Abstract

William B. Clark. DETERMINATION OF THE CYTOTOXICITY OF BORON SULFHYDRYL, BORONOPHENYLALANINE, AND BORIC ACID AND THEIR INTERACTIONS WITH γ-RAYS AND PROTONS IN V-79 CHINESE HAMSTER CELLS. (Under the guidance of Dr. James B. Robertson) Department of Biology East Carolina University. September 1997.

The cytotoxicities and γ-ray interactions of three ^{10}Boron (^{10}B) enriched compounds used in boron neutron capture therapy (BNCT) were tested with V-79 cells. Boron sulfhydral (BSH), borono-phenylalanine (BPA), and boric acid were found to show no toxicity at 30 and 60 ppm of ^{10}B. At 120 ppm, ^{10}B toxicity was observed with boric acid and BSH, but not with BPA. The survival of cells exposed to γ-rays in the presence of boric acid, BSH, and BPA were compared to survival of cells exposed to γ-rays alone. The results show that there is no significant difference in survival between V-79 cells exposed to γ-rays or to γ-rays with 100 ppm ^{10}B of BPA. There is a difference in the survival of V-79 cells exposed to γ-ray and V-79 cells exposed to γ-rays and 100 ppm ^{10}B of boric acid and BSH. These experiments were controls for use with the proton beam at Loma Linda University Medical Center in California. High energy protons collide with atomic nuclei as they travel through matter releasing secondary neutrons. The amount of secondary neutron capture by BPA in V-79 cells exposed to the proton beam did not significantly increase the biological effectiveness of the proton beam.

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List of Symbols and Abbreviations

BNCT-----Boron neutron capture therapy

γ-rays-----gamma rays

BSH-----Boronosulfhydral

BPA-----Boronophenylalanine

$^{7}Li^{+3}$-----Lithium

$^{4}He^{+2}$-----Helium, alpha particle, or α

Introduction

A. Boron Neutron Capture Therapy

Boron neutron capture therapy (BNCT) is a treatment for killing neoplastic tissue involving ^{10}B localized in the tumor cell and thermal neutron radiation. The ^{10}B captures the thermal neutron (0.025 eV) and instantaneously splits into $^{7}Li^{3+}$ and $^{4}He^{2+}$, α-particle (SEE FIGURE 1 and 2). The $^{7}Li^{3+}$ and $^{4}He^{2+}$ particles are direct ionizing particles that only travel the distance of one cell diameter, 7-10 μm, killing the tumor cell and sparing the normal tissue (Locher, 1936). In the past sixty years, considerable research addressed the development of boron compounds capable of delivering ^{10}B to tumor cells and into the development of neutron sources.

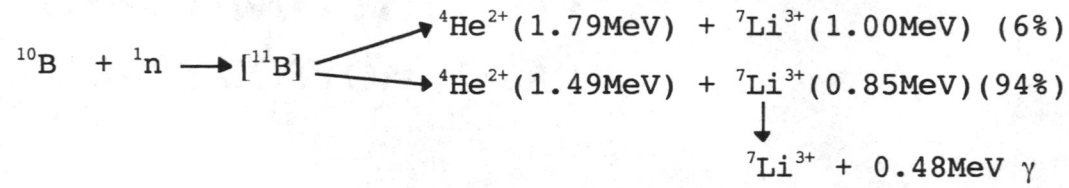

Figure 1. The $^{10}B(n,\alpha)$ ^{7}Li reaction. The n is the neutron. The ^{4}He, α-particle, and the ^{7}Li are high LET particles. Both high LET particles travel only the distance of a cell diameter (Kitao, 1975).

B. Advantage of BNCT over Conventional Cancer Therapy

Boron neutron capture therapy for neoplastic tissue was designed as an alternative to conventional surgery,

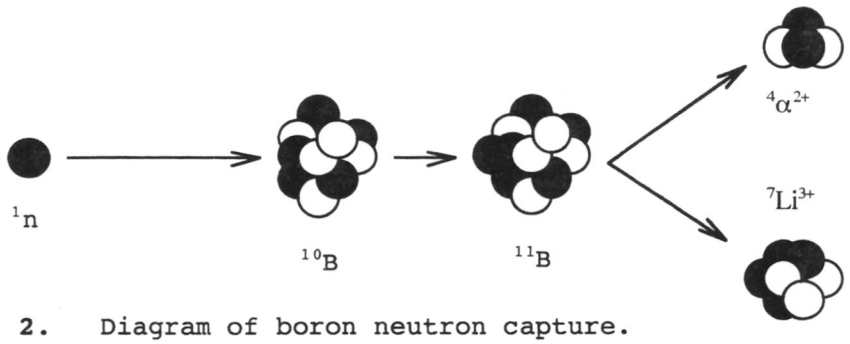

Figure 2. Diagram of boron neutron capture.

chemotherapy, and radiotherapy treatment for glioblastoma multiforme brain tumors. Theoretically, the advantage of BNCT is the localized dose of radiation due to the selective uptake of ^{10}B by tumor cells, and the short range particles generated from the neutron capture reaction, $^{7}Li^{3+}$ and $^{4}He^{2+}$. These are direct ionizing particles and are more biologically effective than indirect ionizing radiation, neutrons, γ-rays and x-rays.

The localization of ^{10}B in tumor cells is an advantage of BNCT. Tumor cells are dividing more rapidly than normal cells, especially in the brain. Cells that are dividing more rapidly have a higher demand for amino acids, phospholipids, nucleotides, and other molecules. The ^{10}B is attached to a molecule selectively localized in the tumor cells. Ideally, the ^{10}B compound is localized in the nucleus. After neutron capture, the direct ionizing particles are generated within the tumor cell increasing the chances of a collision with DNA resulting in cell death. The direct ionizing particles

travel 7-10 μm irradiating neoplastic tissue and sparing normal tissue (Barth *et al.*, 1990).

There are two types of radiation delivered in BNCT, direct ionizing particles and indirect ionizing particles. The direct ionizing particles, $^{7}Li^{3+}$ and $^{4}He^{2+}$, are heavy charged particles. As the heavy charged particles travel through tissue in a straight line, they will lose kinetic energy. The loss of energy is due to ionizations and excitations of electrons within the matter they penetrate. This phenomenon is referred to as linear energy transfer (LET). When heavy charged particles reach the end of their path, they gain electrons and come to rest at a neutral charge. A single 5-MeV α-particle interacts with matter 1.4 x 10^{5} times in 1200 μm before reaching a resting state (Lapp and Andrews, 1972). The high LET of direct ionizing particles to DNA causes double strand breaks leading to cell death in tumor cells. As indirect ionizing particles, γ-rays, x-rays and neutrons, travel through matter they may pass through without a collision or they may interact with one atom at a time. They are considered low LET radiations. An indirect ionizing particle colliding with DNA will usually cause a single strand break. A single strand break of DNA is more likely to be repaired than a double strand break. Thus, the products of BNCT are more biologically effective than indirect ionizing radiation.

C. Boron Compounds

Since before the times of the Roman Empire, borax ($Na_2B_4O(0H)_2$ -8H2O), a boron containing ore, has been used as a detergent and as an internal medicine (Pfeiffer and Jenny, 1950). Borax is still used as a detergent, under the brand name known as "20 Mule Team." Boron has been incorporated into a wide variety of organic molecules for internal use because of its similar structure to carbon (Spielvogel, 1995).

Boron is found next to carbon on the periodic table and has two naturally occurring isotopes, ^{11}B (80%) and ^{10}B (20%). Many types of boron compounds can be easily synthesized because boron is similar in stability and covalent chemistry to carbon. Boron has five electrons in its valence shell. Carbon has four electrons in its valence shell. B and C can bind four hydrogens. However, borohydride (BH_4^-) has a negative charge and methane (CH_4) has a neutral charge (SEE FIGURE 3). Boron can be incorporated into a borazene ring (SEE FIGURE 3), amino acids, nucleotides, and phospholipids in the place of carbon. Boronated biomolecules have been used in the treatment of high cholesterol, osteoporosis, and BNCT for cancer (Spielvogel, 1995).

^{10}Boron was proposed for neutron capture therapy by Locher in 1936. Boron is the ideal element for neutron capture because of its high cross section for thermal neutron capture (SEE TABLE 1) and the low abundance of ^{10}B *in vivo*,

relative to N, C, H, O, and P. The cross section for capture of ^{10}B is dependent on the structure of its nucleus not the size of its nucleus (Locher, 1936).

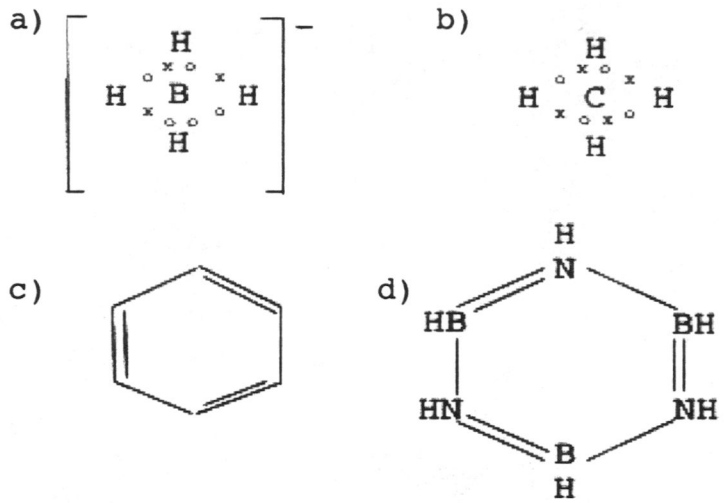

Figure 3. Diagram of a)borohydride b)methane c)benzene d)borazene. (Spielvogel, 1995)

Table 1

Thermal Neutron Capture Cross-Section Values

Nuclide	Neutron Capture Cross Section in barns*
H	0.332
C	0.0034
N	1.82
O	0.00018
P	1.16
^{10}B	3,838
^{7}Li	942

* barns ($b=10^{-24}cm^2$)
(Barth *et al.*, 1990).

There are three considerations in determining the usefulness of new ^{10}boronated molecules for neutron capture therapy: 1) low toxicity 2) selective uptake by tumors, a tumor:normal tissue concentration of ^{10}B of at least 5:1 ^{10}B (Fairchild *et al.*, 1990) and at least 10^9 atoms ^{10}B per cell, 50 μg ^{10}B/g tumor (Fairchild *et al.*, 1985), and 3) photosensitizing potential in humans (Barth *et al.*, 1990).

