0.0000	0.9983	4.4981e-4	0.9962	1.3472e-3	1.1279	0.0392		
510.1250	0.9997	0.0000	0.9986	5.6893e-4	0.9935	1.4065e-3	1.1287	0.0396		
515.5000	0.9997	0.0000	0.9996	7.9609e-4	1.0026	1.9117e-3	1.1292	0.0397		
520.7500	0.9997	0.0000	0.9977	4.7817e-4	1.0022	9.1322e-4	1.1295	0.0396		
525.7650			0.9976	5.3522e-4	0.9925	2.3932e-3	1.1300	0.0398		
531.0620	0.9996	2.8174e-5	0.9987	2.4018e-4	0.9931	1.0584e-3	1.1306	0.0401		
536.3900	0.9997	1.2075e-5	0.9982	6.7522e-4	0.9969	1.8830e-3	1.1314	0.0408		
542.6410	0.9997	4.0249e-6	0.9983	4.8834e-4	1.0019	2.3778e-3	1.1308	0.0401		
547.9690	0.9997	1.3864e-5	0.9985	7.3028e-4	1.0024	1.2539e-3	1.1315	0.0406		
553.3120	0.9997	5.3666e-6	0.9976	3.7109e-4	1.0044	5.8732e-4	1.1317	0.0408		
558.7340	0.9997	2.1019e-5	0.9986	2.4107e-4	0.9971	1.4937e-3	1.1298	0.0388		
564.1870	0.9997	1.3864e-5	0.9976	5.7293e-4	1.0017	9.8739e-4	1.1300	0.0388		
569.5310	0.9997	0.0000	0.9979	6.1909e-4	0.9917	2.7603e-3	1.1296	0.0386		
574.8280	0.9997	2.2361e-6	0.9981	7.2577e-4	0.9985	1.8785e-3	1.1298	0.0387		
580.2970	0.9997	0.0000	0.9975	4.0302e-4	1.0021	1.9145e-3	1.1299	0.0387		
585.7340	0.9997	0.0000	0.9993	6.9526e-4	1.0029	1.0641e-3	1.1303	0.0386		
591.1090	0.9997	2.2360e-	0.9989	9.8560e-	1.0006	7.5237e-	1.1305	0.0386		

		6		4		4				
596.4060	0.9997	0.0000	0.9983	7.4500e-4	0.9970	2.4278e-3	1.1308	0.0385		
601.7190	0.9997	0.0000	0.9978	6.1283e-4	0.9923	1.8200e-3	1.1314	0.0387		
607.0160	0.9997	1.2075e-5	0.9982	4.1415e-4	0.9927	1.7023e-3	0.9976	1.9246e-4	0.9711	4 3
612.3590	0.9997	1.3416e-5	0.9979	8.2094e-4	0.9933	2.2216e-3	0.9979	4.5632e-4	0.9712	4 3
617.6720	0.9997	1.0286e-5	0.9972	7.8047e-4	0.9976	2.1960e-3	0.9968	3.8019e-4	0.9710	4 3
622.9530	0.9997	3.1305e-6	0.9980	6.6537e-4	1.0010	7.9043e-4	0.9975	4.9448e-4	0.9720	4 3
628.2970	0.9997	1.2522e-5	0.9979	7.7894e-4	0.9996	2.0138e-3	0.9977	1.5992e-4	0.9726	3 3
633.6250	0.9997	3.2199e-5	0.9981	4.9499e-4	0.9978	1.6115e-3	0.9976	3.8058e-4	0.9730	4 3
638.9370	0.9997	7.1554e-6	0.9984	6.8486e-4	1.0014	2.1419e-3	0.9971	4.0454e-5	0.9737	3 3
644.2190	0.9997	0.0000	0.9981	6.9782e-4	0.9974	9.8017e-4	0.9977	2.0917e-4	0.9739	3 3
649.7190	0.9997	0.0000	0.9979	2.1311e-4	0.9961	1.4942e-3	0.9982	4.7350e-4	0.9732	4 3

A Appendix 3

A.3 Growth rates of reporters given bladder medium from irradiated mice

A.3.1 Growth rate in reporters exposed to bladder medium from irradiated C57BL6 mice

Fast Growth Colonies – controls

Slow growth colonies - controls

Fast Growth Colonies												
day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 1	day 2	day 3	da
0.00255	0.00321	0.01109	0.01999	0.03913	0.07812	0.16912	0.30145	0.60211	0.0009	0.00134	0.00246	0.0
0.00321	0.00623	0.01213	0.02279	0.04357	0.10999	0.19877	0.35279	0.50145	0.00491	0.00792	0.01215	0.0
0.00423	0.00757	0.01121	0.03299	0.12358	0.29146	0.39512	0.51423	0.70789	0.00412	0.00521	0.00712	0.0
0.00912	0.00131	0.00341	0.00712	0.01233	0.02712	0.07923	0.10146	0.23712	0.00621	0.01015	0.01358	0.0
0.00271	0.00341	0.00678	0.0088	0.01372	0.03678	0.1476	0.22798	0.42779	0.00316	0.00418	0.00912	0.0
0.00715	0.00314	0.00791	0.01218	0.03417	0.08912	0.13479	0.20145	0.39958	0.00121	0.00241	0.00421	0.0
0.00715	0.0102	0.02712	0.04712	0.09218	0.10145	0.22712	0.39178	0.5141	0.00132	0.00421	0.00668	0.0
0.00431	0.00728	0.01371	0.02945	0.06923	0.08961	0.17723	0.19898	0.27556	0.00191	0.00221	0.00651	0.0
0.00421	0.00721	0.01215	0.02413	0.03615	0.05523	0.07898	0.10218	0.18751	0.00157	0.00342	0.00312	0.0
0.00498	0.00721	0.01271	0.02176	0.03142	0.09146	0.16912	0.19786	0.30142	0.00218	0.00361	0.00621	0.0
0.00132	0.00328	0.01412	0.02712	0.03676	0.08498	0.12712	0.19278	0.20141	0.00217	0.00321	0.00421	
0.00271	0.00391	0.00721	0.01271	0.03215	0.04278	0.08146	0.19278	0.20146	0.00157	0.00428	0.00776	0.0
0.00191	0.00414	0.01021	0.01398	0.02712	0.04279	0.05912	0.08872	0.11978	0.00091	0.00104	0.00201	0.0
0.00312	0.00692	0.01721	0.02279	0.04912	0.08218	0.12279	0.19986	0.2294	0.00255	0.00398	0.01345	0.0
0.00428	0.00676	0.01171	0.04279	0.10098	0.18278	0.22712	0.28776	0.44279	0.00211	0.00221	0.00298	0.0
0.00301	0.00772	0.01271	0.01491	0.02012	0.03671	0.05223	0.10817	0.22911	0.001	0.00188	0.002	0.0
0.00528	0.00971	0.01141	0.03217	0.05523	0.10524	0.19774	0.30145	0.65773	0.00228	0.00315	0.00479	0.0
0.00321	0.00728	0.00915	0.01446	0.03268	0.08812	0.24412	0.36912	0.65213	0.00128	0.00368	0.00679	0.0
0.00421	0.00888	0.01316	0.03923	0.07912	0.10992	0.20223	0.40427	0.2778	0.00322	0.00451	0.00671	0.0
0.00142	0.00321	0.00721	0.01142	0.01928	0.03368	0.08815	0.13423	0.31047	0.00142	0.00272	0.00428	0.0
0.00271	0.00428	0.00761	0.01778	0.04723	0.08812	1.13621	0.29346	0.30978				

Fast Growth Colonies – 0 gy

Slow Growth Colonies

0.00091	0.00101	0.00214	0.00891	0.01141	0.05412	0.07213	0.10123	0.2014	0.0012	0.00223	0.00412	0.0
0.00092	0.00201	0.00914	0.00112	0.0321	0.07714	0.1129	0.19987	0.31441	0.00421	0.00612	0.00821	0.0
0.00192	0.00321	0.00441	0.00914	0.0191	0.03298	0.08134	0.1014	0.22912	0.00125	0.02267	0.00413	0.0
0.00291	0.00422	0.00721	0.0121	0.0321	0.0692	0.10092	0.1899	0.2214	0.00271	0.00521	0.00798	0.0
0.00615	0.0072	0.0081	0.00112	0.0362	0.09119	0.1921	0.29156	0.4241	0.00213	0.00421	0.00721	0.0
0.0022	0.00321	0.00562	0.00814	0.0111	0.03312	0.09213	0.19127	0.2914	0.0055	0.00721	0.00912	0.1
0.00321	0.00512	0.00789	0.01121	0.0275	0.08912	0.11298	0.1975	0.3942	0.00099	0.00121	0.0023	0.0
0.00121	0.00321	0.00523	0.00812	0.01123	0.04127	0.07912	0.19712	0.30013	0.00112	0.00321	0.00678	0.0

0.00221	0.00398	0.00577	0.01128	0.02919	0.05148	0.10278	0.22712	0.40912
0.00241	0.00345	0.00612	0.01015	0.02213	0.06712	0.09918	0.20145	0.50501
0.00272	0.00428	0.00578	0.01017	0.02279	0.05678	0.12778	0.29787	0.42712
0.00271	0.00324	0.00441	0.01279	0.03146	0.07812	0.11499	0.27213	0.53278
0.00092	0.00123	0.00339	0.00772	0.02138	0.06693	0.15983	0.31425	0.52923
0.00621	0.00791	0.00899	0.01271	0.03368	0.07912	0.18912	0.32115	0.67147

0.00201	0.00321	0.00572	0.0
0.00278	0.00415	0.00572	0.0
0.00215	0.00446	0.00672	0.0

Fast Growth Colonies – 2 Gy

Slow Growth Colonies – 2 Gy

day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9
0.00301	0.01626	0.04378	0.04837	0.01341	0.01341	0.01445	0.01548	0.12768
0.02383	0.16102	0.2251	0.38788	0.61723	0.72145	0.89145	1.05734	1.97812
0.00631	0.01509	0.03016	0.04178	0.05168	0.04213	0.04008	0.03318	0.04018
0.00624	0.02183	0.04401	0.09704	0.14078	0.17815	0.18815	0.19808	0.30214
0.00186	0.00318	0.00519	0.00714	0.00712	0.00761	0.00799	0.00817	0.00919
0.0081	0.0147	0.02145	0.04318	0.03105	0.01871	0.01998	0.02121	0.02446
0.02142	0.07162	0.09883	0.15271	0.25915	0.26015	0.26145	0.27812	0.33789
0.01556	0.03045	0.05417	0.08023	0.10121	0.09141	0.0814	0.07725	0.11378
0.00366	0.01121	0.01696	0.02666	0.03345	0.03991	0.04152	0.05524	0.057
0.00715	0.02212	0.03291	0.30818	0.04598	0.05213	0.05918	0.10146	0.10422
0.01927	0.03572	0.03691	0.04423	0.08712	0.09915	0.10146	0.10427	0.18769
0.00261	0.00443	0.01184	0.03312	0.05768	0.067	0.08912	0.19278	0.26534
0.00714	0.01607	0.02145	0.03001	0.03361	0.04412	0.05712	0.07809	0.08015
0.00521	0.00549	0.01546	0.03067	0.02768	0.02887	0.02912	0.0322	0.06572

1	day 2	day
0.01169	0.01659	0
0.00151	0.00715	0.0
0.00374	0.00938	0.0
0.00269	0.00489	0.0
0.00261	0.00448	0
0.00199	0.00569	0.0
0.00219	0.00572	0.0
0.00222	0.00577	0.0
0.00348	0.0048	0.0
0.00357	0.00771	0.0
0.00448	0.01039	0.0
0.00701	0.01646	0
0.00143	0.00479	0.0
0.00151	0.00272	0.0
0.00422	0.00814	0.0

Fast Growth Colonies – 20 mGy

Slow Growth Colonies – 20 mGy

day 1	day 2	day 3	day 4	day 5	day 6	day7	day 8	day 9
0.0184	0.0629	0.10681	0.14923	0.31645	0.44213	0.53678	0.60698	0.87289
0.00453	0.01351	0.0328	0.06001	0.10478	0.20012	0.30145	0.32978	0.45457
0.00743	0.02625	0.04888	0.06157	0.07458	0.08155	0.04612	0.11576	0.13779
0.00617	0.01072	0.02168	0.03998	0.05932	0.06912	0.08213	0.18678	0.22806
0.00314	0.00422	0.01046	0.01691	0.01778	0.02146	0.06213	0.08392	0.11571
0.0028	0.00886	0.01667	0.03223	0.03468	0.05213	0.06423	0.07761	0.09023
0.00905	0.02036	0.04198	0.0856	0.13578	0.20678	0.25612	0.39072	0.45679
0.00717	0.01674	0.02969	0.08298	0.14968	0.18912	0.20146	0.26646	0.32812
0.00214	0.00435	0.01099	0.01491	0.02023	0.03216	0.05691	0.08045	0.09915
0.00815	0.03583	0.06443	0.12987	0.26712	0.30146	0.36912	0.44601	0.59915
0.00423	0.00913	0.01996	0.03078	0.05899	0.06721	0.08146	0.11658	0.12299

day 1	day 2	day 3
0.0024 87	0.002617 7	0.00261
0.0025 67	0.003142 3	0.002168 12
0.0025 67	0.004752 3	0.005377 6
0.0044 023	0.006579	0.018338 9
0.0023 856	0.004478	0.010234 5
0.0026 697	0.005803 4	0.012346 7
0.0022 156	0.002999	0.005917 8
0.0024 8	0.002591 4	0.004132 3
0.0074 523	0.009363 12	0.017192 3
0.0021 498	0.002669 8	0.002669 8
0.0027 178	0.005498	0.009893

0.00595	0.01695	0.03198	0.04941	0.13079	0.19412	0.20146	0.22888	0.31434
0.00511	0.00966	0.02759	0.02759	0.07191	0.08721	0.04412	0.05098	0.06115
0.01374	0.05807	0.08805	0.13678	0.33406	0.38712	0.4014	0.47109	0.55678
0.01108	0.0494	0.12358	0.18991	0.29398	0.33409	0.39412	0.47956	0.68712
0.00266	0.00286	0.03278	0.06547	0.11723	0.20145	0.28871	0.3567	0.4895
0.00169	0.00322	0.00859	0.01026	0.0173	0.02141	0.03142	0.04723	0.08324
0.00503	0.01526	0.02038	0.03482	0.06479	0.07815	0.09141	0.18675	0.22578
0.00595	0.01268	0.02539	0.03608	0.07901	0.08141	0.00945	0.10171	0.13212
0.01542	0.02898	0.05938	0.06015	0.05998	0.07712	0.09146	0.13376	0.18798

0.0012789	0.002906	0.005538
0.0040	0.005746	
89	7	0.018698
0.0031998	0.001417	0.006362
0.0021423	0.006106	0.013412

Fast Growth Colonies – 4 Hour group

Slow Growth

colonies – 4 hour group

day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9
0.00382	0.01521	0.03122	0.03212	0.03798	0.06623	0.09912	0.16458	0.26789
0.00164	0.00399	0.00911	0.0184	0.04772	0.07891	0.09872	0.1669	0.29148
0.00169	0.00501	0.01421	0.02936	0.09587	0.20148	0.32145	0.52978	0.86923
0.00431	0.01807	0.04477	0.1	0.22476	0.41678	0.77911	1.14841	1.65117
0.00328	0.01109	0.03532	0.07153	0.18523	0.44123	0.87612	1.04567	1.46856
0.00226	0.00377	0.01985	0.03967	0.09723	0.15923	0.30142	0.43276	0.53768
0.00242	0.00931	0.02181	0.02002	0.08025	0.08765	0.09967	0.18768	0.27546
0.00695	0.02309	0.05237	0.07889	0.16492	0.26712	0.31145	0.42272	0.61476
0.00937	0.02768	0.0523	0.11643	0.22168	0.4014	0.62781	0.00373	1.52005
0.00381	0.01245	0.02641	0.05054	0.10872	0.22131	0.39145	0.54378	1.10723
0.00277	0.00765	0.01815	0.03768	0.08934	0.16688	0.21412	0.38873	0.59877
0.0021	0.00309	0.01318	0.02266	0.06678	0.07146	0.08213	0.15678	0.21079
0.00751	0.00918	0.02759	0.05914	0.16723	0.30127	0.4014	0.5612	0.7029

day 1	day 2	day 3
0.0019	0.00143	0.009
0.00144	0.00482	0.008
0.00202	0.00252	0.008
0.00106	0.00242	0.006
0.00188	0.00333	0.008
0.00176	0.00177	0.001
0.00176	0.00769	0.016
0.00297	0.01197	0.019

Fast Growth – 24 hour group

day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day9
0.00961	0.01024	0.03212	0.10923	0.25623	0.49956	0.82178	1.0876	2.17145
0.03147	0.02914	0.0964	0.32267	0.67056	0.98712	1.00915	1.84267	3.21478
0.00521	0.00862	0.04921	0.19156	0.27287	0.42612	0.56756	0.70012	0.92776
0.00414	0.01013	0.01981	0.04598	0.07798	0.15378	0.27534	0.40123	0.62145
0.00521	0.00825	0.01976	0.03367	0.20378	0.42167	0.43612	0.49931	0.56213
0.01327	0.02054	0.06509	0.21023	0.4013	0.59812	0.8715	1.4468	3.4478
0.00271	0.00541	0.01535	0.03423	0.08678	0.11578	0.27345	0.45712	0.69145
0.00618	0.0097	0.02631	0.11634	0.20567	0.42879	0.6714	0.97123	1.6776
0.00428	0.00889	0.04121	0.0978	.2681	0.60412	0.81435	1.2345	2.1778

day 1	day 2	day 3
0.009145	0.01201489	0.01
0.00321	0.0047145	0.007
0.00143	0.004317	0.01
0.00421	0.0116164	0.01
0.003912	0.0053209	0.013
0.003001	0.0031423	0.00
0.00127	0.003285	0.003
0.00154	0.0034216	0.00
0.001423	0.0024523	0.003

0.02278	0.05567	0.15767	0.60145	1.0223	1.9987	2.0712	4.9912	5.0078	0.002712	0.00337	0.005(
0.01814	0.01863	0.06665	0.232	0.55678	1.0678	2.2756	3.2104	5.92764	0.002178	0.0047523	0.001
0.01003	0.01626	0.05355	0.1338	0.2679	0.5099	0.6917	0.9651	1.6278	0.0042878	0.0037961	0.014
0.01839	0.02839	0.07522	0.19678	0.40819	0.54178	0.77145	0.90331	1.0421	0.00314	0.0046918	0.00{
0.00687	0.00681	0.01631	0.07612	0.09213	0.2031	0.33213	0.46723	0.66723	0.0022134	0.005945	0.01{
0.00621	0.00645	0.03715	0.05568	0.20145	0.33178	0.52178	0.68878	0.89278	0.001291	0.0035217	0.0(
0.00315	0.00553	0.00797	0.0394	0.104	0.21448	0.3671	0.4327	0.7765	0.003912	0.007034	0.02(
0.00271	0.00364	0.02355	0.0625	0.10233	0.16678	0.20756	0.35198	0.50145	0.012789	0.02256612	0.00
0.01041	0.01762	0.04549	0.14808	0.24223	0.36213	0.59167	0.60552	0.82778	0.0015423	0.0015337	0.00{
0.00215	0.00365	0.01151	0.0372	0.05845	0.06615	0.08712	0.1329	0.29968	0.005524	0.007536	0.02{
0.00211	0.00314	0.00596	0.02456	0.04011	0.2314	0.29145	0.3798	0.6601	0.003156	0.0040162	0.008(
0.00168	0.00301	0.0066	0.09005	0.15876	0.29778	0.41478	0.74123	0.9678	0.002412	0.0040523	0.011{
0.00527	0.00804	0.02152	0.05524	0.11005	0.16699	0.21712	0.33045	0.5278	0.00241	0.0046951	0.021{
0.00328	0.00455	0.01651	0.05324	0.11341	0.2267	0.42712	0.60215	0.86923	0.002176	0.006521	0.01(
0.00576	0.01819	0.02991	0.0841	0.18171	0.22956	0.36178	0.55056	0.77067			
0.00914	0.01828	0.05931	0.1453	0.31335	0.5478	0.77912	0.9987	2.0978			
0.00871	0.00962	0.02915	0.1446	0.4935	0.8096	1.314	2.096	4.0421			

Fast growth Colonies – 48 hour group

day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9
0.005213	0.0088992	0.0333456	0.122567	0.18967	0.25767	0.2991675	0.361978	0.50376
0.0010156	0.0014017	0.004223	0.129145	0.050198	0.094123	0.13212	0.24109	0.39213
0.0021456	0.003509	0.015356	0.047234	0.10678	0.18756	0.21435	0.39578	0.50578
0.005278	0.0088502	0.0185567	0.06356	0.114356	0.206787	0.321456	0.4927867	0.57298
0.00898	0.0148445	0.0579923	0.20312	0.29078	0.52712	0.77345	0.96712	1.12478
0.003014	0.0041183	0.01134198	0.044765	0.078198	0.12678	0.23145	0.3099876	0.48734
0.005123	0.0076212	0.032167	0.090208	0.14136	0.2427	0.3217	0.4967	0.5876
0.004712	0.0048556	0.028767	0.098123	0.25505	0.345768	0.5271	0.68871	0.8379
0.006213	0.0094014	0.029423	0.18723	0.2989	0.439	0.56723	0.7376	0.7899
0.022178	0.033949	0.123476	0.371123	0.6373	1.00789	1.9765	2.099	4.001
0.0031445	0.00760298	0.0111523	0.03145	0.25412	0.17589	0.214567	0.35278	0.477567
0.018912	0.02879	0.088387	0.34623	0.59623	0.789122	0.914712	0.20245	1.6909
0.010412	0.01616079	0.046498	0.1467	0.2427	0.49056	0.67899	0.814116	0.99978
0.00614	0.0075621	0.023289	0.00876	0.13998	0.26754	0.31786	0.555178	0.68478
0.011423	0.025596	0.100467	0.28157	0.54712	0.858998	0.97912	1.723678	2.4798
0.004145	0.005907	0.0213481	0.06069	0.10751	0.18691	0.216786	0.37403	0.4271

0.007213	0.0116637	0.029168	0.07891	0.10356	0.1715	0.2271	0.3499	0.4668
0.010145	0.017726	0.0915118	0.38723	0.6375	1.1324	1.8756	2.2478	3.9875
0.00314	0.0051407	0.010906	0.03823	0.08178	0.13256	0.18756	0.24278	0.34723
0.005712	0.008757	0.028128	0.06345	0.188987	0.26876	0.36756	0.57912	0.777234
0.005213	0.006059	0.021187	0.07898	0.16787	0.2975	0.41478	0.72978	1.07739
0.00421	0.0079336	0.020398	0.070213	0.13723	0.20356	0.256123	0.3997567	0.50346
0.003612	0.0110067	0.021267	0.05871	0.1102	0.19345	0.24124	0.3012323	0.39978
0.004234	0.00592167	0.02524998	0.055723	0.1327	0.26678	0.321765	0.411456	0.677565
0.004213	0.0063998	0.016889	0.0977877	0.34723	0.417789	0.721346	0.9014776	0.999125
0.006145	0.008293	0.0300689	0.0868957	0.150789	0.34587	0.501456	0.657008	0.855346
0.006789	0.008397	0.032312	0.08389	0.298765	0.43756	0.60198	0.970089	1.63457
0.00201	0.002622	0.00509	0.061789	0.12378	0.190745	0.22712	0.325987	0.3995678

Slow Growth colonies – 48 hour group

day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9
0.00312	0.00665	0.012576	0.04256	0.100456	0.14644	0.18712	0.26178	0.28667
0.001278	0.0023109	0.0063245	0.023765	0.037567	0.088234	0.101412	0.22678	0.29145
0.00321	0.0056046	0.029445	0.05309	0.11987	0.189678	0.217145	0.319875	0.418756
0.0009124	0.0015967	0.003278	0.02678	0.03387	0.052178	0.07896	0.1046765	0.187956
0.003412	0.002448	0.009891	0.029145	0.03778	0.12476	0.18723	0.23778	0.30156
0.00432	0.0041298	0.013377	0.040978	0.066787	0.09256	0.104213	0.167223	0.169987
0.003412	0.007289	0.01593356	0.099234	0.12823	0.20578	0.26712	0.33786	0.399987
0.005621	0.0164278	0.017378	0.029876	0.0611456	0.085567	0.102178	0.115678	0.20678
0.00217	0.0023389	0.007045	0.016678	0.021078	0.04587	0.082445	0.23345	0.25278
0.002712	0.002736	0.0075623	0.02909	0.05423	0.11567	0.13145	0.16678	0.307892
0.00231	0.002357	0.0056612	0.02678	0.04278	0.06145	0.077234	0.090987	0.0996123
0.002178	0.0030015	0.0059645	0.0118345	0.017823	0.02523	0.029135	0.034678	0.039987
0.00214556	0.004667	0.0157967	0.058456	0.082456	0.15278	0.27109	0.307123	0.033213
0.004213	0.006702	0.015256	0.003912	0.0982	0.12657	0.187123	0.22712	0.26178
0.0014123	0.002972	0.012809	0.01789	0.02897	0.0527	0.0201	0.031457	0.04412
0.00214	0.0039952	0.007623	0.01212	0.01987	0.022131	0.04148287	0.069123	0.031412
0.0021192	0.00481889	0.011707	0.050078	0.08376	0.12278	0.1299982	0.13672	0.2529
0.002178	0.004061	0.0099123	0.037012	0.0506	0.12278	0.189712	0.221487	0.289123
0.004234	0.009136	0.030179	0.100756	0.16856	0.171145	0.2117554	0.33567	0.499123
0.0016123	0.0026745	0.003867	0.011098	0.024768	0.032146	0.056787	0.084765	0.1080078
0.003412	0.004896	0.0140912	0.018912	0.02912	0.062135	0.088912	0.1525987	0.2434
0.002213	0.0035882	0.0058912	0.01734	0.03876	0.05934	0.08145	0.12356	0.169956

A.3.2 Growth rate of reporters exposed to transfer medium from irradiated Balb/c mice

Fast Growth Colonies – controls

day 1	day 2	day 3	day 4	day 5	day6	day7	day 8
0.001874	0.003285	0.007915	0.01586	0.052912	0.101425	0.20712	0.406125
0.011047	0.040614	0.08191	0.10792	0.1976	0.3101	0.6917	1.2917
0.002789	0.005671	0.010194	0.019975	0.091614	0.169742	0.231614	0.38765
0.003143	0.00759	0.026142	0.04753	0.09617	0.19712	0.406652	0.589124
0.011752	0.030078	0.046912	0.057214	0.12102	0.20175	0.31042	0.40275
0.00271	0.07714	0.01001	0.022917	0.07299	0.1001	0.3271	0.5527
0.00294	0.00487	0.00781	0.02733	0.0799	0.10274	0.2016	0.353112
0.008691	0.013271	0.036145	0.071285	0.082145	0.091452	0.112958	0.208857
0.009621	0.019612	0.036154	0.066337	0.089312	0.12912	0.38912	0.55096
0.005914	0.012245	0.031425	0.056601	0.102712	0.2916	0.4591	0.63712
0.004489	0.005011	0.007654	0.029917	0.044178	0.08612	0.1237	0.2997
0.004512	0.007041	0.01191	0.03441	0.089914	0.160124	0.200001	0.29999
0.00361	0.009912	0.019452	0.041725	0.06721	0.16617	0.207525	0.30141
0.00621	0.017254	0.026145	0.039602	0.072912	0.10121	0.202991	0.33147

Slow Growth Colonies – controls

day 1	day 2	day 3
0.00327	0.005681	0.0614
0.002714	0.00259	0.0069
0.005274	0.005991	0.00761
0.00267	0.00391	0.0091
0.005041	0.013141	0.02219
0.00297	0.004971	0.01012
0.003721	0.013292	0.01921
0.003312	0.009975	0.01021
0.007921	0.011271	0.02101
0.001991	0.002469	0.00291
0.002271	0.005327	0.00601

Fast Growth Colonies – 0 gy

day 1	day 2	day 3	day4	day5	day 6	day 7	day 8
0.007712	0.022512	0.04174	0.07961	0.0814	0.1327	0.2617	0.45582
0.003912	0.008176	0.009912	0.017865	0.02657	0.052788	0.081412	0.104478
0.010512	0.030145	0.036912	0.039968	0.056213	0.07301	0.081712	0.113712
0.010912	0.033978	0.059178	0.072912	0.091412	0.101412	0.22712	0.468178
0.005509	0.00875	0.021412	0.04876	0.062113	0.077231	0.092134	0.137721
0.004213	0.012552	0.022178	0.020178	0.029145	0.031712	0.044178	0.157698
0.015213	0.032876	0.07278	0.161678	0.2121	0.39934	0.692134	0.98778
0.002178	0.006145	0.007213	0.008146	0.014213	0.042779	0.092116	0.181445
0.00988	0.030189	0.062156	0.080446	0.136912	0.214912	0.502712	0.751578
0.009381	0.043978	0.05321	0.11511	0.221471	0.36712	0.069214	0.966712
0.005213	0.007623	0.009278	0.017268	0.03214	0.066234	0.08723	0.124712

Slow growth colonies -0 Gy

day 1	day 2	day3
0.00108	0.00406	0.0062
0.00528	0.014378	0.02014
0.004213	0.004998	0.0098
0.002918	0.009324	0.00712
0.003191	0.008145	0.01271
0.003812	0.003267	0.00399
0.014012	0.020067	0.01014
0.003401	0.010045	0.01127
0.002581	0.005812	0.00911
0.001678	0.00541	0.00798
0.003289	0.011609	0.02145
0.002765	0.009412	0.01521
0.003619	0.00514	0.00714
0.00377	0.00921	0.01001

Fast Growth Colonies – 2 Gy

day 1	day 2	day 3	day 4	day 5	day 6	day7	day 8
0.002667	0.004798	0.00567	0.009712	0.011071	0.039978	0.108091	0.244912
0.01278	0.035712	0.060145	0.108078	0.133998	0.17112	0.199678	0.203167
0.001178	0.003176	0.003621	0.005012	0.007912	0.013012	0.036214	0.081478
0.026899	0.061477	0.087298	0.13109	0.147312	0.199987	0.277345	0.588178

Slow Growth Colonies – 2 Gy

day 1	day 2	day 3
0.00399	0.007834	0.02017
0.0032768	0.006812	0.0081
0.004467	0.0066178	0.00791
0.002423	0.006145	0.0071

0.003876	0.010213	0.016214	0.021678	0.04978	0.05278	0.071423	0.138712	0.002678	0.007134	0.00812
0.019218	0.02865	0.042716	0.138712	0.167235	0.199712	0.321712	0.439987	0.002187	0.003467	0.00721
0.005067	0.013258	0.022332	0.049178	0.051231	0.088987	0.145668	0.28834	0.00556912	0.014356	0.02276
0.009907	0.04486	0.069176	0.082612	0.087423	0.099145	0.201723	0.221876	0.004278	0.014523	0.02261
0.007787	0.023712	0.036145	0.063179	0.06996	0.097124	0.152789	0.296712	0.0026678	0.006145	0.00721
0.010312	0.015678	0.033798	0.096156	0.048876	0.050445	0.061456	0.183456	0.005712	0.007278	0.00761
0.00746	0.021612	0.050145	0.070812	0.092712	0.079912	0.10424	0.244789	0.00321	0.003945	0.00399
0.008812	0.019912	0.032178	0.06589	0.077109	0.087213	0.12778	0.272345	0.003278	0.0038145	0.00527
								0.004612	0.006912	0.0069