Early boronated compounds did not meet any of the criteria necessary for BNCT. Borax and boric acid derivatives were the first ^{10}B compound used clinically for BNCT by Sweet from 1951-1962. These compounds were taken into the brain tumors by passive diffusion due to a break down in the blood brain barrier. However, they were not retained in the tumor. The leaking of ^{10}B into the brain's vasculature caused vascular damage during neutron radiation evident during autopsy (Asbury *et al.*, 1972). Since Sweets' early experiments, improvements have been made decreasing the toxicity and increasing the uptake and retention of boronated molecules in tumor tissue for BNCT. The newer molecules are BSH, BPA, bi-specific monoclonal antibody, nucleotides, porphyrins, and low-density lipoproteins.

Boron sulfhydryl, BSH, (SEE FIGURE 4) has been used with some success in clinical trials by Hatanaka. The mean survival of patients treated with BSH and thermal neutron radiation in 1968 was 19.2 months compared to a mean of 12.9 months for patients receiving chemotherapy, immunotherapy,

and photon irradiation (Hatanaka, 1986). An animal study using BSH and rat F98 anaplastic glioma cells as a model for brain tumors, found a 99.9% reduction in tumor growth (Clendenonn *et al.*, 1990). Although there has been success using BSH in clinical trials and animal trials, BSH has a short half-life in tumors and a low tumor:blood ratio. The mean concentrations of ^{10}B in dogs after exposure to 55 mg BSH/kg body weight were 30.6 μg boron/g tumor after two hours and 2.9 μg boron/g tumor after six hours. The concentrations of ^{10}B in the blood, liver, and kidney after two hours were 65.4 μg boron/g, 115.7 μg boron/g, and 101.5 μg boron/g, respectively (Kraft *et al.*, 1994). The tumor:blood concentration ratio of BSH in humans is 1.69 (Hatanaka, 1986).

Boronophenylalanine, BPA, (SEE FIGURE 5) is a boronated derivative of the amino acid phenylalanine. Accumulation of BPA in melanoma cells is due to the binding of BPA to L-DOPA. L-DOPA is selectively taken into melanoma cells as a precursor to melanin. The BPA-L-DOPA complex accumulates in the melanoma cell. In the melanoma cell, the BPA-L-DOPA complex is oxidized to form boric acid and phenylalanine from BPA and dopaquinone from L-DOPA. The phenylalanine is retained in the cell as an amino acid but the boric acid is free to diffuse into the blood (Yoshino *et al.*, 1993). *In vitro*, BPA accumulates in B-16 melanoma cells as much as eleven times the media concentration. Neutron irradiations

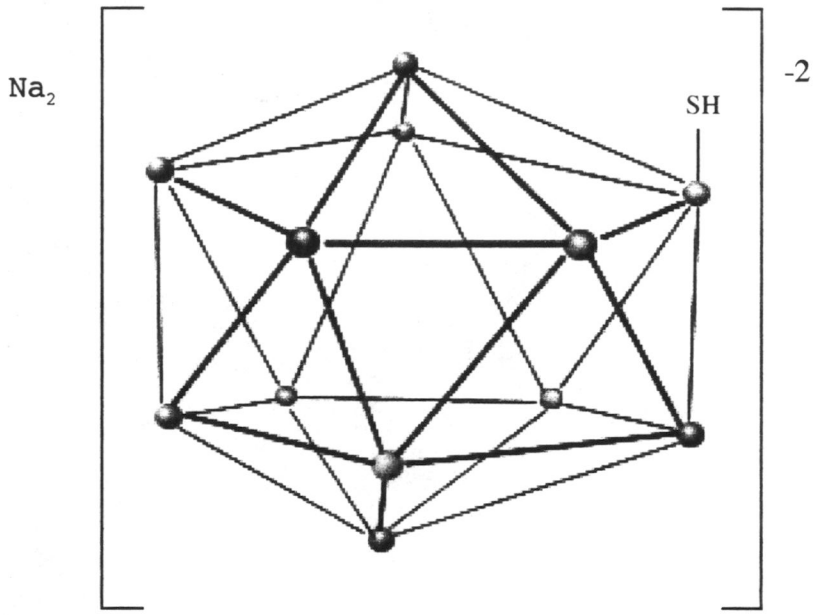

Figure 4. Diagram of BSH. ^{10}B is represented by circles. $Na_2{}^{10}B_{12}SH$

with BPA and B-16 melanoma cells were more biologically effective than neutrons alone (Ichihashi *et al.*, 1982). Balb/c mice with subcutaneous Harding-Passey melanoma were injected with BPA. When the mice were irradiated with high doses of neutrons the tumors stopped growing and completely regressed (Coderre *et al.*, 1988). Tumor:blood ratio of BPA in glioblastoma multiforme has been shown to be approximately 3.5 with a tumor concentration of 3.2-64.5 μg ^{10}B/g (Elowitz *et al.*, 1996). Mishima and colleagues have had success treating several patients with malignant tumors with BPA and neutron radiation (Mishima *et al.*, 1989). Although BPA has

been shown to be effective *in vitro* and results from clinical trials are encouraging, the real success of BNCT will be with the next generation of boronated molecules.

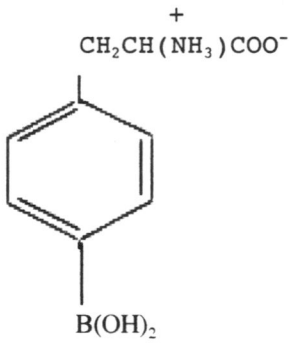

Figure 5. Diagram of BPA. $C_9H_{12}NO_4{}^{10}B$.

The next generation of boronated biomolecules include low-density lipoprotein (LDL), monoclonal antibodies, and nucleotides generated by using more advanced techniques. Low-density lipoproteins accumulate in tumor by receptor mediated endocytosis as high as 240 μg ^{10}B/g tumor. *In Vitro*, the relative biological effectiveness of LDL is ten times greater than BSH (Laster *et al.*, 1991).

D. Neutron Sources

Thermal neutrons were first proposed for BNCT by Locher in 1936. Thermal neutrons deposit little energy in normal tissue, but have a high probability of reacting with ^{10}B. Thermal neutrons are most effective for boron neutron capture because higher energy neutrons may pass through the ^{10}B nucleus without being captured (Locher, 1936).

Neutrons are found in atomic nuclei and must be ejected from the nucleus by disrupting the nucleus with α-particles, protons, γ-rays, or other neutrons. Therefore, the clinical source of neutrons is a nuclear reactor. Neutrons of several energies are generated in nuclear reactors due to the disruption of the nucleus by fission and atomic collisions (SEE TABLE 2)(Locher, 1936). The neutrons generated from a nuclear reactor must be filtered to obtain thermal neutrons. Thermal neutrons lose energy rapidly in tissue. Thermal neutrons only penetrate tissue 3-4 cm. In clinical studies with BNCT and thermal neutrons, the scalp and cranium need to be reflected to expose deep seated tumors to thermal neutron radiation (Mishima *et al.*, 1989).

Since thermal neutrons cannot expose deep seated glioblastoma multiforme to neutron irradiation without surgery, research has gone into developing a neutron source with the ability expose the tumor to thermal neutrons. The most promising source of neutrons for BNCT is epithermal neutrons (1eV) generated from nuclear reactors. As

epithermal neutrons pass through tissue, they lose kinetic energy by colliding with hydrogen and slow to become thermal neutrons. The thermal neutrons are generated within the tissue. These thermal neutrons can reach deep seated tumors. (Barth *et al.*, 1990). Fast neutron beams (>1x10^{7}eV) are a possible source of neutrons to use with BNCT. Fast neutron beams alone have already been shown to be effective in clinical trials treating salivary gland (Griffin *et al.*, 1988) and prostate tumors (Laramore *et al.*, 1993).

Table 2

Energies of neutrons

Type of neutron	Energy
High energy	>1 x 10eV
Fast	1,000eV-10,000,000eV
Intermediate	100eV-1x10^{4}eV
Slow	3.0x10^{-2}eV-100eV
Epithermal	~1eV
Thermal	2.5x10^{-2}eV

Table 2. Energies of various neutrons created in nuclear reactors. (Lapp and Andrews, 1972)

Although neutrons with higher energies than thermal neutrons may pass through ^{10}B without being captured, they have already been shown to be effective treating neoplastic

tissue. Neutrons of higher energies than thermal neutrons slow down to become thermal neutrons as they pass through tissue. A small increase in biological effectiveness due to boron capture of thermal neutrons generated in the tissue would be significant in treating neoplastic tissue with fast neutrons.

E. Possibilities with Protons

The purpose of this study is to determine the efficacy of supplementing proton beam therapy for cancer with BNCT. Although protons are direct ionizing particles, they interact with matter mostly as low LET particles and partially as high LET particles. Up to ninety percent of the energy delivered to tissue by the proton beam are as low LET interactions. Protons behave like low LET particles because of their small mass and small charge relative to heavy charged particles. Their small mass and charge allow protons to travel through tissue with relatively few ionizing interactions. However, protons do behave like high LET as they come to a stop. As the protons slow down, they transfer all of their energy to the tissue. The point where protons deliver their energy and stop is called the Bragg peak. There is no energy delivered by protons past the Bragg peak. The distance the protons travel through tissue before they reach the Bragg peak is determined by the initial energy of the protons. By controlling the energy of protons, shallow or deep seated

tumors can be irradiated sparing normal tissue (Personal Communication with Dr. Robertson).

Proton beam therapy for neoplastic tissue is more effective than γ-ray treatment for neoplastic tissue. As γ-rays travel through tissue, they deliver most of their dose just beneath the skin. Therefore, normal tissue under the skin receives more radiation than deep seated tumors. The amount of dose delivered declines all the way through the tissue. In contrast, the proton beam delivers little dose to the normal tissue shallower than the tumor, most of the dose to the tumor, and no dose past the tumor at the Bragg peak. Delivering the most dose to the tumor is important in prostate and brain tumors because of the critical nature of the surrounding tissues (Personal Communication with Dr. Robertson).

The proton beam may prove to be biologically effective as a source for BNCT for two reasons. First, as protons travel through tissue the protons generate secondary protons and neutrons by colliding with atomic nuclei. The proton beam generates neutrons of all energies as the protons travel through tissue (SEE TABLE 3). Some of these neutrons may be captured by ^{10}B in tumor cells and increase the biological effectiveness of the proton beam. Second, the proton beam is an effective clinical tool in treating neoplastic tissue. A small increase in biological effectiveness of the proton beam due to boron capture of secondary thermal neutrons generated

in the tissue would be significant in treating neoplastic tissue (Personal Communication with Dr. Robertson).

Table 3

Energy generated by direct and secondary products of proton beam in water.

Ionizing particle	% total dose
Direct protons	70%
Secondary protons	20%
Secondary neutrons	6%
Other	4%

(Personal Communication with Jeff Siebers)

F. Purpose

Cytotoxicities and γ-ray interactions of three ^{10}B enriched compounds commonly used for BNCT were tested at East Carolina University as controls for use with the proton beam at Loma Linda Medical University. γ-ray experiments are used as a control for the proton beam experiments because 80%-90% of the dose delivered by protons is low LET. The γ-ray experiments were used as controls to evaluate any radiation-sensitizing effects of boric acid, BSH, and BPA not a result of boron neutron capture. If the regression lines for the group exposed to γ-rays is the same as the regression line

for the group exposed to γ-rays and ^{10}B, then the null hypothesis, there is no interaction between γ-rays and ^{10}B, is accepted. If the regression lines are different, then the null hypothesis is rejected and the alternate hypothesis, there is an interaction between γ-rays and ^{10}B, must be considered. (Personal Communication with Dr. Robertson).

The aim of this study was to make some preliminary investigations into the efficacy of combining proton beam therapy and BNCT. If the regression lines for the group exposed to protons is the same as the regression line for the group exposed to protons and ^{10}B, then the null hypothesis, there is no interaction between protons and ^{10}B, is accepted. If the regression lines are different, then the null hypothesis is rejected and the alternate hypothesis, there is an interaction between protons and ^{10}B, must be considered.

Since γ-rays do not generate neutrons, the controls show the interaction between low LET and ^{10}B. The proton beam experiments show the interaction between protons and ^{10}B. Because the proton beam interacts with matter as low LET and generates secondary neutrons, any significant interaction observed between the proton beam and ^{10}B not observed with γ-rays must be due to BNC. If the null hypothesis is accepted for γ-ray experiments and rejected for proton experiments, then the interaction observed must be due to boron neutron capture and coupling proton beam therapy with BNCT may have clinical possibilities.

Materials and Methods

A. Cell Line

Chinese hamster lung fibroblast, V-79, cells in logarithmic growth were maintained in Eagles MEM (Cellgro stock # 50-010-PB) supplemented with 10% fetal bovine serum, (Hyclone lot # 1111946) 100 units penicillin/L (Sigma stock # P-7539), 40 μg streptomycin/L (Sigma stock # P-7539), and 400 μM L-glutamine (Sigma stock # G-7513).

B. Boron Solutions

All ^{10}B compounds were generously donated by Boron Biologicals Incorporated of Raleigh, NC. All solutions of ^{10}B compounds were prepared in ppm ^{10}B.

Boric acid, $H_3{}^{10}BO_3$ (M.W.=61), went into solution with filter sterilized and autoclaved water. Solutions were made up at concentrations of 300 ppm, 600 ppm, 1000 ppm, and 1200 ppm of ^{10}B. The stock solutions were vacuum sterilized with a 0.22 μm filter. The four stock solutions were diluted 1:10 with media to yield ^{10}B concentrations of 30 ppm, 60 ppm, and 120 ppm ^{10}B for cytotoxicity experiments and 100 ppm ^{10}B for irradiation experiments.

Boron sulfhydral, $Na_2{}^{10}B_{12}SH$ (M.W.=199), went into solution in Hanks balanced salts solution (HBSS). Solutions were made up at concentrations of 300 ppm, 600 ppm, 1000 ppm, and 1200 ppm of ^{10}B. The stock solutions were vacuum sterilized with a 0.22 μm filter. Due to the possibility of

BSH oxidizing with O_2 in the air the BSH solution was bubbled with compressed N_2 gas for at least ten minutes after the bottle was opened (Personal Communication with Dr. Dave Spielvogel). The four stock solutions were diluted 1:10 with media to yield ^{10}B concentrations of 30 ppm, 60 ppm, and 120 ppm ^{10}B for cytotoxicity experiments and 100 ppm ^{10}B for irradiation experiments.

Boronophenylalanine, $C_9H_{12}NO_4{}^{10}B$ (M.W.=208) is highly insoluble in HBSS and water. Therefore, a BPA-fructose complex was made to increase the solubility of BPA in HBSS. The BPA-fructose complex was made by adding equimolar amounts of BPA and fructose, $C_6H_{12}O_6$ (M.W.=180), to sixty-five percent of the total desired volume HBSS. This solution was stirred slowly and the pH was brought up to 9.0-9.5 dropwise, with 10M NaOH. The solution stirred for one hour. The pH was brought up to 9.5-10 with 10M NaOH and allowed to stir thirty minutes. The pH was slowly adjusted with 3N HCl to 7.4. The solution was brought up to full volume with HBSS and stored at 4°C overnight. The solution was vacuum sterilized with a 0.22 μm filter and poured into sterile bottles. Stock solutions of BPA were made at 1200 ppm ^{10}B for cytotoxicity and 1000 ppm ^{10}B for irradiations. The 1200 ppm ^{10}B-BPA stock solution was diluted with HBSS into 300 ppm and 600 ppm ^{10}B-BPA stock solutions. The four stock solutions were diluted 1:10 with media to yield ^{10}B concentrations of 30 ppm, 60 ppm,

and 120 ppm ^{10}B for cytotoxicity experiments and 100 ppm ^{10}B for irradiation experiments.

C. Cytotoxicity Experiments

Two days before the experiment (T=0) V-79 cells in log phase were washed twice with HBSS, trypsinized, diluted in MEM, and counted by hemocytometer. Approximately 1.5 x 10^5 cells were added to the control and experimental flasks, 25 cm^2 Corning tissue culture flasks. The media was aspirated at T=24hr and 5 ml of 0 ppm ^{10}B media was added to the control flask. In a similar manner, 5 ml of 30 ppm, 60 ppm, and 120 ppm ^{10}B media was added to the experimental flasks. At T=48hr all flasks were aspirated, washed two times with HBSS, trypsinized, counted, diluted with 0, 30, 60, and 120 ppm ^{10}B media and plated into three 75 cm^2 petri dishes for each dose. The cells were allowed to attach for six hours. The total exposure was 30 hours. After the cells attached, the 0, 30, 60, and 120 ppm ^{10}B media was aspirated and replaced with fresh media. The cells were allowed to form colonies for seven days. After seven days, the surviving colonies were washed twice with 0.9% NaCl solution, fixed with 1:3 (v/v) acetic acid/methanol solution, and stained with 0.5% crystal violet solution. The colonies were counted. Colonies of greater than fifty members were counted as survivors.

The first ^{10}B cytotoxicity experiments for each compound used 200, 2000, and 20000 cells per dish to ensure some survivors. In all cases, large numbers of survivors were

observed and the following three replicates used about 300 cells per dish.

D. γ Irradiations and Proton Irradiations

Two days before the experiment (T=0) V-79 cells in log phase were washed twice with HBSS, trypsinized, diluted in MEM, and counted by hemocytometer. Approximately 1.5 x 10^5 cells were added to the control and experimental flasks, 25 cm^2 Corning tissue culture flasks. The media was aspirated at T=24hr and 5 ml of 0 ppm ^{10}B media was added to the control flask and 100 ppm ^{10}B media was added to the experimental flask. At T=45hr both flasks were aspirated, washed twice with HBSS, trypsinized, diluted in 0 ppm ^{10}B media and 100 ppm ^{10}B media, and put into three 25 cm^2 flasks for each dose. The cells were allowed to attach for four hours. At T=50, the flasks were filled with 1% media at 0 ppm and 100 ppm ^{10}B. The flasks were irradiated at the East Carolina University Medical School with the cobalt^{60} γ-irradiator at 0, 200, 400, 600, 800, and 1000 rads. After all irradiations, the flasks were brought back to lab and the 1% FBS media was removed. Total exposure was 30 hours. The 1% media was replaced with fresh 10% FBS media at 0 ppm ^{10}B. The cells were allowed to form colonies for seven days. After seven days, the surviving colonies were washed twice with 0.9% NaCl solution, fixed with 1:3 (v/v) acetic acid/methanol solution, and stained with 0.5% crystal violet solution. The colonies were

counted. Colonies of greater than fifty members were counted as survivors.

E. Data Analysis

Plating efficiency (PE) was determined by counting the three flasks in each experiment with no treatment. The survivors of the three flasks were averaged and divided by the number of cells plated based on serial dilutions multiplied by 100. Plating efficiency varied from 27-88%.

Survival (S/So) was determined by multiplying the inverse of the PE (1/0.6) by the inverse of the cells plated based on serial dilutions (1/300) by the number of colonies observed (153) equals the normalized (N) S/So (0.85) for the flask (1/0.6 x 1/300 x 153 = 0.85). The S/So values for three flasks at each dose were averaged and plotted on semi-log paper with standard deviations (S/So vs. Dose). The PE, cells plated, cells observed, N values, means, and standard deviations can be found in Appendix I-VII for both cytotoxicity and γ-ray experiments.

Survival curves due to γ-rays were exponential. To transform the survival curves to linear relationships we used the following formula:

$$S/So = e^{-(\alpha D + \beta D\text{^}2)} \qquad D = \text{Dose(Rads)}$$

$$\ln S/So = -(\alpha D + \beta D^2) \qquad \beta = \text{constant}$$

$$\frac{-\ln S/So}{D} = \alpha + \beta D \qquad \alpha = \text{constant}$$

The transformed equation is the same as the equation of a line, $y=b + mx$. In this case $y=-\ln S/So/D$, $b=\alpha$ and $m=\beta$.

The null hypothesis is: the regression line for γ-rays or the proton beam is the same as the regression line for γ-rays or proton beam and boric acid, BSH and BPA. The null hypothesis was tested by using the two sample t test for comparing two straight regression lines (Kleinbaum and Kupper, 1978). The t values were calculated by subtracting the means ($\alpha_1-\alpha_2$ or $\beta_1-\beta_2$) and dividing by the difference of the respective standard deviations. Probabilities were then calculated corresponding to the t test values.