Fast Growth Colonies - 20mGy

Slow Growth Colonies – 20 mGy

day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 1	day 2	day 3	day
0.006901	0.010567	0.009876	0.009123	0.050214	0.07898	0.139912	0.3585	0.003689	0.008667	0.009912	
0.009146	0.017167	0.036145	0.111978	0.209912	0.32171	0.46978	0.65412	0.011017	0.0261456	0.0296912	
0.018345	0.026198	0.032156	0.041278	0.094123	0.204967	0.32198	0.53378	0.003123	0.014812	0.023456	
0.003223	0.007746	0.007789	0.007745	0.01321	0.03145	0.074998	0.18723	0.010612	0.0291	0.025342	
0.005357	0.006178	0.008915	0.023078	0.027278	0.035145	0.081467	0.111256	0.00591	0.01356	0.013016	
0.01701	0.025598	0.036145	0.05712	0.027145	0.030945	0.088491	0.24456	0.008167	0.00823	0.008835	
0.00401	0.004198	0.00467	0.005455	0.009614	0.024167	0.06321	0.150568	0.0026178	0.004356	0.0056912	
0.00787	0.00859	0.01456	0.025212	0.034414	0.021217	0.052712	0.106128	0.002146	0.0046234	0.00566412	
0.008023	0.014623	0.026912	0.042278	0.05765	0.07523	0.16579	0.325781	0.010612	0.016912	0.0199612	
0.010511	0.039915	0.042789	0.052778	0.06678	0.08167	0.198675	0.255765	0.004221	0.001978	0.00199987	(
0.006787	0.009987	0.01345	0.023657	0.066912	0.09423	0.205469	0.301098	0.01089	0.013067	0.012278	
0.017235	0.02987	0.039423	0.069765	0.071598	0.07791	0.08476	0.18734	0.015223	0.019912	0.0202987	
								0.005923	0.009067	0.001127	

Fast Growth Colonies – 4 Hour group

Slow Growth Colonies- 4 Hour group

day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 1	day 2	day 3
0.00299	0.00757	0.012756	0.04278	0.07356	0.13658	0.26512	0.606687	0.004112	0.013723	0.0
0.00917	0.03197	0.05621	0.16287	0.19723	0.24712	0.769178	1.07412	0.0037278	0.0103367	0.0
0.012212	0.039714	0.07987	0.21956	0.29912	0.306712	0.897312	1.89967	0.006678	0.01789	0.(
0.014712	0.028612	0.0333278	0.133756	0.199786	0.23445	0.36978	0.7716	0.004612	0.011512	0.0
0.003712	0.009634	0.011278	0.03167	0.201478	0.32178	0.815412	1.4989	0.002912	0.003719	0.00
0.006012	0.010089	0.0301456	0.052178	0.07367	0.13978	0.206678	0.487412	0.00145	0.001998	0.0(
0.003145	0.006267	0.016789	0.04123	0.087145	0.187045	0.26145	0.50145	0.007912	0.0180067	0.(
0.004912	0.0126012	0.02412	0.115576	0.189612	0.43756	0.80423	1.01567	0.010412	0.02244	0.02
0.006267	0.010698	0.026912	0.086145	0.099145	0.17523	0.39278	0.816912	0.003578	0.009557	0.0
0.007723	0.023798	0.036213	0.104213	0.19978	0.26956	0.53912	0.88069	0.002188	0.002567	0.0(
0.00602	0.014934	0.022756	0.030145	0.089967	0.2605668	0.53278	0.71809	0.003012	0.00389	0.0
0.0030012	0.0073124	0.016912	0.066912	0.100456	0.22456	0.56156	0.99612	0.003178	0.0087	0.0
								0.00458	0.01298	0.02

Fast Growth Colonies – 24 hour group Slow Growth Colonies – 24 hour groups

fast Growth Colonies							Slow Growth Colonies			
day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 1	day 2	day 3	day 4
0.00289	0.015876	0.00798	0.010489	0.036912	0.127998	0.25712	0.005723	0.012456	0.029156	0.044
0.003998	0.016109	0.017992	0.029467	0.079912	0.26356	0.67987	0.001198	0.006687	0.00712	0.009
0.00776	0.018509	0.026178	0.038167	0.10398	0.36912	0.29967	0.002178	0.00432	0.0127567	0.063
0.009612	0.023712	0.060512	0.113228	0.26912	0.5099	1.20734	0.009612	0.017897	0.0271567	0.078
0.00789	0.018078	0.03067	0.055172	0.099123	0.17998	0.67567	0.0011456	0.02089	0.039912	0.064
0.004412	0.012663	0.031056	0.079115	0.169178	0.437234	1.68167	0.0034198	0.0055412	0.009723	0.019
0.007498	0.019198	0.026957	0.088912	0.100968	0.25109	0.53578	0.003412	0.00321	0.0041612	0.0056
0.005012	0.013078	0.020145	0.035612	0.139278	0.359167	0.788178	0.003378	0.0110145	0.021498	0.047
0.009146	0.018298	0.037145	0.102178	0.260567	0.494698	1.79167	0.005467	0.010078	0.015598	0.022
0.008234	0.015956	0.029145	0.109279	0.29145	0.5699	1.9967	0.004978	0.009145	0.009967	0.020
0.018667	0.010476	0.015923	0.018712	0.17998	0.43712	1.27145	0.001712	0.002178	0.0010141	0.020
0.007589	0.020812	0.051056	0.073213	0.179123	0.76512	1.28897	0.009612	0.01478	0.022712	0.0
0.003225	0.007812	0.016178	0.036998	0.130145	0.307156	0.541912	0.93156			

Fast Growth Colonies – 48 hour group Slow Growth Colonies – 48 hour group

day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	Day 1	day 2	day 3
0.009678	0.02309	0.063245	0.1104178	0.132712	0.187912	0.20767	0.30178	0.003278	0.01987	0.(
0.005167	0.006657	0.012345	0.047213	0.096778	0.23712	0.40178	0.79723	0.001956	0.005118	0.0(
0.008712	0.017223	0.023712	0.090045	0.156278	0.206978	0.72767	1.06712	0.021678	0.037298	0.0
0.002689	0.009798	0.012712	0.077798	0.102768	0.32078	0.82714	1.9697	0.00329	0.00401	0.0(
0.002978	0.007823	0.008145	0.0499678	0.073521	0.187156	0.30776	0.57623	0.00223	0.003341	0.0(
0.034078	0.069198	0.078912	0.11752	0.30146	0.76271	1.03376	2.0619	0.002567	0.005168	0.0(
0.00405	0.007145	0.0102712	0.048123	0.099678	0.20014	0.327145	0.86478	0.00181	0.00398	0.0(
0.014223	0.04856	0.080412	0.206912	0.52798	0.83723	1.11298	2.39124	0.00337	0.00567	0.0
0.003765	0.009223	0.012768	0.061423	0.106712	0.23712	0.66712	1.61178	0.00979	0.019978	0.0
0.0255467	0.053089	0.080445	0.162412	0.36512	0.52756	0.77987	2.4712	0.004398	0.006213	0.0(
0.002999	0.009	0.0121789	0.050145	0.100278	0.369412	0.601423	0.951912	0.002712	0.003712	0.0(
0.002123	0.0066734	0.011423	0.029117	0.066567	0.167145	0.271412	0.516756	0.010007	0.02312	0.(
0.005412	0.019078	0.017278	0.091167	0.081678	0.207789	0.332778	0.861178			

A Appendix 4

A.4 Size Distribution of Reporters Given Medium from Bladders from C57Bl6 and Balb Mice

A.4.1 Size Distribution of reporter colony size given bladder medium from irradiated C57BL6 mice

Area(mm2)	Control- # of colonies	0 Gy- # of colonies	2 Gy- # of colonies	20 mGy- # of colonies	4 Hour group- # of colonies	24 Hour Group- # of colonies	48 Hour Group- # of colonies
0.0100				2.0000		1.0000	
0.0200					1.0000	8.0000	
0.0300					1.0000	5.0000	
0.0400					1.0000	4.0000	2.0000
0.0500					1.0000	7.0000	
0.0600					1.0000	2.0000	
0.0700						2.0000	3.0000
0.0800	2.0000			1.0000	1.0000	4.0000	4.0000
0.0900	5.0000			1.0000			
0.1000	4.0000				2.0000	1.0000	1.0000
0.1100	2.0000				2.0000		
0.1200	3.0000				1.0000	3.0000	
0.1300	2.0000				2.0000	1.0000	
0.1400	1.0000				1.0000	1.0000	1.0000
0.1500	1.0000				1.0000		
0.1600	3.0000				3.0000		1.0000
0.1700	3.0000	2.0000			2.0000	1.0000	
0.1800	1.0000	1.0000		1.0000	5.0000	2.0000	
0.1900	1.0000	1.0000		2.0000	3.0000		2.0000
0.2000		2.0000		1.0000	5.0000	1.0000	
0.2100	1.0000				1.0000		3.0000
0.2200					2.0000	3.0000	1.0000
0.2300	3.0000		1.0000		1.0000		3.0000
0.2400	1.0000	1.0000			1.0000		
0.2500	1.0000				1.0000	1.0000	
0.2600					2.0000	1.0000	
0.2700	1.0000	1.0000		1.0000	2.0000		
0.2800	2.0000		1.0000	1.0000	1.0000	1.0000	
0.2900	4.0000	3.0000			3.0000		
0.3000	1.0000	1.0000		1.0000	2.0000	3.0000	1.0000
0.3100					1.0000		1.0000

0.3200				1.0000		2.0000	
0.3300		1.0000			1.0000	2.0000	2.0000
0.3400	1.0000			3.0000	1.0000	1.0000	1.0000
0.3500	1.0000	2.0000		2.0000	1.0000	4.0000	
0.3600		1.0000			2.0000		
0.3700	1.0000	2.0000		1.0000	1.0000	1.0000	1.0000
0.3800	1.0000			1.0000	2.0000	2.0000	1.0000
0.3900	1.0000	1.0000			1.0000		
0.4000	3.0000				1.0000	1.0000	1.0000
0.4100	2.0000	1.0000			2.0000	3.0000	5.0000
0.4200	1.0000	2.0000			1.0000	5.0000	1.0000
0.4300					2.0000	1.0000	1.0000
0.4400	1.0000			2.0000	2.0000	1.0000	3.0000
0.4500	1.0000				5.0000		
0.4600	1.0000	1.0000					1.0000
0.4700				1.0000	1.0000	2.0000	
0.4800	1.0000			1.0000	4.0000	2.0000	1.0000
0.4900				1.0000	2.0000		
0.5000	2.0000	1.0000					1.0000
0.5100	1.0000	1.0000					1.0000
0.5200	2.0000	1.0000		1.0000	3.0000	1.0000	
0.5300	2.0000				2.0000	2.0000	
0.5400		1.0000		1.0000		1.0000	1.0000
0.5500	2.0000			1.0000		1.0000	
0.5600	1.0000			2.0000	2.0000	1.0000	
0.5700				1.0000			4.0000
0.5800				1.0000	2.0000	1.0000	1.0000
0.5900		1.0000		1.0000	1.0000		
0.6000	1.0000	1.0000			1.0000	1.0000	
0.6100							
0.6200	1.0000	2.0000				1.0000	
0.6300		1.0000		2.0000	1.0000	1.0000	2.0000
0.6400	1.0000	1.0000			1.0000		1.0000
0.6500	2.0000			2.0000	3.0000		1.0000
0.6600	1.0000				1.0000		1.0000
0.6700						1.0000	
0.6800	1.0000			2.0000	2.0000		2.0000
0.6900				3.0000			
0.7000						1.0000	2.0000
0.7100							
0.7200	1.0000	2.0000			1.0000	1.0000	
0.7300				1.0000	1.0000	1.0000	1.0000

0.7400	1.0000	2.0000			2.0000	1.0000	
0.7500							
0.7600		1.0000			2.0000		
0.7700	2.0000			1.0000	2.0000		
0.7800	1.0000			3.0000	3.0000		
0.7900				1.0000	1.0000		
0.8000				1.0000			
0.8100	1.0000					2.0000	
0.8200	1.0000			1.0000			
0.8300	1.0000		1.0000			1.0000	1.0000
0.8400	1.0000			1.0000	1.0000		
0.8500	1.0000					1.0000	2.0000
0.8600	1.0000			1.0000	1.0000	1.0000	1.0000
0.8700	1.0000	1.0000			1.0000		3.0000
0.8800	1.0000				1.0000	1.0000	
0.8900	1.0000	3.0000		1.0000	1.0000	1.0000	
0.9000		1.0000				2.0000	
0.9100	1.0000	1.0000		1.0000	2.0000		1.0000
0.9200							
0.9300	1.0000						
0.9400	4.0000						1.0000
0.9500			1.0000				
0.9600		1.0000			1.0000		
0.9700						4.0000	
0.9800				1.0000	1.0000	9.0000	
0.9900						3.0000	
1.0000	7.0000	1.0000	1.0000	5.0000	5.0000	4.0000	4.0000
1.1000	5.0000	1.0000	2.0000	4.0000	2.0000		
1.2000	5.0000	1.0000		1.0000	8.0000	5.0000	2.0000
1.3000	7.0000	1.0000		2.0000	6.0000	6.0000	1.0000
1.4000	2.0000	2.0000			8.0000	4.0000	1.0000
1.5000	1.0000	3.0000		2.0000	6.0000	2.0000	1.0000
1.6000	1.0000			1.0000	1.0000	4.0000	2.0000
1.7000	8.0000	3.0000				1.0000	3.0000
1.8000	1.0000	3.0000			4.0000	1.0000	
1.9000	2.0000				3.0000	1.0000	
2.0000	4.0000	1.0000				4.0000	1.0000
2.1000	1.0000			1.0000	1.0000		
2.2000	2.0000	2.0000		2.0000	2.0000	1.0000	4.0000
2.3000	5.0000				2.0000		2.0000
2.4000	1.0000				2.0000	2.0000	1.0000
2.5000	1.0000	1.0000			1.0000		

2.6000	2.0000				1.0000	1.0000	
2.7000		1.0000			3.0000	2.0000	1.0000
2.8000	1.0000	2.0000				2.0000	2.0000
2.9000	3.0000						
3.0000	1.0000						
3.1000	1.0000	2.0000			3.0000	1.0000	
3.2000	1.0000	1.0000		1.0000		1.0000	
3.3000		1.0000			1.0000		2.0000
3.4000	1.0000				2.0000	1.0000	1.0000
3.5000	1.0000					1.0000	1.0000
3.6000	1.0000	1.0000				1.0000	
3.7000		1.0000				2.0000	1.0000
3.8000	2.0000				2.0000	1.0000	1.0000
3.9000	1.0000			1.0000			
4.0000	2.0000				1.0000	1.0000	
4.1000	2.0000				1.0000	1.0000	
4.2000	1.0000	1.0000				3.0000	
4.3000	2.0000	1.0000			1.0000		
4.4000	1.0000						3.0000
4.5000							
4.6000							
4.7000						3.0000	
4.8000	1.0000	1.0000					
4.9000						1.0000	
5.0000						2.0000	1.0000
5.1000							
5.2000	1.0000					1.0000	
5.3000	1.0000				2.0000		1.0000
5.4000						1.0000	1.0000
5.6000							
5.7000							
5.8000	1.0000						
5.9000							
6.0000		1.0000			1.0000		
6.1000	2.0000						2.0000
6.2000							
6.3000							
6.4000							
6.5000						2.0000	
6.6000							
6.7000	1.0000						
6.8000							