Results

A. Cytotoxicity

The cytotoxicity of boric acid to V-79 cells was measured at 30, 60, and 120 ppm ^{10}B. Boric acid was not toxic at 30 and 60 ppm ^{10}B. Boric acid was toxic at 120 ppm ^{10}B with 71% survivors (SEE TABLE 4, FIGURE 6, and Appendix I).

The cytotoxicity of BSH was measured at 30, 60, and 120 ppm ^{10}B. BSH was not toxic at 30 and 60 ppm ^{10}B. BSH was toxic at 120 ppm ^{10}B with 69% survivors (SEE TABLE 5, FIGURE 7, and Appendix II).

The cytotoxicity of BPA was measured at 30, 60, and 120 ppm ^{10}B. BPA was not toxic at any concentration tested. Cell cultures exposed to BPA had the same survival rate as control cell cultures (SEE TABLE 6, FIGURE 8, and Appendix III).

The data shown below in the tables and figures are the pooled data for the cytotoxicity experiments 2,3, and 4 for boric acid, BSH, and BPA. All of the data for the cytotoxicity experiments is shown in Appendix I, II, and III.

Table 4

Data from Boric Acid Cytotoxicity Pooled

ppm $^{10}B-H_3{}^{10}BO_3$	Mean	s.d.	+-s.d.
0	1.00	0.05	0.95-1.05
30	0.96	0.09	0.87-1.05
60	0.98	0.13	0.85-1.11
120	0.71	0.06	0.71-0.77

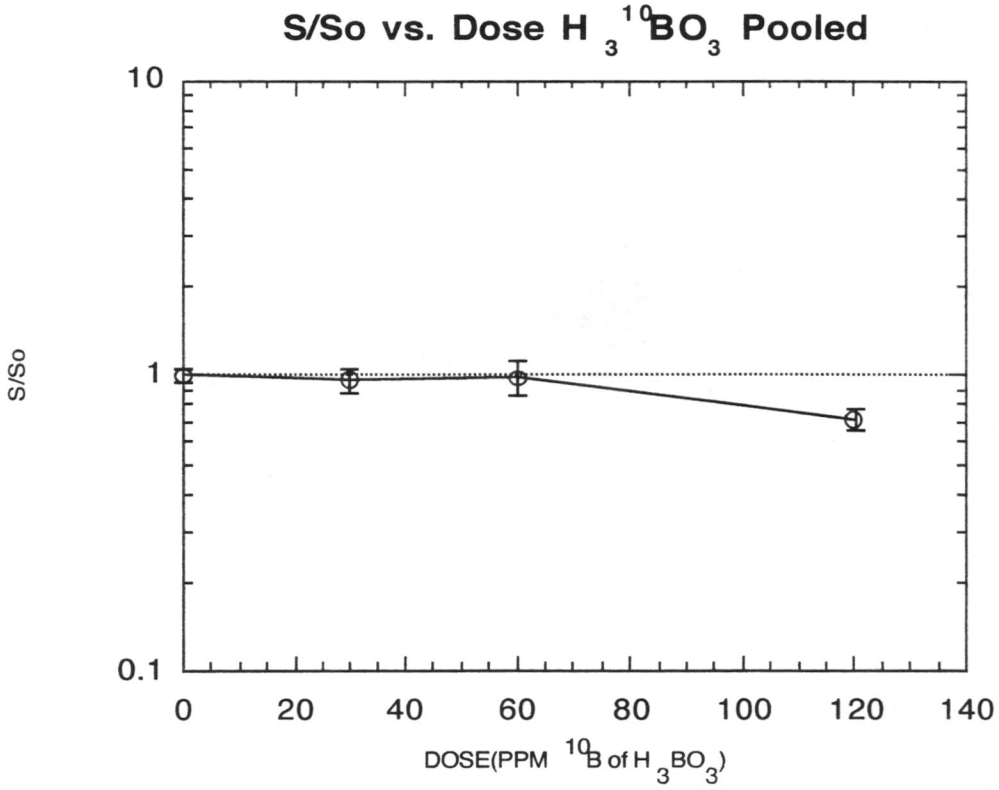

Figure 6. Graph of pooled data from experiments 2,3, and 4 S/So for boric acid cytotoxicity. There is no toxicity at 30 ppm and 60 ppm ^{10}B. The survival at 120 ppm ^{10}B is 0.71.

Table 5

Data from BSH Cytotoxicity Pooled

ppm ^{10}B-BSH	Mean	s.d.	+-s.d.
0	1.00	0.05	0.95-1.05
30	1.03	0.31	0.72-1.34
60	0.98	0.24	0.74-1.22
120	0.67	0.19	0.86-0.48

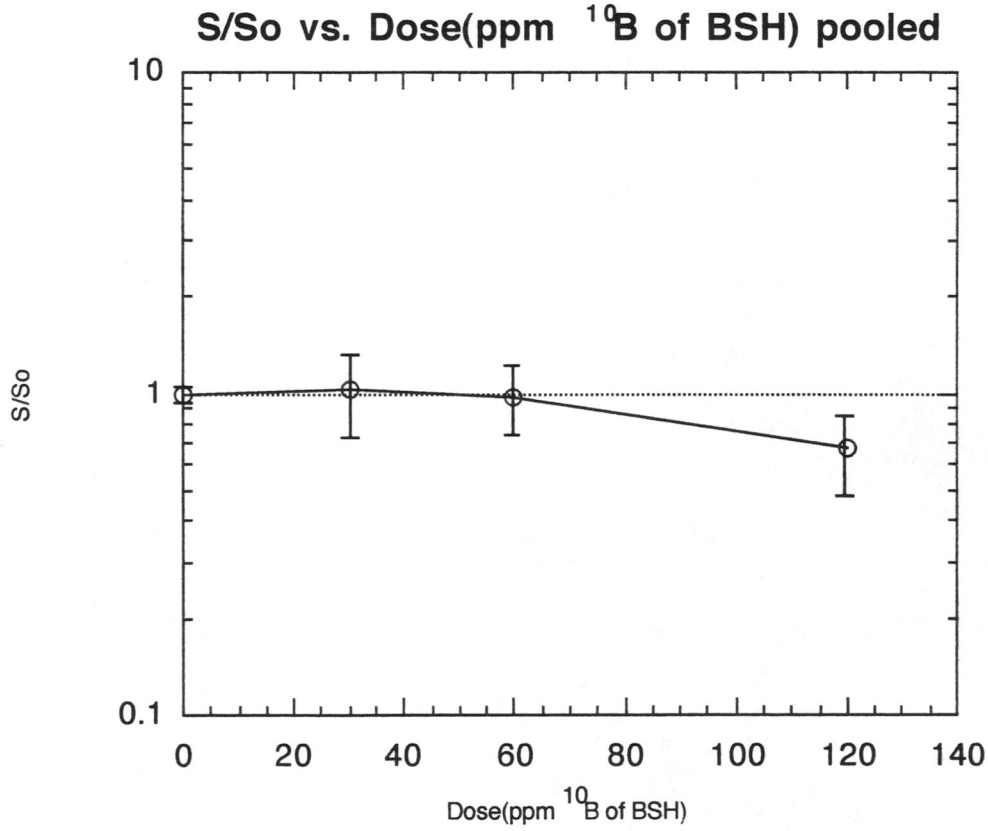

Figure 7. Plot of the pooled data for experiments 2,3, and 4 S/So for BSH cytotoxicity tests. There is no toxicity at 30 ppm and 60 ppm ^{10}B. The survival at 120 ppm ^{10}B is 0.69.

Table 6

Data from BPA Cytotoxicity Pooled

ppm ^{10}B-BSH	Mean	s.d.	+-s.d.
0	1.01	0.07	0.94-1.08
30	1.06	0.27	0.79-1.33
60	0.99	0.18	0.81-1.17
120	0.99	0.16	0.83-1.15

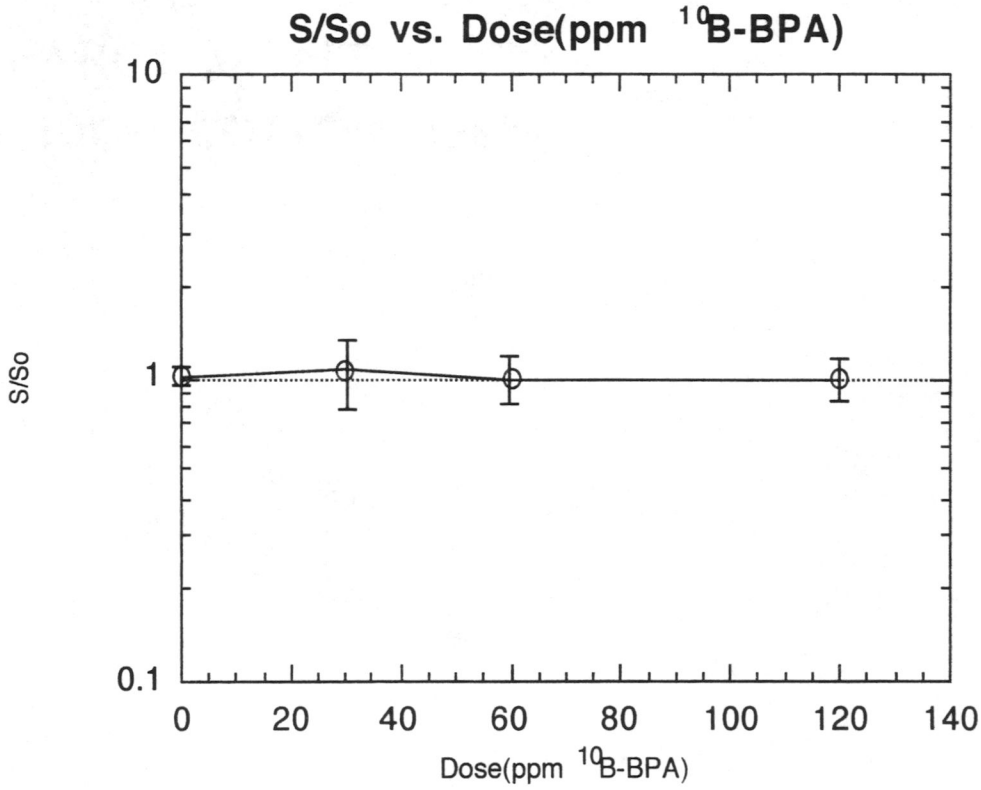

Figure 8. Plot of the pooled data for experiments 2,3, and 4 S/So for BPA cytotoxicity tests. There is no toxicity at any concentration.

B. 100 ppm ^{10}Boron γ Irradiations

The interaction of ^{10}B and γ-rays was measured as controls for ^{10}B and proton irradiations. The α, y-intercept, and β, slope, for γ-rays alone and γ-rays with boric acid, BSH, and BPA were compared using t ratios to determine if the regression lines were the same. If the regression lines are the same, then there is not a significant interaction between γ-rays and boric acid, BSH, and BPA.

B_1. Boric Acid and γ Irradiations

Cells were exposed to 100 ppm ^{10}B boric acid media 24 h before irradiation with γ-rays. The survival curves of these irradiations were compared to cells exposed to γ-rays without boric acid. The survival curves of pooled experiments are shown in FIGURE 9, TABLE 7, and Appendix IV. The two curves show a similar shape with the boric acid group showing less cell survival. The two survival curves were compared by transforming the mean survival (S/So) for each group of data at each radiation dose to fit a linear equation. A linear regression line was fit to each group. Results of the linear transformation are plotted in FIGURE 10 and tabulated in Appendix IV. The regression lines are:

γ-rays only

$-\ln S/So/D = 3.73\times10^{-4} + 3.11\times10^{-6}D$ ($\alpha=3.73\times10^{-4}$; $\beta= 3.11\times10^{-7}$)

γ-rays with 100 ppm ^{10}B boric acid

$-\ln S/So/D = 1.98\times10^{-3} + 3.75\times10^{-6}D$ ($\alpha=1.98\times10^{-3}$; $\beta= 3.75\times10^{-6}$)

The two regression lines were compared and the t value for α=-3.11 (P=0.0045) and the t value for β=0.82 (P=0.42). The results of the t-test showed the differences in the intercepts were significant and the differences in the slopes were not significant. Therefore, the intercepts are the different and the slopes are the same (SEE FIGURE 10).

Table 7

Pooled N(S/S0) for γ Irradiations for 100 ppm ^{10}B

	Dose					
Experiment	**0**	**200**	**400**	**600**	**800**	**1000**
γ only B.A.						
(Mean)	1.00	0.84	0.51	0.25	0.94	0.36
(s.d.)	0.07	0.07	0.03	0.01	0.013	0.009
γ + B.A.						
(Mean)	1.00	0.53	0.33	0.091	0.019	0.0036
(s.d.)	0.15	0.15	0.12	0.039	0.0096	0.0024
γ only BSH						
(Mean)	1.00	0.83	0.51	0.27	0.11	0.035
(s.d.)	0.13	0.1	0.05	0.05	0.03	0.008
γ + BSH						
(Mean)	1.00	0.61	0.43	0.17	0.079	0.036
(s.d.)	0.14	0.15	0.13	0.092	0.052	0.017
γ only BPA						
(Mean)	1.00	0.76	0.57	0.31	0.15	0.065
(s.d.)	0.12	0.11	0.10	0.048	0.025	0.016
γ + BPA						
(Mean)	1.00	0.69	0.53	0.31	0.14	0.06
(s.d)	0.09	0.14	0.082	0.057	0.029	0.014

Boric Acid and γ-ray experiments S/So pooled

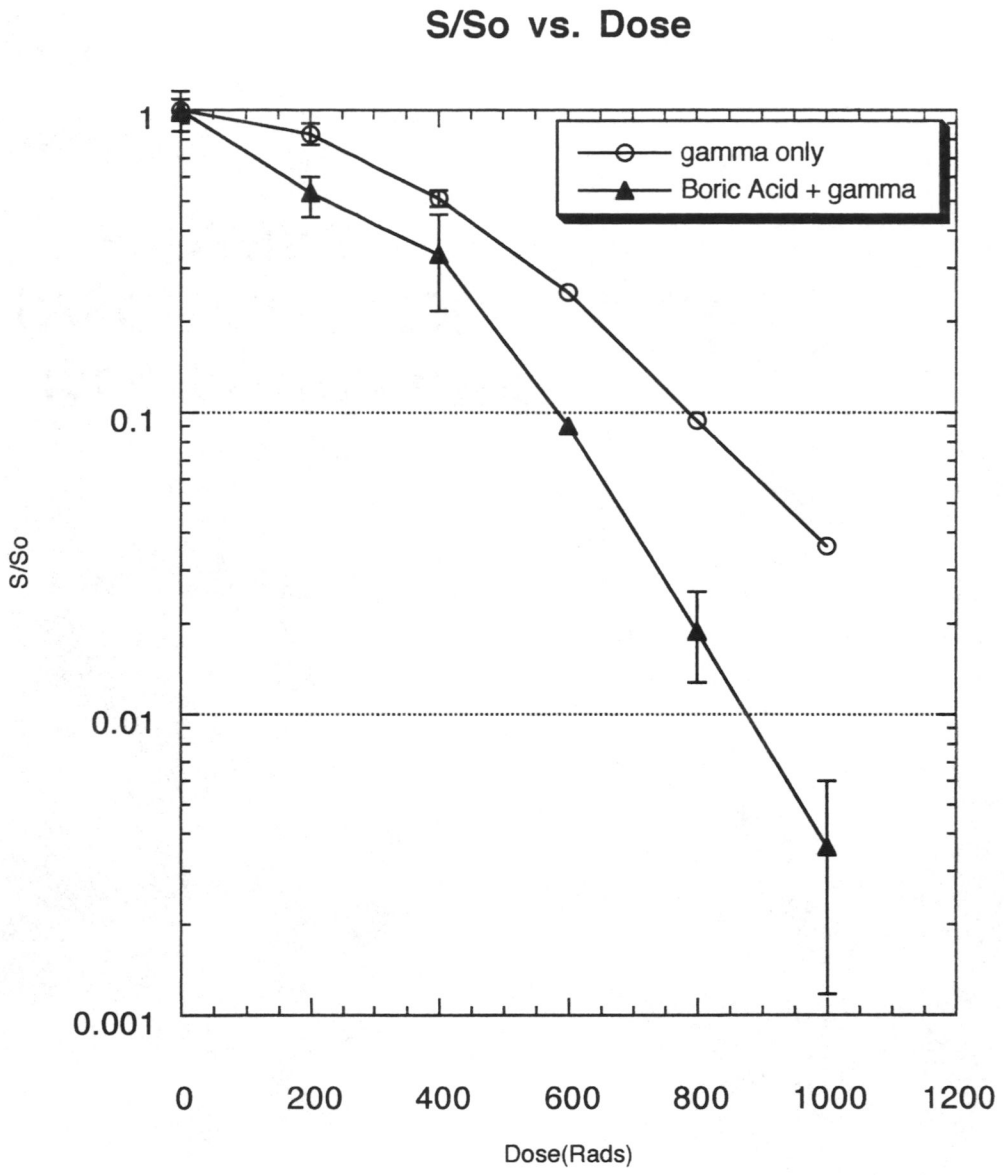

Figure 9. S/So vs. dose for boric acid and γ-ray experiment S/So pooled. Data from table 7.

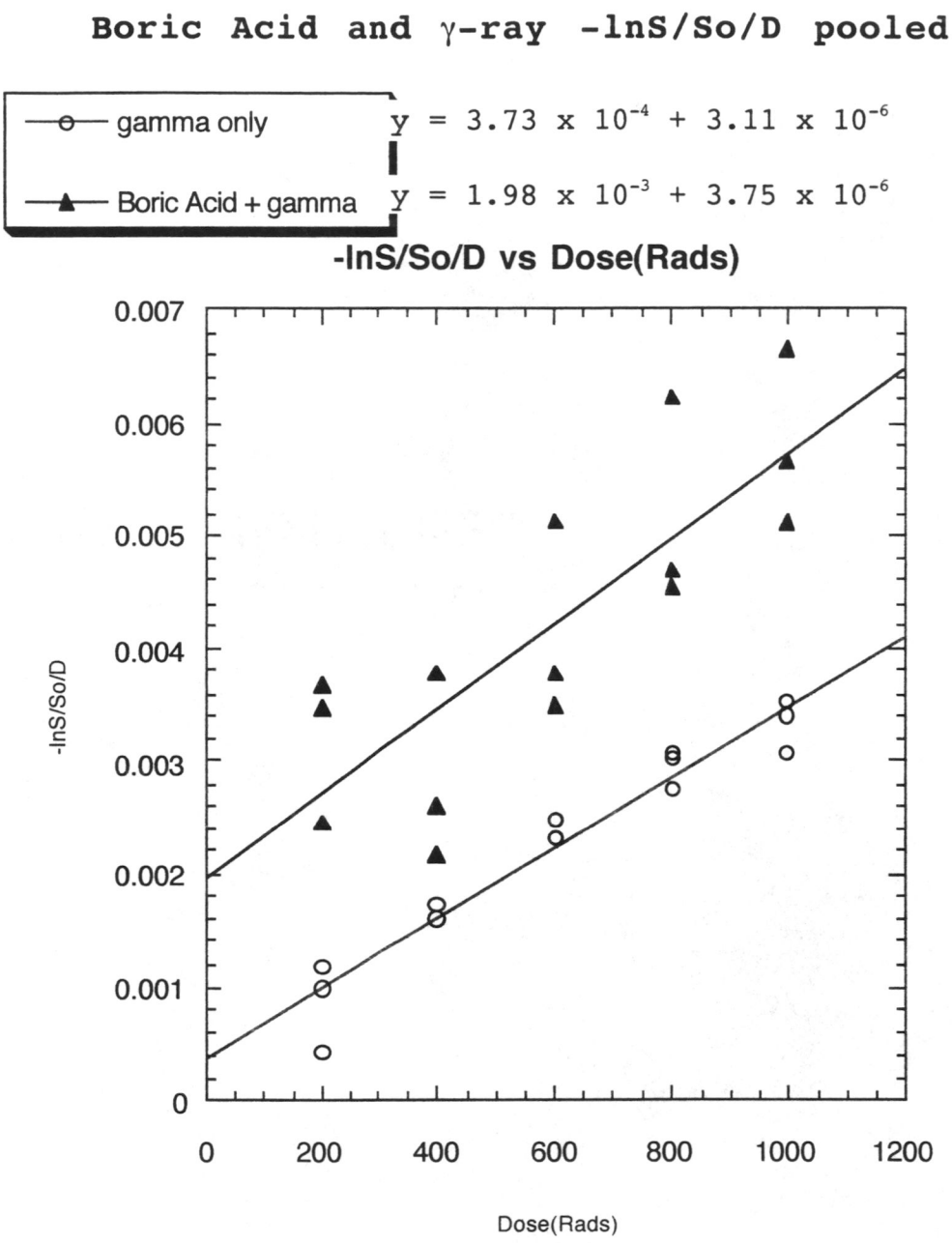

Figure 10. -lnS/So/D vs. Dose for boric acid and γ-ray experiments. All of the points are -lnS/So/D from tables