6.9000							
7.0000						2.0000	
7.1000							
7.2000	2.0000						
7.3000						1.0000	
7.4000							
7.5000					1.0000	1.0000	
7.6000					1.0000	1.0000	
7.7000			1.0000				
7.8000	1.0000						
7.9000	1.0000						1.0000
8.0000		1.0000					
8.1000							
8.2000			1.0000				
8.3000		1.0000			1.0000		
8.4000							
8.5000							
8.6000						1.0000	
8.7000		1.0000					
8.8000							
8.9000							
9.0000					1.0000	1.0000	2.0000
9.1000		1.0000			1.0000		
9.2000					1.0000		
9.3000					1.0000		
9.4000					1.0000		
9.5000							
9.6000					1.0000	1.0000	
9.7000						2.0000	
9.8000					2.0000	1.0000	
9.9000					2.0000	2.0000	
10.0000	2.0000	1.0000			1.0000	1.0000	
11.0000					2.0000		1.0000
12.0000	1.0000	1.0000			1.0000		
13.0000	1.0000				1.0000		
14.0000	2.0000				3.0000		1.0000
15.0000		1.0000			2.0000	1.0000	
16.0000		1.0000			5.0000		1.0000
17.0000	1.0000				3.0000		
18.0000				2.0000	5.0000	2.0000	
19.0000					1.0000	1.0000	
20.0000					2.0000	8.0000	1.0000

21.0000	1.0000	1.0000			1.0000	5.0000	
22.0000					1.0000	4.0000	

A.4.2 Size distribution of reporter colonies given bladder medium from irradiated Balb/c Mice

Area (mm2)	Contols- # of colonies	20 mGy- # of colonies	Area (mm2)	0 Gy- # of colonies	2 Gy - # of colonies	Area (mm2)	4 Hour Group- # of colonie	24 Hor Group- # of colonies	48 Hour group- # of colonies		
0.1000	1.0000	8.0000	0.0140		9.0000	5.0000e-3	6.0000				
0.1100	1.0000	79.0000	0.0150		10.0000	6.0000e-3	10.0000				
0.1200		63.0000	0.0160		4.0000	7.0000e-3	6.0000				
0.1300		39.0000	0.0170		6.0000	8.0000e-3	5.0000				
0.0140		15.0000	0.0180		3.0000	9.0000e-3	5.0000				
0.0150		27.0000	0.0190		2.0000	0.0100	22.0000		1.0000		
0.0160		13.0000	0.0200		7.0000	0.0200	14.0000		1.0000		
0.0170		12.0000	0.0210		4.0000	0.0300	1.0000				
0.0180		15.0000	0.0220	1.0000	4.0000	0.0400	4.0000				
0.0190	1.0000	17.0000	0.0230		1.0000	0.0500	5.0000		1.0000		
0.0200		16.0000	0.0240	1.0000	1.0000	0.0600	4.0000				
0.0210		12.0000	0.0250		2.0000	0.0700	5.0000		2.0000		
0.0220		11.0000	0.0260			0.0800	1.0000		1.0000		
0.0230		10.0000	0.0270		2.0000	0.0900	1.0000		1.0000		
0.0240		11.0000	0.0280		1.0000	0.1000			1.0000		
0.0250		12.0000	0.0290		1.0000	0.1100	1.0000				
0.0260	1.0000	11.0000	0.0300		1.0000	0.1200	1.0000		4.0000		
0.0270	2.0000	8.0000	0.0310		4.0000	0.1300	2.0000		1.0000		
0.0280		13.0000	0.0320		2.0000	0.1400			1.0000		
0.0290	1.0000	4.0000	0.0330		1.0000	0.1500					
0.0300		7.0000	0.0340		2.0000	0.1600	1.0000	1.0000	1.0000		
0.0310	1.0000	11.0000	0.0350		2.0000	0.1700	1.0000		3.0000		
0.0320		4.0000	0.0360		1.0000	0.1800		1.0000			
0.0330		3.0000	0.0370			0.1900					
0.0340		4.0000	0.0380	1.0000		0.2000		2.0000	2.0000		
0.0350	2.0000	5.0000	0.0390			0.2100		1.0000	2.0000		
0.036	1.0000	3.0000	0.0400		1.0000	0.2200					
0.0370		4.0000	0.0410	1.0000	1.0000	0.2300	2.0000	1.0000	2.0000		
0.0380		4.0000	0.0420			0.2400	1.0000	3.0000			

0.0390	1.0000	4.0000	0.0450	2.0000	2.0000	0.2500	1.0000	1.0000			
0.0400	1.0000	3.0000	0.0460		1.0000	0.2600		2.0000			
0.0410	2.0000	3.0000	0.0470		2.0000	0.2700	1.0000	2.0000	2.0000		
0.0420		9.0000	0.0480		5.0000	0.2800	1.0000		2.0000		
0.0450	1.0000	2.0000	0.0490		1.0000	0.2900			1.0000		
0.0460		2.0000	0.0500		1.0000	0.3000			1.0000		
0.0470		6.0000	0.0510		1.0000	0.3100		1.0000			
0.0480	1.0000	1.0000	0.0520		1.0000	0.3200		1.0000			
0.0490	2.0000	1.0000	0.0530		2.0000	0.3300					
0.0500	2.0000	2.0000	0.0540		1.0000	0.3400					
0.0510	3.0000	1.0000	0.0550		3.0000	0.3500					
0.0520	2.0000	2.0000	0.0560			0.3600			1.0000		
0.0530	1.0000	1.0000	0.0560		1.0000	0.3770		1.0000			
0.0540	3.0000		0.0570		1.0000	0.3800			1.0000		
0.0550	2.0000	2.0000	0.0580	1.0000		0.3900					
0.0560		3.0000	0.0590			0.4000	1.0000	2.0000	2.0000		
0.0560	2.0000	1.0000	0.0600			0.4100					
0.0570		1.0000	0.0610	1.0000	1.0000	0.4200	1.0000		1.0000		
0.0580	1.0000	1.0000	0.0620			0.4300			1.0000		
0.0590	1.0000		0.0630	2.0000		0.4400			1.0000		
0.0600			0.0640			0.4500					
0.0610	1.0000	5.0000	0.0650		1.0000	0.4600		1.0000			
0.0620			0.0660			0.4700		1.0000			
0.0630	2.0000	1.0000	0.0670	1.0000	1.0000	0.4800		2.0000	1.0000		
0.0640		2.0000	0.0680		1.0000	0.4900		1.0000			
0.0650		1.0000	0.0690	1.0000		0.5000		1.0000			
0.0660	1.0000	1.0000	0.0700			0.5100			1.0000		
0.0670			0.0710	2.0000	1.0000	0.5200					
0.0680	1.0000		0.0720			0.5300		2.0000	1.0000		
0.0690	1.0000	1.0000	0.0730	1.0000	2.0000	0.5400		1.0000	1.0000		
0.0700	1.0000		0.0740		1.0000	0.5500	1.0000	1.0000	1.0000		
0.0710	2.0000		0.0750			0.5600	1.0000	1.0000	2.0000		
0.0720	1.0000		0.0760	1.0000		0.5700	1.0000		1.0000		
0.0730	1.0000	2.0000	0.0770	1.0000		0.5800					
0.0740	1.0000	3.0000	0.0780			0.5900					
0.0750			0.0790	1.0000		0.5900					
0.0760		3.0000	0.0800	1.0000		0.6000					
0.0770		2.0000	0.0810			0.6100					
0.0780		1.0000	0.0820	1.0000		0.6200	1.0000		2.0000		
0.0790		1.0000	0.0830			0.6300		1.0000	2.0000		
0.0800		2.0000	0.0840	1.0000	2.0000	0.6400		1.0000			
0.0810	3.0000	1.0000	0.0850		1.0000	0.6500			1.0000		

0.0820			0.0860	1.0000		0.6600					
0.0830		1.0000	0.0870			0.6700			1.0000		
0.0840		2.0000	0.0880	1.0000		0.6800					
0.0850		1.0000	0.0890	1.0000		0.6900					
0.0860	3.0000	3.0000	0.0900	2.0000	1.0000	0.7000	1.0000		2.0000		
0.0870	1.0000	1.0000	0.0910			0.7100					
0.0880	1.0000	6.0000	0.0920	4.0000		0.7200	1.0000				
0.0890	1.0000		0.0930			0.7300		1.0000			
0.0900	3.0000	5.0000	0.0940	1.0000	1.0000	0.7400					
0.0910	1.0000	2.0000	0.0950			0.7500					
0.0920	4.0000	1.0000	0.0960		1.0000	0.7600					
0.0930		1.0000	0.0970	1.0000		0.7700			2.0000		
0.0940	6.0000	2.0000	0.0980	2.0000		0.7800			1.0000		
0.0950		1.0000	0.0990		1.0000	0.7900					
0.0960		2.0000	0.1000	13.0000		0.8000					
0.0970	3.0000		0.1100	13.0000		0.8100			1.0000		
0.0980	3.0000	1.0000	0.1200	9.0000	1.0000	0.8200					
0.0990	4.0000	2.0000	0.1300	7.0000	3.0000	0.8300	1.0000		1.0000		
0.1000	15.0000		0.1400	11.0000		0.8400	1.0000		4.0000		
0.1100	8.0000		0.1500	4.0000	2.0000	0.8500			2.0000		
0.1200	2.0000	2.0000	0.1600	9.0000		0.8600	1.0000	1.0000	1.0000		
0.1300	8.0000		0.1700	6.0000	1.0000	0.8700	1.0000		1.0000		
0.1400	10.0000	1.0000	0.1800	7.0000	1.0000	0.8800			1.0000		
0.1500	11.0000		0.1900	3.0000		0.8900		1.0000			
0.1600	12.0000	1.0000	0.2000	4.0000		0.9000	1.0000	1.0000	1.0000		
0.1700	7.0000	2.0000	0.2100	7.0000		0.9100	1.0000	1.0000			
0.1800	9.0000	2.0000	0.2200	4.0000		0.9200		1.0000			
0.1900	9.0000	1.0000	0.2300	8.0000		0.9300	1.0000	1.0000			
0.2000	9.0000		0.2400	6.0000		0.9400					
0.2100	10.0000	6.0000	0.2500	10.0000		0.9500	1.0000	1.0000			
0.2200	6.0000	7.0000	0.2600	6.0000	1.0000	0.9600	1.0000				
0.2300	3.0000	7.0000	0.2700	4.0000	2.0000	0.9700	1.0000	1.0000			
0.2400	5.0000	4.0000	0.2800	10.0000	1.0000	0.9800	1.0000				
0.2500	9.0000	7.0000	0.2900	1.0000	1.0000	0.9900					
0.2600	3.0000	4.0000	0.3000	4.0000	1.0000	1.0000	4.0000	5.0000	9.0000		
0.2700	6.0000	4.0000	0.3100	5.0000	4.0000	1.1000	4.0000	2.0000	4.0000		
0.2800	5.0000	4.0000	0.3200	6.0000	2.0000	1.2000	3.0000	2.0000	2.0000		
0.2900	9.0000	2.0000	0.3300	4.0000	1.0000	1.3000	1.0000	3.0000	2.0000		
0.3000	4.0000	2.0000	0.3400	4.0000	2.0000	1.4000	2.0000	5.0000	4.0000		
0.3100	7.0000	3.0000	0.3500	8.0000	2.0000	1.5000	4.0000	3.0000	3.0000		
0.3200	6.0000	5.0000	0.3600	6.0000	1.0000	1.6000	4.0000		1.0000		
0.3300	2.0000	3.0000	0.3700	7.0000		1.7000	6.0000	1.0000	3.0000		

0.3400	2.0000	2.0000	0.3870	5.0000		1.8000	3.0000	1.0000	1.0000		
0.3500	2.0000	3.0000	0.3900	1.0000		1.9000	2.0000	1.0000	1.0000		
0.3600	3.0000		0.4000	5.0000	1.0000	2.0000	3.0000	3.0000	6.0000		
0.3700	8.0000		0.4100	3.0000	1.0000	2.1000	1.0000		5.0000		
0.3870	4.0000	1.0000	0.4200	4.0000		2.2000	1.0000	5.0000	6.0000		
0.3900	3.0000	1.0000	0.4300	3.0000	2.0000	2.3000	4.0000	2.0000	3.0000		
0.4000	6.0000	2.0000	0.4400		1.0000	2.4000	3.0000		6.0000		
0.4100	1.0000		0.4500	1.0000	2.0000	2.5000	1.0000	2.0000			
0.4200	6.0000		0.4600		5.0000	2.6000		1.0000	4.0000		
0.4300	4.0000	1.0000	0.4700	1.0000	1.0000	2.7000	2.0000	1.0000	1.0000		
0.4400	6.0000		0.4800	1.0000	1.0000	2.8000	2.0000	3.0000	3.0000		
0.4500	4.0000	1.0000	0.4900	1.0000	1.0000	2.9000		1.0000			
0.4600	5.0000		0.5000	2.0000	1.0000	3.0000	3.0000	1.0000			
0.4700	1.0000	1.0000	0.5100	6.0000	2.0000	3.1000	3.0000	2.0000	4.0000		
0.4800	3.0000	1.0000	0.5200	3.0000	1.0000	3.2000	3.0000	2.0000	3.0000		
0.4900	2.0000		0.5300	2.0000	3.0000	3.3000	1.0000	2.0000	1.0000		
0.5000	4.0000		0.5400	2.0000		3.4000		4.0000	1.0000		
0.5100	3.0000		0.5500	2.0000	1.0000	3.5000	3.0000	1.0000			
0.5200			0.5600	3.0000	1.0000	3.6000	2.0000		1.0000		
0.5300			0.5700	1.0000		3.7000		3.0000	2.0000		
0.5400	2.0000		0.5800			3.8000		2.0000	2.0000		
0.5500	4.0000		0.5900			3.9000	1.0000	2.0000	1.0000		
0.5600	2.0000		0.6000	4.0000	1.0000	4.0000	1.0000		4.0000		
0.5700	1.0000		0.6100			4.1000	1.0000		1.0000		
0.5800	6.0000		0.6200			4.2000	5.0000	3.0000	1.0000		
0.5900			0.6300	2.0000		4.3000			2.0000		
0.6000	1.0000		0.6400	2.0000	1.0000	4.4000		1.0000	3.0000		
0.6100	3.0000		0.6500	5.0000		4.5000	3.0000		1.0000		
0.6200	3.0000		0.6600	3.0000	1.0000	4.6000	4.0000		3.0000		
0.6300	1.0000		0.6700	1.0000	1.0000	4.7000	3.0000		1.0000		
0.6400			0.6800			4.8000	1.0000	1.0000	1.0000		
0.6500	1.0000		0.6900	1.0000		4.9000			1.0000		
0.6600	3.0000		0.7000	1.0000	1.0000	5.0000	1.0000	1.0000	3.0000		
0.6700	2.0000	1.0000	0.7100	2.0000		5.1000			1.0000		
0.6800	4.0000		0.7200	2.0000	2.0000	5.2000	1.0000		3.0000		
0.6900	2.0000		0.7300		1.0000	5.3000		1.0000	2.0000		
0.7000	2.0000		0.7400	3.0000		5.4000	1.0000	1.0000	1.0000		
0.7100			0.7500	4.0000		5.5000		4.0000	2.0000		
0.7200	1.0000		0.7600			5.6000	1.0000	1.0000	2.0000		
0.7300	3.0000		0.7700	1.0000		5.7000	2.0000	1.0000	2.0000		
0.7400			0.7800	1.0000		5.8000	2.0000	3.0000	1.0000		
0.7500	3.0000		0.7900	3.0000		5.9000	2.0000	1.0000	3.0000		

0.7600	1.0000		0.8000	4.0000		6.0000			1.0000		
0.7700	1.0000	2.0000	0.8100			6.1000		1.0000	1.0000		
0.7800	2.0000		0.8200			6.2000			1.0000		
0.7900	2.0000		0.8300	1.0000	2.0000	6.3000	1.0000				
0.8000	1.0000		0.8400	2.0000	1.0000	6.4000					
0.8100	1.0000		0.8500			6.5000	1.0000	1.0000			
0.8200			0.8600	2.0000		6.6000					
0.8300	2.0000		0.8700	1.0000		6.7000	1.0000	1.0000			
0.8400	2.0000		0.8800	2.0000		6.8000		1.0000	1.0000		
0.8500	2.0000		0.8900	2.0000	1.0000	6.9000		1.0000	1.0000		
0.8600	1.0000		0.9000	1.0000		7.0000	2.0000				
0.8700			0.9100			7.1000	2.0000	1.0000			
0.8800			0.9200	1.0000		7.2000	1.0000		2.0000		
0.8900			0.9300		1.0000	7.3000	1.0000	1.0000			
0.9000	1.0000		0.9400	1.0000		7.4000		1.0000	1.0000		
0.9100	1.0000		0.9500	3.0000	1.0000	7.5000	1.0000		3.0000		
0.9200	1.0000		0.9600	2.0000		7.6000	1.0000				
0.9300			0.9700			7.7000		1.0000			
0.9400	1.0000		0.9800		1.0000	7.8000		1.0000	1.0000		
0.9500	1.0000		0.9900	2.0000		7.9000			1.0000		
0.9600	1.0000		1.0000	12.0000		8.0000			2.0000		
0.9700			1.1000	5.0000	1.0000	8.1000		3.0000	1.0000		
0.9800			1.2000	6.0000	3.0000	8.2000					
0.9900			1.3000	4.0000		8.3000	1.0000	1.0000	2.0000		
1.0000	5.0000		1.4000	1.0000	2.0000	8.4000		1.0000			
1.1000	4.0000		1.5000	2.0000		8.5000		1.0000	1.0000		
1.2000	1.0000		1.6000	4.0000	1.0000	8.6000	1.0000				
1.3000	3.0000		1.7000	2.0000	1.0000	8.7000		3.0000	1.0000		
1.4000	2.0000		1.8000	3.0000		8.8000					
1.5000	7.0000		1.9000	1.0000		8.9000		1.0000	1.0000		
1.6000	3.0000		2.0000			9.0000	2.0000				
1.7000	1.0000		2.1000	1.0000		9.1000		1.0000			
1.8000	1.0000		2.2000			9.2000					
1.9000	2.0000		2.3000	2.0000		9.3000		3.0000	1.0000		
2.0000	3.0000		2.4000			9.4000	1.0000		2.0000		
2.1000	2.0000		2.5000	2.0000	1.0000	9.5000	1.0000	2.0000			
2.2000			2.6000	1.0000		9.6000					
2.3000			2.7000			9.7000			1.0000		
2.4000	2.0000		2.8000			9.8000		1.0000			
2.5000			2.9000	2.0000		9.9000					
2.6000			3.0000			10.0000	1.0000	3.0000	2.0000		
2.7000	1.0000		3.1000	2.0000		11.0000		4.0000	4.0000		

2.8000			3.2000			12.0000	2.0000	2.0000	2.0000		
2.9000			3.3000			13.0000	3.0000	6.0000	4.0000		
3.0000	1.0000		3.4000	2.0000		14.0000	4.0000	1.0000	2.0000		
3.1000			3.5000			15.0000	1.0000	3.0000	3.0000		
3.2000	1.0000		3.6000	1.0000		16.0000		2.0000			
3.3000			3.7000	1.0000		17.0000	2.0000		2.0000		
3.4000			3.8700			18.0000	1.0000	3.0000	1.0000		
3.5000			3.9000			19.0000	1.0000		1.0000		
3.6000			4.0000			20.0000		2.0000			
3.7000			4.1000			21.0000		2.0000			
3.8700			4.2000			22.0000		1.0000			
3.9000			4.3000			23.0000		1.0000			
4.0000			4.4000			24.0000		1.0000			
4.1000			4.5000			25.0000		1.0000			
4.2000			4.6000			26.0000			1.0000		
4.3000			4.7000			27.0000					
4.4000			4.8000			28.0000		1.0000			
4.5000			4.9000			29.0000		3.0000			
4.6000			5.0000			30.0000					
4.7000			0.0140		9.0000	31.0000					
4.8000			0.0150		10.0000	32.0000					
4.9000			0.0160		4.0000	33.0000					
5.0000			0.0170		6.0000	34.0000					
						35.0000					
						36.0000					
						37.0000					
						38.0000					
						39.0000					
						40.0000		4.0000			
						41.0000					
						42.0000					
						43.0000					
						44.0000					
						45.0000					
						46.0000					
						47.0000					
						48.0000					
						49.0000					
						50.0000		2.0000			

B APPENDIX 1

B.1 Clonogenic survival measured in reporters exposed to transfer medium from contaminated and control Mink frogs

Treatment	Cells Plated	Colony Count		PE (%)		SF		SD
		Man	Auto	M	A	M	A	
Controls n=1	1000	175	193	18	19	66.79	73.384	
Females from control sites N=1	1000	262	263	26	26	100	100	
Males from control sites N=1	1000	254	243	25	24	98.0915	92.3954	
Females from contaminated sites N=1	1000	46	25	4.6	2.5	17.55	9.505703	
Males from contaminated sites N= 1	1000	122	132	12	13	46.56	50.19011	

B.2 Growth Rate Data for Mink Frogs

Controls – Fast Growth Colonies

day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10
0.00581352	0.0152191	0.0399229	0.08489	0.1585513	0.2902023	0.3953609	1.08289	2.4443321	3.788654
0.0060467	0.0091883	0.0228638	0.0507437	0.189576	0.417153	0.6478559	1.433867	2.617625	4.818261
0.00603775	0.0081879	0.010289	0.0614685	0.1762412	0.299912	0.3792498	1.6763456	2.617625	4.991827
0.0094843	0.0318828	0.0663488	0.4415361	0.3475748	0.6536778	0.9919856	1.67634	2.9918826	5.199827
0.0052404	0.0136625	0.0268649	0.0519978	0.086978	0.130115	0.1883745	0.3725308	0.518726	1.01298
0.00576251	0.0091883	0.0152817	0.0256191	0.084454	0.140612	0.215183	0.4361889	0.8918827	1.21892
0.0034056	0.0051773	0.0119842	0.0208561	0.0881498	0.188017	0.2779123	0.594249	0.819928	2.002917
0.00560589	0.0089918	0.0151268	0.0277555	0.086543	0.110478	0.1756013	0.545914	0.9019289	2.19287
0.0050245	0.0061883	0.0159876	0.0305875	0.2510112	0.5056171	0.761563	1.547378	2.88192	3.910028
0.00502454	0.0071766	0.0202318	0.0288761	0.0714216	0.102409	0.1516678	0.2925467	0.5188267	1.020192
0.00607097	0.2188264	0.0442467	0.1091862	0.3081996	0.6153112	0.9723167	2.201879	4.287123	6.199823
0.0040278	0.0061773	0.016444	0.0298317	0.0933236	0.139707	0.2009612	0.4262333	0.6188723	1.899276
0.02841498	0.0717725	0.1377141	0.221354	0.3839287	0.526987	0.6178278	0.979367	1.418827	2.381872
0.00527386	0.0188272	0.0341005	0.0874967	0.22686	0.347034	0.4653178	1.231845	2.819287	4.100928
0.0117267	0.0166773	0.0244256	0.038643	0.0813229	0.1492245	0.233567	0.636345	0.9928172	1.9992763
0.00458378	0.0091883	0.0188734	0.0377248	0.143469	0.348245	0.569987	1.129987	2.219982	4.928172
0.00352198	0.0061883	0.0191016	0.0360775	0.0741557	0.1274156	0.1744223	0.3966912	0.7188276	1.8271672
0.0040867	0.0071726	0.0156388	0.0356267	0.1088961	0.246601	0.3580461	0.7275956	1.0188267	2.118276
0.006262	0.0091883	0.0164198	0.0319836	0.162096	0.2945298	0.4045623	0.651321	0.8192837	1.012726
0.00877891	0.0188276	0.0314967	0.0493961	0.1123961	0.243803	0.323949	0.699174	1.5182763	2.81726
0.00269913	0.0091883	0.0212397	0.0502168	0.0888228	0.127873	0.2157879	0.4072378	0.8172653	1.281253
0.00377219	0.0129993	0.0398897	0.0590268	0.1630398	0.2856161	0.3176257	0.89576	1.4172635	2.318723
0.00276557	0.002766	0.0027658	0.002768	0.002765	0.002765	0.002765	0.002765	0.002777	0.002777
0.0035798	0.0071727	0.0333698	0.0511589	0.183879	0.290809	0.4036023	1.048612	2.228712	4.239122
0.00391988	0.0061727	0.014484	0.0217178	0.0359278	0.0358965	0.25179	0.489556	0.6172763	0.9928712
0.00317725	0.0041773	0.0071829	0.0128872	0.0318827	0.0612883	0.0991883	0.2112898	0.5199237	0.9188723
0.00417725	0.0081993	0.0099183	0.0199282	0.0318827	0.0718828	0.1667275	0.2278172	0.8199284	1.4417623
0.00418827	0.0071776	0.0092874	0.0122818	0.0318829	0.0881728	0.1617789	0.2288172	0.6177283	1.277612
0.00218826	0.0031883	0.0041787	0.0091883	0.0188199	0.0418828	0.0992817	0.1982716	0.6187234	1.0293843
0.0018827	0.0031879	0.0041883	0.0102992	0.0219993	0.0551883	0.0718873	0.1688173	0.4198928	0.9182732
0.00218826	0.0041877	0.0061773	0.0091993	0.0218828	0.0517727	0.0991883	0.1678975	0.5188723	0.9188237

N= 30 colonies Tracked

Data Source: Data 2 in Notebook1
Equation: Linear

R Rsqr Adj Rsqr Standard Error of Estimate

0.8280 0.6855 0.6462 0.4687

	Coefficient	Std. Error	t	P	VIF
y0	-0.6552	0.3202	-2.0462	0.0749	4.6667<
a	0.2155	0.0516	4.1759	0.0031	4.6667<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	2	6.6409	3.3204
Residual	8	1.7577	0.2197
Total	10	8.3986	0.8399

Controls- slow growth colonies

day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10
0.002258356	0.0062552	0.0088614	0.0168256	0.0501456	0.0888559	0.1276645	0.254415	0.3179976	0.5991828
0.00342167	0.0122873	0.0250979	0.0587417	0.1726888	0.324932	0.456912	1.1589	2.2188727	0.4188276
0.00234201	0.0082776	0.0113113	0.0234867	0.0700861	0.0700861	0.0817726	0.0918827	0.1299181	0.1992826
0.00235032	0.0061773	0.0176567	0.0130057	0.034461	0.034409	0.0448896	0.0521412	0.0618826	0.0719277
0.0024256	0.0068873	0.0147915	0.0219589	0.2000815	0.2204198	0.2491423	0.474349	0.6177265	0.8299182
0.0032476	0.0032888	0.033204	0.006256	0.0355876	0.0490498	0.0739047	0.148675	0.2218862	0.4102887
0.00461723	0.0072662	0.0140356	0.0137947	0.013795	0.0137998	0.01138	0.01138	0.01138	0.01138
0.005049	0.0078817	0.0196745	0.0314428	0.0947367	0.1142089	0.1336772	0.2551881	0.4100287	0.9188278
0.0018769	0.0051983	0.0139198	0.0240014	0.025668	0.0296712	0.0299906	0.0310115	0.0388193	0.0419928
0.00338623	0.0041883	0.0157623	0.026352	0.092534	0.1345612	0.1947213	0.3160398	0.4419982	0.7188287
0.0032798	0.0051883	0.019889	0.0394416	0.056289	0.132188	0.1593578	0.307834	0.51098	0.8199278
0.003447151	0.0061883	0.020015	0.0366667	0.0666198	0.0669912	0.0699945	0.1412278	0.2189827	0.4419893
0.0034056	0.0061773	0.0148609	0.0208045	0.035969	0.038909	0.048069	0.046378	0.0501282	0.0599183
0.0048335	0.0051883	0.0105978	0.0200681	0.0211432	0.0236753	0.0242569	0.0299614	0.0328172	0.0419928
0.00245823	0.0061773	0.0187623	0.0262778	0.0559267	0.0685198	0.0770576	0.1190002	0.202182	0.319823
0.00290678	0.0062882	0.0140567	0.0236523	0.0517569	0.0593917	0.0626367	0.150002	0.218827	0.2999182
0.005298	0.0071826	0.0262691	0.0405022	0.0466441	0.0491017	0.053498	0.101845	0.2100283	0.392881
0.005888876	0.0081727	0.0180884	0.0265979	0.0890698	0.117549	0.145619	0.284379	0.3199827	0.5190927
0.00246059	0.0031826	0.0086123	0.0107716	0.014276	0.01467	0.0147765	0.0207192	0.0271828	0.030103
0.005713967	0.0081773	0.0157567	0.0264183	0.027423	0.0333687	0.0376134	0.102768	0.1782873	0.261726
0.00392827	0.0071883	0.0146578	0.0228961	0.0610891	0.0870118	0.08999	0.221745	0.3188723	0.5012736
0.0022838	0.0032155	0.007546	0.0125409	0.0226145	0.0314023	0.0421615	0.079198	0.0998732	0.1567662
0.0020765	0.0061776	0.0201973	0.0377214	0.0566402	0.0715323	0.1106506	0.268912	0.4198283	0.7188273
0.0106636	0.0188272	0.0491498	0.0799743	0.106979	0.141798	0.0708009	0.079961	0.1299832	0.2414723

0.00948456	0.0166727	0.0401499	0.0516291	0.097396	0.1000916	0.250143	0.510496	0.7182873	0.9182736
0.00418826	0.0051883	0.0061773	0.0081883	0.0199188	0.0318999	0.0518983	0.0718883	0.128732	0.4187234
0.00218826	0.0031773	0.0041879	0.0067782	0.0091883	0.0161773	0.0277165	0.0817725	0.1442761	0.3127887
0.003188267	0.0038882	0.0038865	0.0081993	0.0191883	0.0388173	0.0718828	0.1022887	0.3188723	0.5128834
0.0031544	0.0044567	0.0071883	0.0091883	0.0188272	0.0551773	0.1288178	0.1998927	0.4199824	0.8177236

N=28 colonies tracked
Data Source: Data 1 in Notebook1
Equation: Linear

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.8904	0.7929	0.7670	0.0677

	Coefficient	Std. Error	t	P	VIF
y0	-0.1089	0.0462	-2.3554	0.0463	4.6667<
a	0.0412	0.0075	5.5340	0.0006	4.6667<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	2	0.2793	0.1396
Residual	8	0.0366	0.0046
Total	10	0.3159	0.0316

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	1	0.1403	0.1403	30.6247	0.0006
Total	9	0.1769	0.0197		

Reporters given bladder medium from contaminated female frogs – Fast Growth Colonies