B_2. BSH and γ Irradiations

Cells were exposed to 100 ppm ^{10}B BSH media 24 h before irradiation with γ-rays. The survival curves of these irradiations were compared to cells exposed to γ-rays without BSH. Results of the survival curves are shown in FIGURE 11, TABLE 7, and Appendix V. The two curves show a similar shape with the BSH group showing slightly less cell survival. The two survival curves were compared by transforming the mean survival (S/So) for each group of data at each radiation dose to fit a linear equation. A linear regression line was fit to each group. Results of the linear transformation are plotted in FIGURE 12 and tabulated in Appendix V. The regression lines are:

γ-rays only

$$-\ln S/So/D = 4.18\times10^{-4} + 2.98\times10^{-6}D \quad (\alpha=4.18\times10^{-4} ; \beta= 2.98\times10^{-6})$$

γ-rays with 100 ppm ^{10}B BSH

$$-\ln S/So/D = 1.88\times10^{-3} + 1.95\times10^{-6}D \quad (\alpha=1.18\times10^{-3} ; \beta= 1.95\times10^{-6})$$

The two regression lines were compared and the t value for α=-2.52 (P=0.018) and the t value for β=1.17 (P=0.25). The results of the t-test showed the differences in the intercepts were significant and the differences in the slopes were not significant. Therefore, the intercepts for the two groups are different and the slopes for the two groups are the same (SEE FIGURE 12).

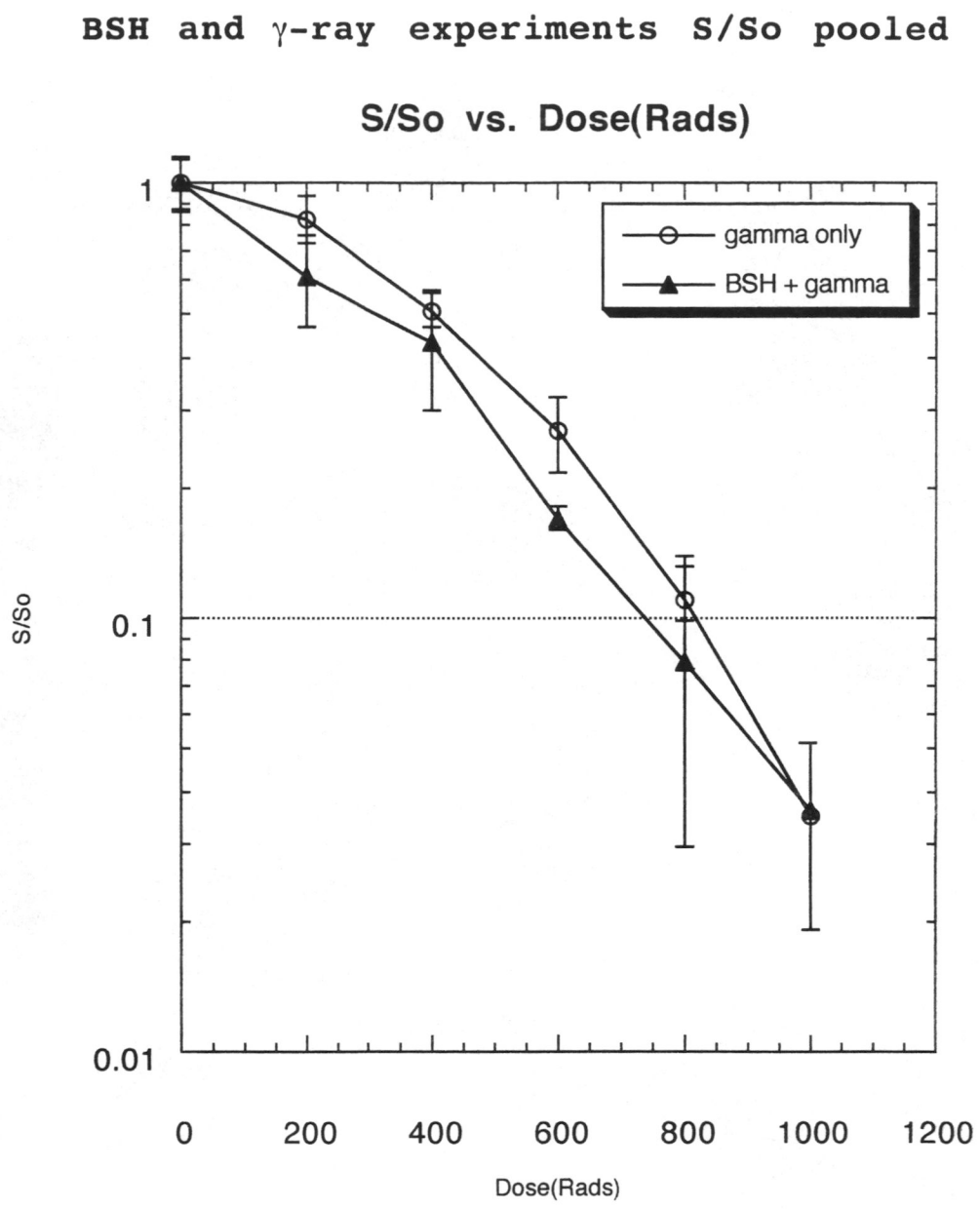

Figure 11. S/So vs. dose for BSH and γ-ray experiment S/So pooled. Data from table 7.

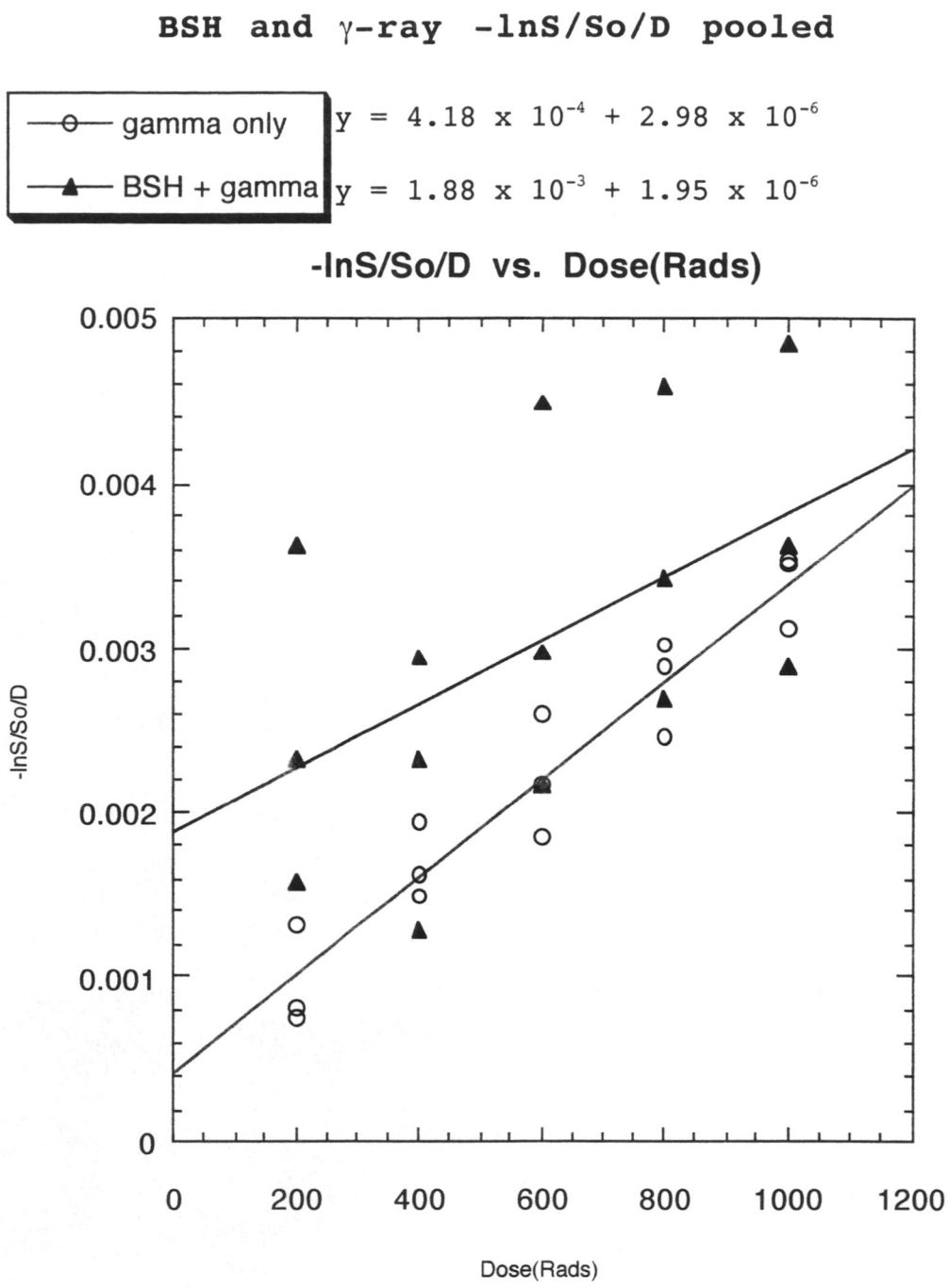

Figure 12. -lnS/So/D vs. Dose for BSH and γ-ray experiments. All of the points are -lnS/So/D from tables in Appendix V.