Day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10
0.0027406	0.002746	0.0064012	0.0093745	0.0072416	0.0107135	0.011234	0.0100145	0.0100015	0.0100042
0.004335	0.0052199	0.008761	0.0088813	0.0091232	0.0199307	0.0207044	0.0333867	0.0666612	0.0716624
0.006884867	0.0087652	0.0127398	0.0180302	0.0175563	0.0183712	0.0185783	0.0235723	0.020776	0.0233871
0.00272404	0.004698	0.0145005	0.0176738	0.018032	0.0190356	0.0271123	0.0474142	0.0399567	0.0399183
0.00187693	0.0039142	0.0099167	0.0187768	0.0101404	0.0163422	0.0166423	0.0133623	0.013367	0.0133678
0.0026409	0.0041923	0.0097412	0.0111121	0.0189232	0.0064198	0.006444	0.0132467	0.0153398	0.0167782
0.00173578	0.0046178	0.008289	0.0093118	0.0128478	0.0123334	0.0114198	0.0204567	0.0118345	0.0128837
0.001943378	0.00200001	0.0020149	0.0134624	0.0138779	0.0070097	0.0093534	0.0350978	0.0419987	0.0551883
0.00137032	0.0042198	0.006471	0.008567	0.0089988	0.0124008	0.0281378	0.0281378	0.026913	0.0281726
0.00346319	0.0091423	0.0143921	0.0272689	0.028779	0.0211138	0.0210786	0.0200089	0.028967	0.0318823
0.002184223	0.00561923	0.0094812	0.0188735	0.0191098	0.0122712	0.0077778	0.0066759	0.0091423	0.0119982
0.003380124	0.003999	0.0059049	0.018435	0.016423	0.0267899	0.0399865	0.0677543	0.0214612	0.0317725

0.00285698	0.003416	0.0202192	0.0142261	0.0201423	0.0307546	0.0367598	0.0543067	0.0591999	0.0632772
0.00218823	0.02819924	0.0033188	0.0051882	0.0081772	0.0119983	0.0192884	0.0288173	0.0319925	0.0437764
0.003188272	0.00418825	0.0067173	0.0091883	0.0142881	0.0218823	0.0299183	0.0318825	0.0381726	0.0412884
0.00218823	0.02991823	0.0029918	0.0031993	0.0039182	0.0051887	0.0100298	0.0141762	0.0199287	0.0221883
0.00716263	0.007163	0.0081726	0.0081883	0.0091883	0.0192389	0.0218982	0.0281992	0.0319924	0.0358817
0.00129823	0.00318823	0.0041882	0.0041898	0.0051883	0.005199	0.0067882	0.0069918	0.0072623	0.0078764
0.002281872	0.00299183	0.0031993	0.0038873	0.0041882	0.0048183	0.0057172	0.0061882	0.0091824	0.0103883
0.00418823	0.00423771	0.0048273	0.0071882	0.0089937	0.008994	0.0091884	0.0128387	0.0289918	0.0319928
0.00518823	0.00599183	0.0061772	0.0068287	0.0078838	0.0081872	0.0098387	0.0102999	0.0181772	0.0281726
0.006128723	*0.00677724*	*0.0069919*	*0.0078824*	*0.0081724*	*0.0101282*	*0.0188271*	*0.0202881*	*0.0237187*	*0.026172*

N= 21 colonies tracked
Data Source: Data 2 in Notebook1
Equation: Linear

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9829	0.9661	0.9619	0.0017

	Coefficient	Std. Error	t	P	VIF
y0	-0.0004	0.0012	-0.3619	0.7268	4.6667<
a	0.0029	0.0002	15.1051	<0.0001	4.6667<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	2	0.0031	0.0015
Residual	8	2.3924E-005	2.9905E-006
Total	10	0.0031	0.0003

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	1	0.0007	0.0007	228.1642	<0.0001
Total	9	0.0007	7.8473E-005		

Reporters given bladder medium from contaminated female frogs – Slow Growth Colonies

day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10
0.009192	0.0098879	0.0102233	0.0122345	0.0111345	0.0109876	0.0100098	0.0099987	0.0081123	0.0081232
0.002449	0.0024498	0.0076812	0.0076823	0.007104	0.007258	0.0076823	0.0076856	0.006912	0.006912
0.001536	0.0021114	0.0024498	0.0064867	0.008567	0.0086655	0.009654	0.0070012	0.0070011	0.007001
0.001586	0.001566	0.0013967	0.0013867	0.001386	0.0013786	0.0013867	0.0013867	0.002457	0.0025163
0.002476	0.0026168	0.002956	0.002956	0.002942	0.002951	0.0029512	0.002951	0.002223	0.0022817
0.004086	0.0073145	0.013546	0.0210298	0.0164107	0.0164112	0.0168841	0.018046	0.006999	0.0069918
0.00206	0.0055986	0.0087919	0.0108967	0.0110623	0.0099979	0.0073124	0.0072146	0.0072112	0.007212

0.002209	0.0020913	0.0020913	0.0020913	0.0022568	0.003534	0.003534	0.00353	0.0024612	0.0024612
0.001503	0.001503	0.003903	0.0059967	0.00599	0.00599	0.009142	0.009198	0.0034623	0.0034612
0.002284	0.0024145	0.0038367	0.0035698	0.003567	0.003567	0.0035666	0.0031167	0.0026145	0.0027716
0.007931	0.0094912	0.008898	0.008127	0.0076779	0.0078999	0.0070012	0.0069988	0.0069345	0.0069918
0.001154	0.002462	0.0092018	0.0188773	0.0051219	0.0051298	0.0041498	0.0041498	0.0031461	0.0034887
0.002882	0.0029182	0.0031002	0.0031625	0.0037188	0.0041827	0.0044187	0.0061726	0.0068172	0.0071882
0.002188	0.0021889	0.0031877	0.0034281	0.0041882	0.0048813	0.0068817	0.0071882	0.0079928	0.0081893
0.002187	0.0028172	0.0031882	0.0037189	0.0039182	0.0044713	0.0051882	0.0058298	0.0068173	0.0081727
0.001998	0.0019928	0.0020129	0.0022818	0.0028199	0.0038817	0.0039918	0.0061724	0.0066177	0.0071882
0.005189	0.0052189	0.0059918	0.0063882	0.0078829	0.0080199	0.0082377	0.0091872	0.0099239	0.0099994
0.002188	0.0028817	0.0031824	0.0038172	0.0041882	0.0048827	0.0051882	0.0059918	0.0061824	0.0068173
0.001924	0.0020192	0.0029918	0.0029998	0.0032188	0.0039182	0.0049189	0.0051992	0.0055182	0.0056172
0.002188	0.0022198	0.0028924	0.0031882	0.0038199	0.0041882	0.0049918	0.049923	0.0510024	0.0052188
0.003103	*0.0032199*	*0.003229*	*0.0032991*	*0.0031992*	*0.0031993*	*0.0032103*	*0.0032221*	*0.0032317*	*0.0037718*

N=21 colonies tracked

Nonlinear Regression

Data Source: Data 1 in Notebook1
Equation: Linear

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.7905	0.6249	0.5780	0.0011

	Coefficient	Std. Error	t	P	VIF
y0	0.0034	0.0008	4.4729	0.0021	4.6667<
a	0.0004	0.0001	3.6509	0.0065	4.6667<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	2	0.0004	0.0002
Residual	8	9.6980E-006	1.2123E-006
Total	10	0.0004	3.6206E-005

Reporters given bladder medium from contaminated male frogs - Fast Growth Colonies

day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10
0.081357	0.181472	0.202153	0.237345	0.336204	0.468472	0.820507	1.419823	2.996215	5.11292
0.0041775	0.006149	0.013946	0.014932	0.016336	0.033528	0.035372	0.064738	0.177636	0.212882
0.0032164	0.005102	0.010114	0.00722	0.02869	0.028698	0.029988	0.033197	0.09615	0.201299
0.0236481	0.034619	0.079301	0.093742	0.147916	0.183862	0.286789	0.481276	0.983922	1.899281
0.0047089	0.006219	0.015829	0.017654	0.019478	0.021576	0.054548	0.057326	0.097908	0.192771

0.0255131	0.036291	0.059803	0.071208	0.081456	0.112758	0.144047	0.275005	0.631364	0.918825
0.0025603	0.006422	0.0098	0.011309	0.014973	0.023936	0.040481	0.091929	0.226287	0.518825
0.0056089	0.007042	0.016888	0.014136	0.02097	0.030214	0.031991	0.082405	0.106878	0.310288
0.0124991	0.021497	0.055496	0.051259	0.060812	0.077543	0.078675	0.103381	0.131817	0.281723
0.0069763	0.008915	0.034139	0.035225	0.036668	0.036409	0.044926	0.087947	0.147921	0.233881
0.0034715	0.004292	0.0074	0.0076	0.008928	0.012057	0.020032	0.035499	0.101703	0.210028
0.0060543	0.007815	0.009177	0.015198	0.018805	0.018995	0.019065	0.047953	0.102916	0.310023
0.009077	0.01242	0.027008	0.028506	0.031011	0.032265	0.043934	0.063857	0.097998	0.200192
0.0054216	0.007142	0.010014	0.015564	0.015698	0.020756	0.042568	0.062048	0.090707	0.232881
0.0032389	0.00422	0.005801	0.010589	0.022324	0.02462	0.035463	0.039999	0.090691	0.182991
0.0066606	0.009621	0.022731	0.027166	0.033992	0.052621	0.073388	0.105299	0.139962	0.317725
0.0069929	0.007892	0.012707	0.01985	0.025389	0.044507	0.05451	0.086936	0.21725	0.322125
0.0083132	0.009422	0.014323	0.036168	0.036178	0.073723	0.091407	0.181107	0.468712	0.617725
0.0076325	0.008915	0.015523	0.028365	0.03229	0.042687	0.079652	0.163842	0.367007	0.918827
0.0044657	0.00522	0.011868	0.016701	0.018097	0.036086	0.055742	0.125015	0.270488	0.418825
0.0034217	0.004142	0.005813	0.010634	0.0124	0.018453	0.028092	0.073765	0.165921	0.318825
0.0033588	0.004292	0.008862	0.0143	0.017872	0.017989	0.033153	0.077452	0.167928	0.399183
0.0038038	0.008149	0.010396	0.006215	0.014193	0.017479	0.025665	0.044142	0.072818	0.182761
0.0067271	0.007815	0.009654	0.014194	0.015002	0.02328	0.028257	0.040022	0.059509	0.189827
0.0063533	0.008915	0.026659	0.039658	0.040441	0.058492	0.07253	0.141458	0.312168	0.617725
0.0052772	0.008993	0.012388	0.031229	0.051287	0.081278	0.123883	0.251278	0.418788	0.716625
0.0081277	0.008229	0.010224	0.021009	0.031229	0.038872	0.044487	0.081229	0.124	0.281928
0.0051289	0.008728	0.011002	0.022129	0.044487	0.071887	0.099929	0.155873	0.333998	0.517725
0.0051289	0.007128	0.009128	0.010289	0.018873	0.021201	0.026718	0.041287	0.071727	0.188827

N= 28 colonies tracked
Data Source: Data 2 in Notebook1
Equation: Linear

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.8098	0.6559	0.6128	0.1151

	Coefficient	Std. Error	t	P	VIF
y0	-0.1384	0.0786	-1.7606	0.1163	4.6667<
a	0.0495	0.0127	3.9046	0.0045	4.6667<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	2	0.3806	0.1903
Residual	8	0.1059	0.0132
Total	10	0.4865	0.0487

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	1	0.2019	0.2019	15.2459	0.0045
Total	9	0.3078	0.0342		

Reporters given bladder medium from contaminated male frogs- Slow Growth Colonies

day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10
0.002292189	0.0029145	0.0028134	0.0028178	0.0026677	0.0034654	0.0054322	0.0066777	0.0087654	0.010023
0.004177423	0.0041467	0.0033467	0.003467	0.003467	0.003467	0.003467	0.003467	0.003467	0.003467
0.00331378	0.0081491	0.0149407	0.0155775	0.0155781	0.0158798	0.0196198	0.0290509	0.0432968	0.071262
0.00411929	0.0049145	0.0049999	0.005267	0.0042117	0.0042167	0.0042114	0.004216	0.0053812	0.0071626
0.00232409	0.0061926	0.0116436	0.0159045	0.027228	0.0156636	0.0166142	0.016678	0.0299316	0.0577812
0.0045598	0.0046654	0.0047787	0.0050125	0.0056141	0.0056141	0.0076141	0.0142187	0.0293987	0.0388123
0.0045926	0.0081945	0.0103178	0.0121497	0.014606	0.014606	0.014606	0.0155564	0.0155567	0.015557
0.0031559	0.0040117	0.0049248	0.0079812	0.0109867	0.0122978	0.017656	0.0346078	0.0517321	0.0728812
0.0022098	0.0041612	0.0057345	0.0070923	0.0077765	0.0078999	0.0079999	0.0065412	0.0066178	0.0069188
0.004285398	0.0052618	0.00704	0.0092434	0.012823	0.0129917	0.0129916	0.0129916	0.0116423	0.012882
0.00362929	0.003987	0.004098	0.0041423	0.0049098	0.004709	0.0071675	0.010564	0.0274487	0.0418825
0.00792298	0.0082147	0.01267	0.0139608	0.0188928	0.0199155	0.0221568	0.0319576	0.0621478	0.1029893
0.005024	0.0060141	0.0065367	0.0098562	0.0103362	0.0180468	0.0219578	0.0224432	0.056776	0.0661723
0.002516423	0.0040412	0.0082887	0.0083062	0.0155388	0.0210098	0.0218876	0.0236754	0.0276653	0.0318992
0.002865467	0.0028612	0.0074823	0.0126658	0.0138867	0.0188909	0.0188967	0.0191015	0.0201191	0.0418825
0.0032879	0.0040425	0.0056723	0.007929	0.0164856	0.0244856	0.0478208	0.0803967	0.1570478	0.3199283
0.005763612	0.0070215	0.015572	0.0126657	0.0127887	0.0127999	0.0260809	0.0298765	0.045745	0.081723
0.00235867	0.0023661	0.0029234	0.002923	0.002923	0.0029565	0.006651	0.0070141	0.0134623	0.0162512
0.0074913	0.0134967	0.0136687	0.0136698	0.0136777	0.0138988	0.0149388	0.0150987	0.0344321	0.0728124
0.00255794	0.0026791	0.0032978	0.0032917	0.003292	0.003292	0.003292	0.003292	0.003292	0.0039918
0.0047508	0.0052191	0.0061709	0.0094767	0.0254467	0.0302768	0.0346871	0.0372147	0.0660998	0.0817725
0.00888634	0.0110422	0.0218432	0.0218383	0.0295987	0.031798	0.0374016	0.0514967	0.0717421	0.0918825
0.007914987	0.080015	0.0089023	0.0089023	0.008962	0.008902	0.0089558	0.0129725	0.0211458	0.0299183
0.0099876	0.013464	0.1998765	0.0211664	0.0229986	0.0229876	0.0298765	0.0316764	0.0388765	0.0618825
0.003261234	0.0036612	0.0033344	0.0035432	0.0039988	0.003999	0.0040125	0.004012	0.0040123	0.004012
0.0041276	0.0049993	0.0061625	0.0098828	0.0129988	0.0188828	0.0199929	0.0212288	0.0388772	0.0418825
0.00212887	0.0042187	0.0061278	0.0078876	0.0091883	0.010229	0.0122883	0.0210021	0.0432881	0.0617725
0.00333871	0.0061776	0.010339	0.0110009	0.0211092	0.0238387	0.0299817	0.0388871	0.0412298	0.0519925
0.01119982	0.0122871	0.0188873	0.0228871	0.0255662	0.0299982	0.0312998	0.0388277	0.0521223	0.0817263

0.0022334	0.0042122	0.0051299	0.0068812	0.0078827	0.0099289	0.0138889	0.0328882	0.0552887	0.0717632
0.003878872	0.0051276	0.0072762	0.0091287	0.0098898	0.010229	0.0177265	0.0223387	0.0441289	0.0677291