B_3. BPA and γ Irradiations

Cells were exposed to 100 ppm ^{10}B BPA media 24 h before irradiation with γ-rays. The survival curves of these irradiations were compared to cells exposed to γ-rays without BPA. Results of the survival curves are shown in FIGURE 13, TABLE 7, and Appendix VI. The two survival curves are similar. The two survival curves were compared by transforming the mean survival (S/So) for each group of data at each radiation dose to fit a linear equation. A linear regression line was fit to each group. Results of the linear transformation are plotted in FIGURE 14 and tabulated in Appendix VI. The regression lines for the two lines are:

γ-rays only

$-\ln S/So/D = 8.98\times10^{-4} + 1.82\times10^{-6}D$ ($\alpha=8.98\times10^{-4}$; $\beta= 1.82\times10^{-6}$)

γ-rays with 100 ppm ^{10}B BPA

$-\ln S/So/D = 1.35\times10^{-3} + 1.37\times10^{-6}D$ ($\alpha=1.35\times10^{-3}$; $\beta= 1.37\times10^{-6}$)

The two regression lines were compared and the t value for α=-1.39 (P=0.18) and the t value for β=0.93 (P=0.36). The results of the t-test showed differences in the intercepts were not significant and the differences in the slopes were not significant. The regression lines for the two groups are the same (SEE FIGURE 14).

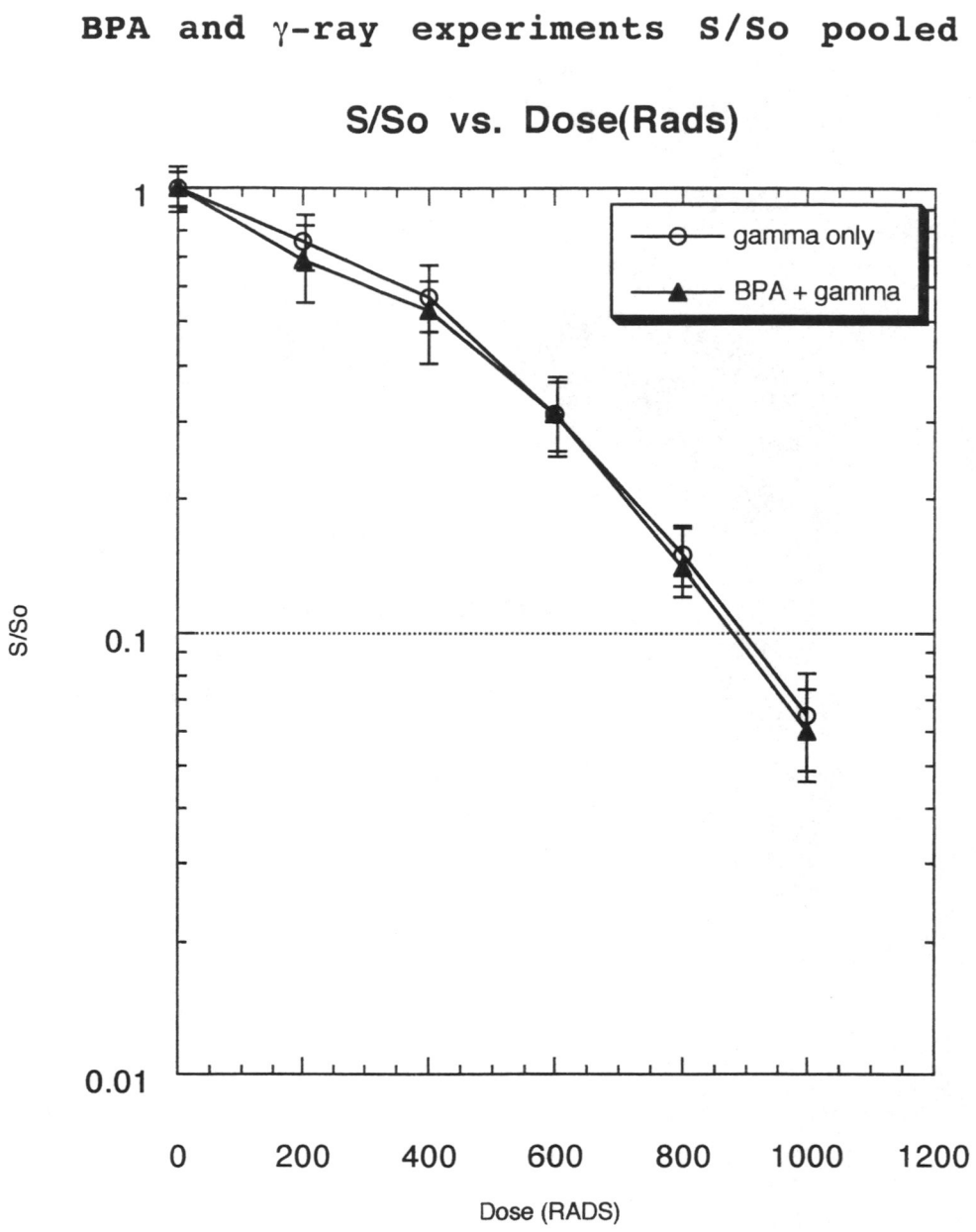

Figure 13. S/So vs. dose for BPA and γ-ray experiment S/So pooled. Data from table 7.

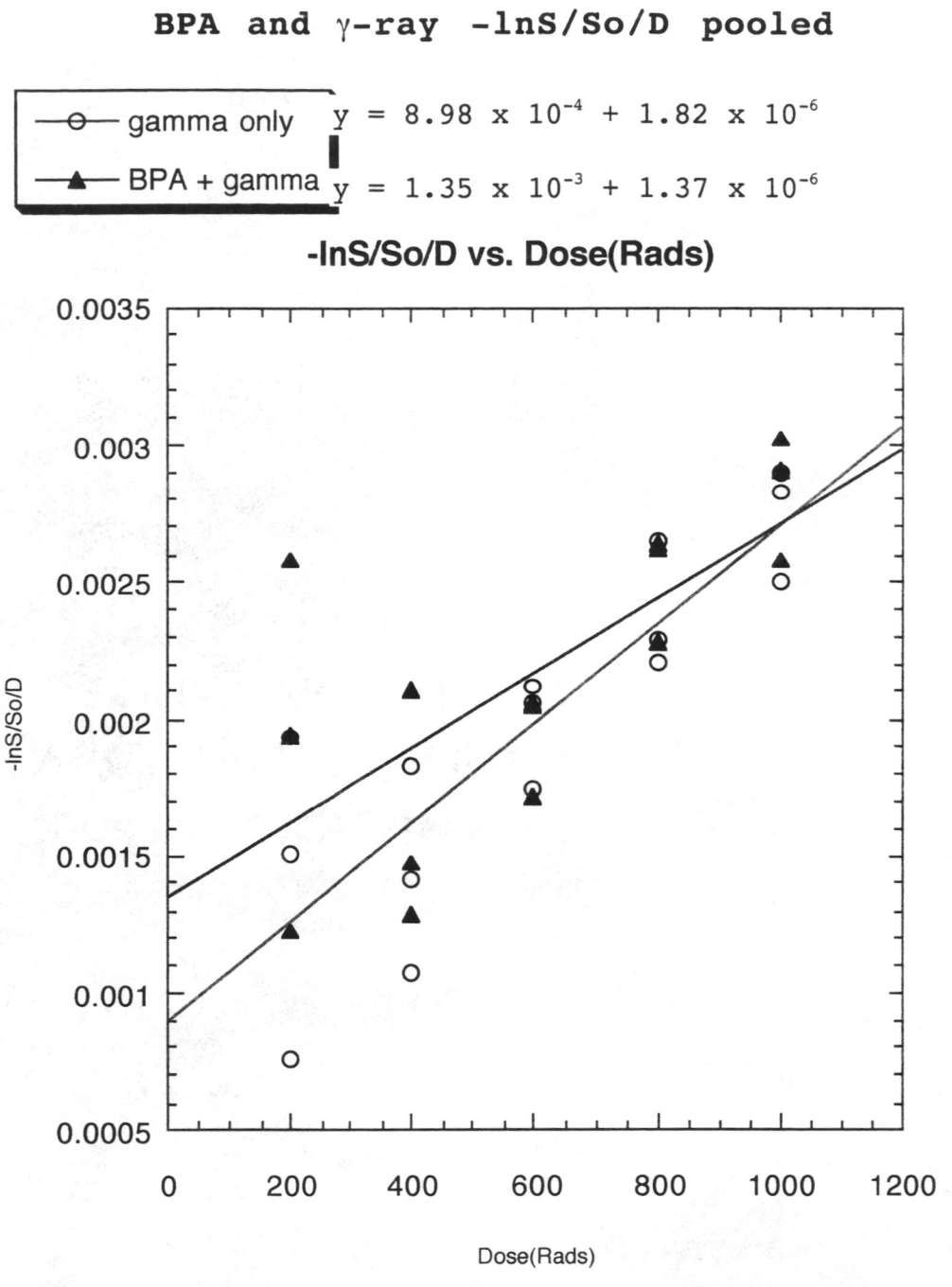

Figure 14. -lnS/So/D vs. Dose for BPA and γ-ray experiments. All of the points are -lnS/So/D from tables from Appendix VI.

C. 100 ppm ^{10}Boron Proton Irradiations

Cells were exposed to 100 ppm ^{10}B BPA media 24 h before irradiation with the proton beam. The survival curves of these irradiations were compared to cells exposed to γ-rays and protons without BPA. Results of the survival curves are shown in FIGURE 15, TABLE 8, and Appendix VII. To determine if boron neutron capture had a significant increase in the biological effectiveness of the proton beam, the survival curve for proton beam control was compared to the survival curve for proton beam with 100 ppm ^{10}B BPA. The two survival curves were compared by transforming the mean survival (S/So) for each group of data at each radiation dose to fit a linear equation. A linear regression line was fit to each group. Results of the linear transformation are plotted in FIGURE 16 and tabulated in Appendix VII. The regression lines are:

Protons only

$-\ln S/So/D = 1.02\times10^{-3} + 2.31\times10^{-6}D$ ($\alpha=8.98\times10^{-4}$; $\beta= 1.82\times10^{-6}$)

Protons with 100 ppm ^{10}B BPA

$-\ln S/So/D = 2.17\times10^{-3} + 4.53\times10^{-6}D$ ($\alpha=1.35\times10^{-3}$; $\beta= 1.37\times10^{-6}$)

The two regression lines were compared and the t value for $\alpha=0.16$ ($P=0.87$) and the t value for $\beta=0.54$ ($P=0.60$). The results of the t-test showed differences in the intercepts were not significant and the differences in the slopes were not significant. The regression lines for the two groups are the same (SEE FIGURE 16).