N= 31 colonies tracked

Data Source: Data 1 in Notebook1
Equation: Linear

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.8582	0.7365	0.7036	0.0084

	Coefficient	Std. Error	t	P	VIF
y0	-0.0045	0.0057	-0.7788	0.4585	4.6667<
a	0.0044	0.0009	4.7286	0.0015	4.6667<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	2	0.0054	0.0027
Residual	8	0.0006	7.0774E-005
Total	10	0.0060	0.0006

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	1	0.0016	0.0016	22.3594	0.0015
Total	9	0.0021	0.0002		

Reporters given bladder medium from female frogs in control sites- Slow Growth Colonies

day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10
0.002533	0.0054213	0.0088534	0.0221245	0.0488256	0.0742987	0.108745	0.1835679	0.2817725	0.66288
0.0042607	0.0081412	0.016626	0.0420234	0.0739478	0.1322161	0.3131167	0.3974789	0.5188236	0.88271
0.0029812	0.00649125	0.012117	0.0236361	0.033645	0.0493098	0.0771209	0.1176159	0.1992837	0.40281
0.007026	0.01049823	0.0238856	0.0485172	0.076096	0.1192518	0.2096423	0.2966456	0.4188237	0.71002
0.004858	0.00961412	0.023278	0.0511258	0.0700861	0.0790625	0.1488178	0.2215789	0.3827126	0.60182

0.0081467	0.0014219	0.0498723	0.0855912	0.1587257	0.1709567	0.290765	0.4067734	0.6127343	0.80123
0.0049245	0.01129267	0.0295571	0.0560723	0.1056987	0.1401967	0.222039	0.433956	0.5818824	0.71882
0.00205134	0.00401423	0.0099923	0.0179056	0.0295256	0.0392745	0.0997435	0.1761998	0.2819924	0.71662
0.00914423	0.00136145	0.024067	0.037773	0.0410986	0.0504568	0.063409	0.1119356	0.2388172	0.38192
0.00235667	0.00798412	0.0136956	0.0316786	0.048291	0.0665305	0.1713908	0.325467	0.4199237	0.61992
0.0059234	0.00699723	0.0224069	0.0224678	0.0372817	0.0446735	0.067387	0.140379	0.2188236	0.43312
0.011637	0.0314912	0.0799521	0.1200077	0.2652189	0.3790498	0.657189	1.05976	2.3128824	4.41878
0.0020914	0.0074213	0.0113198	0.1653567	0.0268193	0.0428298	0.0899356	0.1472489	0.2881724	0.37812
0.0255612	0.05291467	0.127956	0.2525976	0.4213876	0.5212765	0.8735778	1.346723	2.8991824	4.99182
0.0375534	0.0664912	0.1181141	0.2291274	0.453809	0.4848067	0.7900408	1.0014623	2.8899173	4.6887
0.002973	0.008999	0.0316172	0.0774195	0.1744478	0.2109105	0.385646	0.6422567	1.327887	3.19237
0.0044847	0.00521491	0.082067	0.0374691	0.0566777	0.0744045	0.1487923	0.233289	0.3188237	0.39918
0.00835786	0.02492134	0.0553617	0.1238278	0.2332645	0.254798	0.523127	0.8877267	1.4331343	3.51243
0.0027074	0.00521546	0.009276	0.0298645	0.0663612	0.070053	0.1644512	0.2541923	0.3678123	0.50128
0.0276387	0.04421678	0.0414398	0.1074006	0.1769304	0.1999877	0.272512	0.4392867	0.5991824	0.80923
0.01403567	0.0216729	0.0485371	0.0673879	0.1207764	0.14905	0.212798	0.288967	0.5100239	0.81992
0.00382031	0.0124698	0.0210117	0.0335278	0.086289	0.1254309	0.230398	0.432978	0.6881723	1.02883
0.0112296	0.01992978	0.0396	0.056988	0.098759	0.1330217	0.255479	0.3709623	0.7188237	1.51772
0.0021598	0.0046213	0.0097412	0.0138612	0.0353218	0.0391414	0.0462998	0.0883227	0.2019824	0.60029
0.01137014	0.02462156	0.0535278	0.1083723	0.294322	0.372897	0.773987	1.05768	2.441243	4.51877
0.00827762	0.0122287	0.0228726	0.0552872	0.0888187	1.1288736	1.9928837	3.23412	5.6188237	8.17728
0.00527881	0.00889982	0.0110909	0.0222813	0.0882876	0.5265125	1.227615	2.661726	3.1002934	5.19928
0.00817726	0.0128786	0.0277615	0.0477628	0.0899827	0.1677887	0.3387816	0.7166257	0.9918873	1.82764
0.011208928	0.01787251	0.0312287	0.0612652	0.1322761	0.2277618	0.5128927	0.8177262	2.1882742	5.19928

N= 29 colonies tracked

Data Source: Data 2 in Notebook1
Equation: Linear

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.8429	0.7104	0.6742	0.3735

	Coefficient	Std. Error	t	P	VIF
y0	-0.5362	0.2551	-2.1015	0.0688	4.6667<
a	0.1822	0.0411	4.4301	0.0022	4.6667<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	2	4.9060	2.4530
Residual	8	1.1158	0.1395

Total	10	6.0218	0.6022

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	1	2.7374	2.7374	19.6257	0.0022
Total	9	3.8532	0.4281		

Reporters given bladder medium from female frogs from control sites – Slow Growth Colonies

day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 1(
0.00283201	0.008142	0.019185	0.03322	0.050955	0.075136	0.085298	0.098706	0.189284	0.3188
0.0033784	0.005922	0.010626	0.019358	0.033698	0.031618	0.037766	0.038877	0.041238	0.0517
0.00195998	0.003615	0.005864	0.017117	0.026261	0.032691	0.039989	0.039477	0.051724	0.0718
0.0030064	0.007215	0.011918	0.029176	0.06425	0.06429	0.066915	0.096288	0.102998	0.1566
0.0050245	0.007999	0.018903	0.03246	0.044174	0.049988	0.052723	0.104467	0.199183	0.2417
0.00748289	0.001249	0.029652	0.039955	0.030314	0.031335	0.029948	0.029948	0.033177	0.0391
0.008545	0.010492	0.032568	0.032671	0.047876	0.041124	0.049999	0.070567	0.078817	0.0823
0.0023669	0.003215	0.004626	0.006661	0.019963	0.014887	0.020298	0.048378	0.089887	0.2012
0.00364	0.003654	0.003645	0.003657	0.004015	0.004322	0.005675	0.007621	0.008716	0.0089
0.0024338	0.002434	0.002434	0.002434	0.002434	0.002412	0.002412	0.002412	0.002788	0.0029
0.0033552	0.006143	0.009908	0.011229	0.031078	0.022423	0.034227	0.085168	0.172664	0.2517
0.0023337	0.002965	0.003521	0.010297	0.019609	0.019606	0.019606	0.019606	0.019606	0.0196
0.00521557	0.006992	0.016229	0.042322	0.029527	0.029056	0.055071	0.064912	0.102281	0.2182
0.00521556	0.007815	0.018247	0.027216	0.048756	0.052247	0.062788	0.090877	0.188199	0.3188
0.0041086	0.005218	0.012799	0.021297	0.020215	0.030411	0.039649	0.050693	0.06002	0.0817
0.00372067	0.006142	0.00892	0.01167	0.020861	0.022033	0.05351	0.129467	0.199284	0.2918
0.00881163	0.009146	0.016434	0.025571	0.03082	0.035669	0.047577	0.066798	0.071872	0.0982
0.0048086	0.009791	0.025409	0.03837	0.034549	0.048915	0.049968	0.091555	0.100283	0.1728
0.00275	0.002275	0.002276	0.002275	0.002956	0.002957	0.002957	0.002957	0.002957	0.0029
0.0027998	0.005692	0.008446	0.015097	0.024409	0.024409	0.040125	0.065056	0.102884	0.2788
0.004094378	0.005621	0.012848	0.015106	0.010123	0.013097	0.020996	0.031247	0.044187	0.049
0.00632012	0.008147	0.0172	0.022631	0.02789	0.02882	0.036215	0.037132	0.043093	0.0567
0.004128872	0.006129	0.007718	0.009992	0.013887	0.031278	0.055188	0.091883	0.192884	0.2918
0.00218872	0.004129	0.007127	0.012872	0.0212	0.038887	0.051233	0.071265	0.102998	0.2188
0.001287872	0.002123	0.004122	0.007163	0.009129	.01123iu1	0.021287	0.031228	0.078827	0.1427
0.00412872	0.006128	0.007713	0.009129	0.011029	0.023123	0.041387	0.071276	0.128884	0.2617
0.00918826	0.010287	0.012202	0.023313	0.033313	0.041288	0.071773	0.099129	0.167725	0.3188
0.0016762	0.003129	0.006128	0.019883	0.041289	0.082776	0.212989	0.499813	0.928784	0.2188
0.006419	0.00942	0.016412	0.0289	0.09913	0.21423	0.313472	0.643923	1.077695	2.8918
0.01792256	0.032144	0.069181	0.137271	0.237681	0.347111	0.533378	0.84331	1.102398	3.2881
0.00193507	0.00422	0.010078	0.015016	0.036142	0.046118	0.076631	0.139965	0.221772	0.3716

N=30 Colonies tracked
Data Source: Data 1 in Notebook1
Equation: Linear

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.8498	0.7221	0.6874	0.0620

	Coefficient	Std. Error	t	P	VIF
y0	-0.0840	0.0424	-1.9808	0.0830	4.6667<
a	0.0311	0.0068	4.5595	0.0019	4.6667<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	2	0.1563	0.0782
Residual	8	0.0308	0.0039
Total	10	0.1871	0.0187

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	1	0.0800	0.0800	20.7889	0.0019
Total	9	0.1108	0.0123		

Growth rate of reporters given bladder medium from male frogs from control sites- fast growth colonies

Fast Growth Colonies - Absolute Area Growth

day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9
0.0028321	0.0069142	0.0172765	0.03493096	0.164157	0.310787	0.436429	0.8342845	1.442881
0.01731598	0.02362198	0.0441827	0.0752967	0.16211412	0.2675623	0.4118798	0.8459256	2.001925
0.0068625	0.02321498	0.046847	0.1080312	0.265534	0.485603	0.6336974	0.4981209	1.12882
0.004983012	0.008613412	0.0137945	0.0897213	0.2520089	0.2835723	0.5606723	1.497768	2.19925
0.01583312	0.0234912	0.0407026	0.10213967	0.22989	0.346326	0.5463178	1.1182978	2.333871
0.012042298	0.0091423	0.0386189	0.07061645	0.186178	0.2902192	0.4194456	1.0898456	3.19982
0.0093876	0.0103466	0.0307956	0.06128278	0.1612567	0.33080516	0.5076445	1.1519278	3.448812
0.0045845	0.00926198	0.0341689	0.0587998	0.164379	0.276432	0.457258	1.095198	2.551826
0.0031978	0.00361923	0.00431689	0.03152498	0.0904163	0.11984187	0.1886232	0.6418045	0.8199285
0.0086289	0.01246145	0.0468156	0.1118023	0.1962292	0.2622191	0.4248768	0.8699498	2.18825
0.0045845	0.00926198	0.0341689	0.0587998	0.164379	0.276432	0.457258	1.095198	2.551826
0.0031978	0.00361923	0.00431689	0.03152498	0.0904163	0.11984187	0.1886232	0.6418045	0.8199285
0.0060467	0.0081423	0.012906	0.0332259	0.10577234	0.1332209	0.24215598	0.52293456	3.102958
0.0103467	0.0222612	0.0222256	0.0583012	0.164746	0.1900024	0.237145	0.627789	1.028815

0.00258234	0.0026141	0.0079491	0.0166931	0.038688	0.0234209	0.1229398	0.4023378	0.6177825
0.042181456	0.0891423	0.1755507	0.4158962	0.7123556	0.9009378	1.559078	2.42123	4.19925
0.0052076	0.0072912	0.014907	0.0212078	0.0613576	0.068665	0.11791487	0.2892178	0.518825
0.0099925	0.0104213	0.0137569	0.0699546	0.1452278	0.2931098	0.3925967	0.7069145	0.998825
0.009788332	0.0114216	0.0420816	0.0670137	0.2763312	0.4224812	0.728767	1.62756	4.18825
0.0126712	0.0414267	0.07035198	0.1141698	0.1949167	0.245606	0.3115461	0.659889	1.42615
0.0056808	0.0081914	0.00645308	0.0568912	0.1720089	0.1900932	0.2648178	0.647343	0.918825
0.005423	0.0079956	0.0214623	0.0456278	0.1745387	0.2454634	0.4378823	1.121409	2.318825
0.052454	0.10241435	0.17300206	0.27650712	0.5151298	0.7757726	1.1117809	1.996213	4.992815
0.03644245	0.07142198	0.1158053	0.2439167	0.561287	0.00369912	1.4556912	3.314634	5.1925
0.00556435	0.00999412	0.03493267	0.06324414	0.208945	0.3449567	0.5775234	1.09678	2.129925
0.01875217	0.029142	0.05662978	0.1486849	0.3888589	0.6206412	0.9011456	1.508867	3.210925
0.009128734	0.01273643	0.02287123	0.0502387	0.1656271	0.299181	0.5188237	1.0128732	2.17725
0.00417236	0.006188237	0.009128723	0.015418725	0.0341812	0.08192398	0.172883	0.3102983	0.518825
0.0034882	0.00418725	0.00618825	0.01002935	0.02287125	0.04198825	0.13388725	0.318925	0.51787825
0.004188725	0.00677812	0.00928812	0.02318825	0.06772812	0.1229983	0.251612	0.3918273	1.01882
0.0021882	0.00318825	0.00449925	0.01022985	0.03288715	0.05669812	0.1556285	0.3199925	0.6177285
0.00310025	0.0051815	0.00919925	0.019928785	0.0519925	0.16278925	0.22881523	0.488253	0.992812

N= 33

Data Source: Data 2 in Notebook1
Equation: Linear

R	**Rsqr**	**Adj Rsqr**	**Standard Error of Estimate**
0.7956	0.6330	0.5871	0.8398

	Coefficient	**Std. Error**	**t**	**P**	**VIF**
y0	-1.0714	0.5737	-1.8675	0.0988	4.6667<
a	0.3434	0.0925	3.7142	0.0059	4.6667<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	**SS**	**MS**
Regression	2	16.4124	8.2062
Residual	8	5.6427	0.7053
Total	10	22.0551	2.2055

Corrected for the mean of the observations:

	DF	**SS**	**MS**	**F**	**P**
Regression	1	9.7305	9.7305	13.7956	0.0059
Total	9	15.3732	1.7081		

Growth rate of reporters given bladder medium from male frogs from control sites- slow growth colonies

day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9
0.002258356	0.00625517	0.0088614	0.0168256	0.0501456	0.0888559	0.1276645	0.254415	0.317
0.00342167	0.0122873	0.02509788	0.05874167	0.17268876	0.324932	0.456912	1.1589	2.218
0.00234201	0.00827761	0.01131127	0.0234867	0.07008612	0.0700861	0.0817726	0.0918827	0.12
0.00235032	0.00617726	0.0176567	0.01300567	0.034461	0.034409	0.0448896	0.0521412	0.061
0.0024256	0.00688726	0.0147915	0.0219589	0.2000815	0.2204198	0.2491423	0.474349	0.617
0.0032476	0.00328876	0.033204	0.006256	0.0355876	0.0490498	0.07390467	0.148675	0.221
0.00461723	0.00726615	0.0140356	0.01379469	0.01379498	0.0137998	0.01138	0.01138	0.0
0.005049	0.007881726	0.0196745	0.0314428	0.0947367	0.1142089	0.13367723	0.25518812	0.41
0.0018769	0.00519826	0.0139198	0.02400139	0.025668	0.0296712	0.0299906	0.03101145	0.03881
0.00338623	0.004188267	0.01576234	0.02635198	0.092534	0.1345612	0.1947213	0.3160398	0.441
0.0032798	0.00518826	0.019889	0.03944156	0.056289	0.132188	0.1593578	0.307834	0.5
0.003447151	0.00618826	0.020015	0.036666709	0.0666198	0.0669912	0.0699945	0.1412278	0.21
0.0034056	0.00617726	0.0148609	0.0208045	0.035969	0.038909	0.048069	0.046378	0.05
0.0048335	0.00518827	0.0105978	0.02006812	0.0211432	0.02367532	0.0242569	0.0299614	0.032
0.00245823	0.00617725	0.01876234	0.0262778	0.0559267	0.0685198	0.0770576	0.1190002	0.2
0.00290678	0.00628816	0.0140567	0.0236523	0:0517569	0.0593917	0.0626367	0.150002	0.21
0.005298	0.0071826	0.0262691	0.04050223	0.04664412	0.0491017	0.053498	0.101845	0.21
0.005888876	0.00817265	0.0180884	0.0265979	0.0890698	0.117549	0.145619	0.284379	0.31
0.00246059	0.0031826	0.0086123	0.010771623	0.014276	0.01467	0.0147765	0.0207192	0.0271
0.005713967	0.00817726	0.0157567	0.026418299	0.027423	0.0333687	0.0376134	0.102768	0.17
0.00392827	0.007188276	0.0146578	0.02289612	0.0610891	0.08701178	0.08999	0.221745	0.31
0.0022838	0.00321552	0.007546	0.0125409	0.0226145	0.0314023	0.0421615	0.079198	0.09
0.0020765	0.006177625	0.02019734	0.037721446	0.05664023	0.0715323	0.11065062	0.268912	0.41
0.0106636	0.01882716	0.0491498	0.0799743	0.106979	0.141798	0.0708009	0.079961	0.12
0.00948456	0.01667265	0.04014987	0.05162912	0.097396	0.1000916	0.250143	0.510496	0.71
0.00418826	0.00518826	0.00617726	0.008188276	0.019918826	0.031899927	0.05189827	0.07188826	0.1
0.00218826	0.003177256	0.004187873	0.006778216	0.00918826	0.01617726	0.0277165	0.08177254	0.144
0.003188267	0.003888173	0.00388654	0.00819927	0.01918826	0.03881726	0.07188276	0.10228871	0.31
0.0031544	0.00445674	0.00718827	0.009188276	0.018827156	0.05517725	0.128817826	0.199892716	0.419

N= 29 colonies tracked
Data Source: Data 1 in Notebook1
Equation: Linear

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.8465	0.7165	0.6811	0.0705

	Coefficient	Std. Error	t	P	VIF
y0	-0.0974	0.0482	-2.0215	0.0779	4.6667<
a	0.0349	0.0078	4.4967	0.0020	4.6667<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	2	0.1902	0.0951
Residual	8	0.0398	0.0050
Total	10	0.2300	0.0230

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	1	0.1006	0.1006	20.2203	0.0020
Total	9	0.1404	0.0156		

B.3 ANOVA and T test Results for growth rates of reporters given bladder medium from Mink Frogs

ANOVA RESULTS - treatment comparison on each day

VARIABLE	MEAN	SD	
SCF1	2.967E-03	2.071E-03	b
SCM1	4.521E-03	2.437E-03	a
SBF1	4.615E-03	3.264E-03	a
SBM1	5.195E-03	3.038E-03	a
SCTL1	3.856E-03	2.066E-03	a,b
SCF2	3.571E-03	2.484E-03	b
SCM2	8.315E-03	0.0136	a
SBF2	6.696E-03	5.386E-03	a,b
SBM2	6.670E-03	3.854E-03	a,b
SCTL2	6.842E-03	3.610E-03	a,b
SCF3	4.988E-03	3.350E-03	b
SCM3	0.0143	0.0348	a,b
SBF3	0.0139	0.0128	a,b
SBM3	0.0124	8.103E-03	a,b
SCTL3	0.0170	0.0102	a
SCF4	6.363E-03	5.357E-03	a
SCM4	9.888E-03	5.461E-03	a
SBF4	0.0233	0.0240	b
SBM4	0.0220	0.0152	b
SCTL4	0.0257	0.0168	a
SCF5	5.723E-03	3.680E-03	c
SCM5	0.0128	7.736E-03	c
SBF5	0.0364	0.0422	b
SBM5	0.0364	0.0315	b
SCTL5	0.0575	0.0456	a
SCF6	5.971E-03	3.443E-03	c
SCM6	0.0141	8.572E-03	c
SBF6	0.0488	0.0670	b
SBM6	0.0445	0.0368	b
SCTL6	0.0779	0.0672	a
SCF7	6.205E-03	3.406E-03	c
SCM7	0.0175	0.0111	c
SBF7	0.0720	0.1047	a,b
SBM7	0.0657	0.0487	b
SCTL7	0.1014	0.0933	a
SCF8	8.579E-03	0.0101	b
SCM8	0.0226	0.0165	b
SBF8	0.1214	0.1887	a

SBM8	0.1366	0.1045	a
SCTL8	0.1627	0.1308	a
SCF9	7.792E-03	0.0102	c
SCM9	0.0365	0.0300	c
SBF9	0.1906	0.2896	b
SBM9	0.2290	0.1649	a,b
SCTL9	0.3151	0.4087	a
SCF10	5.827E-03	2.297E-03	b
SCM10	0.0556	0.0574	b
SBF10	0.3555	0.7401	a
SBM10	0.3880	0.2703	a
SCTL10	0.4140	0.2857	a
FCF1	3.270E-03	1.708E-03	c
FCM1	9.618E-03	0.0148	a,b
FBF1	8.716E-03	8.359E-03	a,b,c
FBM1	0.0107	0.0118	a
FCTL1	5.636E-03	4.739E-03	b,c
FCF2	7.055E-03	7.354E-03	a
FCM2	0.0158	0.0328	a
FBF2	0.0157	0.0156	a
FBM2	0.0189	0.0244	a
FCTL2	0.0183	0.0393	a
FCF3	7.973E-03	4.420E-03	b
FCM3	0.0255	0.0382	a,b
FBF3	0.0364	0.0311	a
FBM3	0.0370	0.0429	a
FCTL3	0.0244	0.0252	a,b
FCF4	0.0114	6.212E-03	c
FCM4	0.0320	0.0440	b,c
FBF4	0.0706	0.0599	a
FBM4	0.0811	0.0859	a
FCTL4	0.0568	0.0823	a,b
FCF5	0.0123	6.247E-03	c
FCM5	0.0432	0.0626	c
FBF5	0.1253	0.1112	b
FBM5	0.1945	0.1565	a
FCTL5	0.1248	0.0980	b
FCF6	0.0141	7.278E-03	b
FCM6	0.0595	0.0865	b
FBF6	0.2095	0.2293	a
FBM6	0.2685	0.2033	a
FCTL6	0.2255	0.1752	a
FCF7	0.0176	9.957E-03	b
FCM7	0.0885	0.1504	b
FBF7	0.3886	0.4217	a

FBM7	0.4736	0.3536	a
FCTL7	0.3353	0.2493	a
FCF8	0.0242	0.0162	c
FCM8	0.1557	0.2605	c
FBF8	0.6377	0.7272	b
FBM8	0.9739	0.6599	a
FCTL8	0.7350	0.5447	a,b
FCF9	0.0261	0.0158	c
FCM9	0.3197	0.5524	c
FBF9	1.1397	1.2667	b
FBM9	2.0430	1.3388	a
FCTL9	1.3624	1.0257	b
FCF10	0.0299	0.0175	c
FCM10	0.5872	0.9392	c
FBF10	2.0246	2.1131	b
FBM10	4.0731	1.8671	a
FCTL10	2.4123	1.6297	b

T-TEST RESULT - comparison of slow and fast data each day

VARIABLE	MEAN	SD	
SCF1	2.967E-03	2.071E-03	a
FCF1	3.270E-03	1.708E-03	a
SCF2	3.571E-03	2.484E-03	b
FCF2	7.055E-03	7.354E-03	a
SCF3	4.988E-03	3.350E-03	b
FCF3	7.973E-03	4.420E-03	a
SCF4	6.363E-03	5.357E-03	b
FCF4	0.0114	6.212E-03	a
SCF5	5.723E-03	3.680E-03	b
FCF5	0.0123	6.247E-03	a
SCF6	5.971E-03	3.443E-03	b
FCF6	0.0141	7.278E-03	a
SCF7	6.205E-03	3.406E-03	b
FCF7	0.0176	9.957E-03	a
SCF8	8.579E-03	0.0101	b
FCF8	0.0242	0.0162	a
SCF9	7.792E-03	0.0102	b
FCF9	0.0261	0.0158	a
SCF10	5.827E-03	2.297E-03	b
FCF10	0.0299	0.0175	a

SCM1	4.521E-03	2.437E-03	a
FCM1	9.618E-03	0.0148	a
SCM2	8.315E-03	0.0136	a
FCM2	0.0158	0.0328	a
SCM3	0.0143	0.0348	a
FCM3	0.0255	0.0382	a
SCM4	9.888E-03	5.461E-03	b
FCM4	0.0320	0.0440	a
SCM5	0.0128	7.736E-03	b
FCM5	0.0432	0.0626	a
SCM6	0.0141	8.572E-03	b
FCM6	0.0595	0.0865	a
SCM7	0.0175	0.0111	b
FCM7	0.0885	0.1504	a
SCM8	0.0226	0.0165	b
FCM8	0.1557	0.2605	a
SCM9	0.0365	0.0300	b
FCM9	0.3197	0.5524	a
SCM10	0.0556	0.0574	b
FCM10	0.5872	0.9392	a
SBF1	4.615E-03	3.264E-03	b
FBF1	8.716E-03	8.359E-03	a
SBF2	6.696E-03	5.386E-03	b
FBF2	0.0157	0.0156	a
SBF3	0.0139	0.0128	b
FBF3	0.0364	0.0311	a
SBF4	0.0233	0.0240	b
FBF4	0.0706	0.0599	a
SBF5	0.0364	0.0422	b
FBF5	0.1253	0.1112	a
SBF6	0.0488	0.0670	b
FBF6	0.2095	0.2293	a
SBF7	0.0720	0.1047	b
FBF7	0.3886	0.4217	a
SBF8	0.1214	0.1887	b
FBF8	0.6377	0.7272	a

SBF9	0.1906	0.2896	b
FBF9	1.1397	1.2667	a
SBF10	0.3555	0.7401	b
FBF10	2.0246	2.1131	a
SBM1	5.195E-03	3.038E-03	b
FBM1	0.0107	0.0118	a
SBM2	6.670E-03	3.854E-03	b
FBM2	0.0189	0.0244	a
SBM3	0.0124	8.103E-03	b
FBM3	0.0370	0.0429	a
SBM4	0.0220	0.0152	b
FBM4	0.0811	0.0859	a
SBM5	0.0364	0.0315	b
FBM5	0.1945	0.1565	a
SBM6	0.0445	0.0368	b
FBM6	0.2685	0.2033	a
SBM7	0.0657	0.0487	b
FBM7	0.4736	0.3536	a
SBM8	0.1366	0.1045	b
FBM8	0.9739	0.6599	a
SBM9	0.2290	0.1649	b
FBM9	2.0430	1.3388	a
SBM10	0.3880	0.2703	b
FBM10	4.0731	1.8671	a
SCTL1	3.856E-03	2.066E-03	a
FCTL1	5.636E-03	4.739E-03	a
SCTL2	6.842E-03	3.610E-03	a
FCTL2	0.0183	0.0393	a
SCTL3	0.0170	0.0102	a
FCTL3	0.0244	0.0252	a
SCTL4	0.0257	0.0168	b
FCTL4	0.0568	0.0823	a
SCTL5	0.0575	0.0456	b
FCTL5	0.1248	0.0980	a
SCTL6	0.0779	0.0672	b
FCTL6	0.2255	0.1752	a

SCTL7	0.1014	0.0933 b
FCTL7	0.3353	0.2493 a
SCTL8	0.1627	0.1308 b
FCTL8	0.7350	0.5447 a
SCTL9	0.3151	0.4087 b
FCTL9	1.3624	1.0257 a
SCTL10	0.4140	0.2857 b
FCTL10	2.4123	1.6297 a

B.4 Colony area distribution of reporters given bladder medium from Mink Frogs

Controls			
Object # (automated counts assign each colony a number)	Area ($mm^{2)}$	Reporters associated with bladder medium from female frogs from control sites	
Obj.#	Area	Number of Colonies detected	Area ($mm^{2)}$
10	0.01		
27	0.02	16	0
6	0.03	4	0.01
5	0.04	0	0.02
6	0.05	1	0.03
4	0.06	1	0.04
5	0.07	1	0.05
3	0.08	0	0.06
2	0.09	1	0.07
13	0.1	1	0.08
9	0.2	1	0.09
5	0.3	5	0.1
4	0.4	14	0.2
4	0.5	6	0.3
5	0.6	11	0.4
4	0.7	6	0.5
3	0.8	6	0.6
4	0.9	7	0.7
18	1	3	0.8
20	2	4	0.9
9	3	27	1
4	4	26	2
4	5	16	3
7	6	11	4
7	7	7	5
2	8	12	6
3	9	7	7
3	10	8	8
3	11	5	9
5	12	4	10
1	13	3	11
3	14	5	12
2	15	2	13
2	16	4	14
1	17	4	15
3	18	1	16
3	19	4	17
	20	2	18

1	21	5	19
	22	4	20
1	23	0	21
1	24	1	22
1	25	1	23
	26	1	24
	27	2	25
	28	0	26
	29	0	27
1	30	1	28
	31	1	29
	32	0	30
	33	0	31
		1	32
		0	33
		0	34
		6	35

bladder medium from male frogs from control sites.			
Number of colonies detected)	Area ($mm^{2)}$	Reporters associated with bladder medium from male frogs from contaminated sites	
# of colonies	Area (mm^2)	Number of Colonies detected	Area ($mm^{2)}$
0	0.01		0
0	0.02	1	0.01
0	0.03		0.02
0	0.04	1	0.03
0	0.05		0.04
1	0.06		0.05
0	0.07	1	0.06
9	0.08	1	0.07
8	0.09	1	0.08
48	0.1	2	0.09
30	0.2	4	0.1
8	0.3	8	0.2
8	0.4	4	0.3
3	0.5	8	0.4
4	0.6	7	0.5
6	0.7	14	0.6
5	0.8	8	0.7
3	0.9	8	0.8
27	1	12	0.9
14	2	42	1
19	3	17	2
11	4	10	3
5	5	11	4
2	6	10	5
5	7	3	6
2	8	1	7
9	9	4	8
4	10	1	9
4	11	2	10
3	12	2	11
3	13		12
1	14		13
3	15	1	14
2	16		15
3	17		16
1	18		17
2	19		18
3	20	1	19
1	21		20
1	22		21
3	23		22
0	24		23
1	25		24
2	26		25
2	27		26
1	28		27
0	29		28
0	30		29
0	31		30
1	32		31
1	33		32
0	34		33
3	35		34
1	36		35
			0
		1	0.01
			0.02

Reporters associated with bladder medium from female frogs from contaminated sites.	
Number of colonies detected)	Area ($mm^{2)}$
# of colonies	Area (mm^2)
0.001	
0.002	
0.003	
0.004	
0.005	
0.006	
0.007	
0.008	1
0.009	
0.01	
0.02	
0.03	
0.04	2
0.05	1
0.06	2
0.07	1
0.08	2
0.09	2
0.1	4
0.2	1
0.3	3
0.4	3
0.5	
0.6	
0.7	
0.8	
0.9	
1	1
2	
3	
4	
5	
6	
7	