Table 8

γ-ray, Proton beam, and BPA + Proton Beam Pooled

	RADS					
Experiment	**0**	**200**	**400**	**600**	**800**	**1000**
γ only						
(Mean)	1.00	0.778	0.429	0.211	0.101	0.0422
(s.d.)	0.057	0.054	0.047	0.031	0.008	0.0085
Proton only						
(Mean)	1.00	0.708	0.259	0.0916	0.0441	0.0152
(s.d.)	0.21	0.071	0.155	0.0769	0.0336	0.0132
BPA + Proton						
(Mean)	1.00	0.667	0.205	0.077	0.0342	0.0081
(s.d.)	0.039	0.068	0.154	0.0806	0.0365	0.008

γ-ray, Proton Beam, and BPA + Proton Beam Pooled

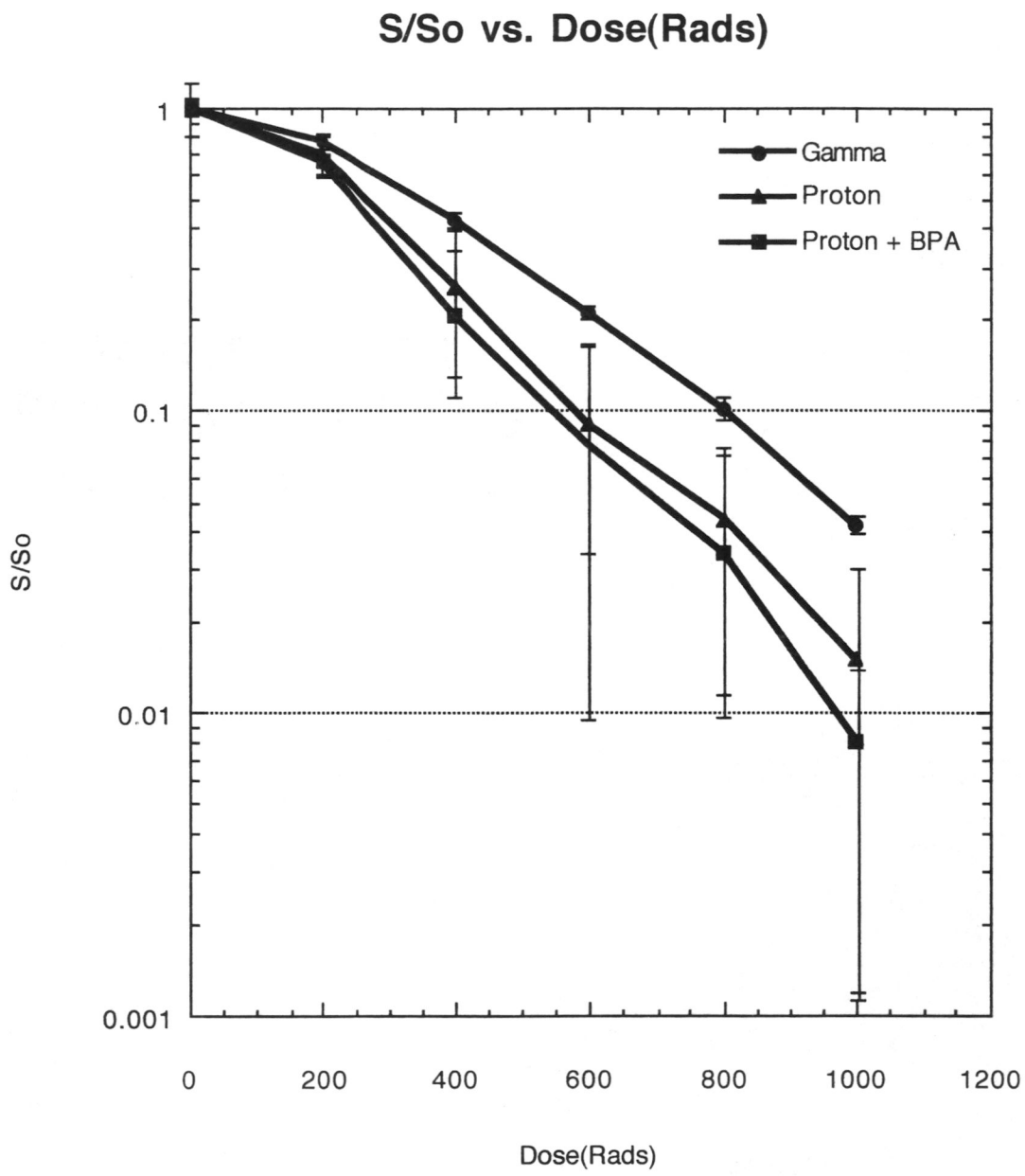

Figure 15. S/So vs. dose for BPA, γ-ray, and proton experiment S/So pooled. Data from table 8.

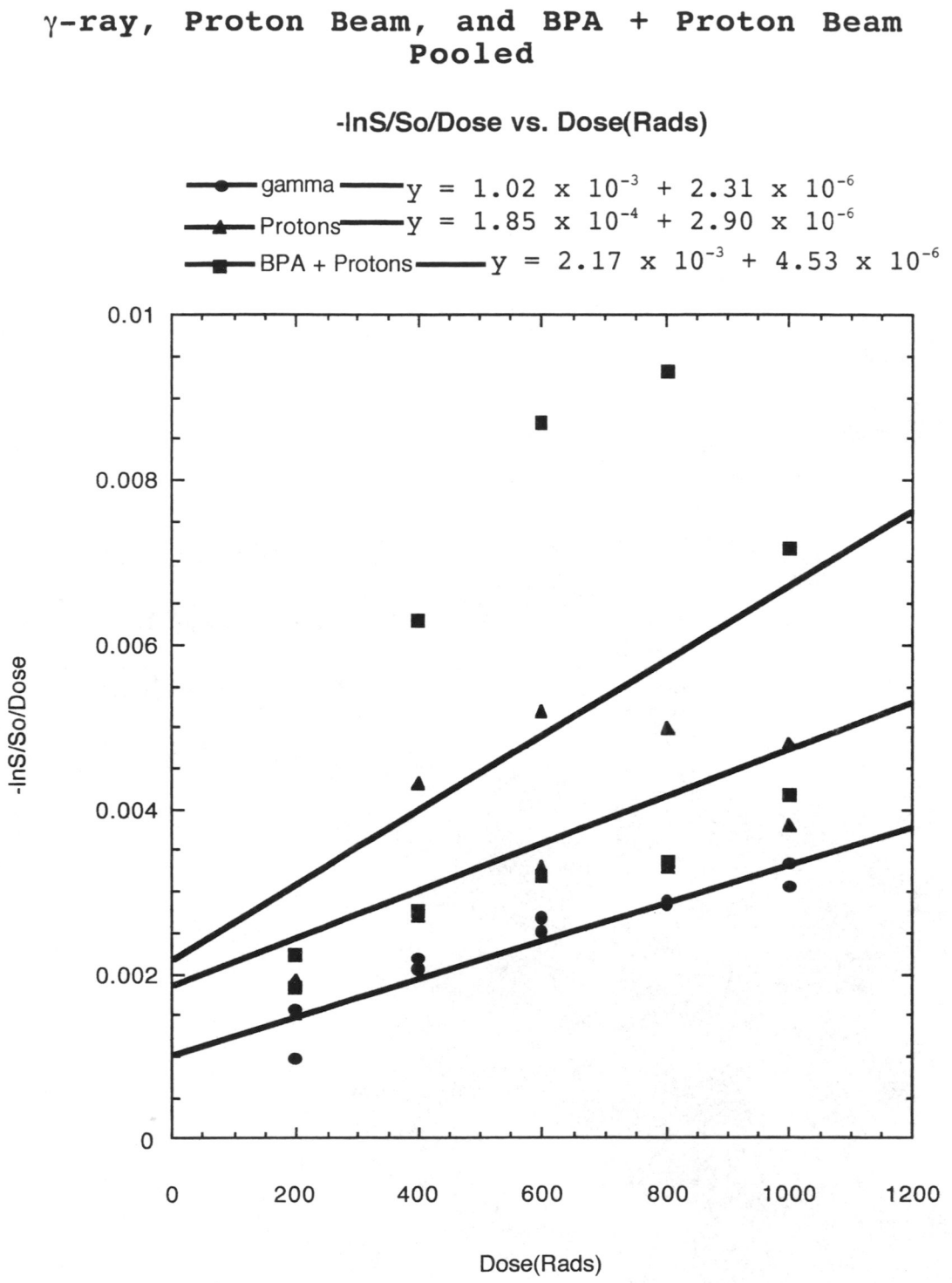

Figure 16. -lnS/So/D vs. Dose for BPA, γ-ray, and proton experiments. All of the points are -lnS/So/D from tables from Appendix VII.

Conclusions

A. Cytotoxicity

Boric acid and BSH were toxic at 120 ppm ^{10}B. The cytotoxicity of BSH at 30 ppm and 60 ppm ^{10}B was consistent with the results published by Lindstrom and colleagues. Lindstrom and colleagues showed BSH was not toxic in melanoma B16 and U-343MGa cells at concentration up to 50 ppm ^{10}B (Lindstrom *et al.*, 1994). In this study, BSH was toxic in V-79 cells at 120 ppm ^{10}B.

The cytotoxicity of BPA was consistent with results published by Pettersson and colleagues. They showed BPA was not toxic at any concentration tested up to 10 μg/g ^{10}B, 10 ppm ^{10}B (Pettersson *et al.*, 1994). There are two reasons BPA may not show toxicity in the V-79 cell line. First, BPA is a derivative of the amino acid phenylalanine and is not toxic. Second, BPA may not be taken up by the V-79 cells. BPA has been shown to be incorporated into melanoma cells (Yoshino *et al.*, 1993). However, the V-79 cell line is not a melanoma cell line and may not incorporate BPA as well as a melanoma cell.

B. 100 ppm ^{10}Boron γ Irradiations

Boric acid and BSH showed similar interactions with γ-rays in the V-79 cell line. The interactions of boric acid and BSH with γ-rays may be similar due their similar cytotoxicity. When the V-79 cells were exposed to 100 ppm ^{10}B

boric acid or BSH, the cytotoxicity was due to the concentration of compound. When the V-79 cells were exposed to 100 ppm ^{10}B boric acid or BSH and radiation, the cytotoxicity was due to the concentration of compound and the dose of radiation. The effect of cytotoxicity due to the boron compound was the same at every radiation dose. Since the ^{10}B compound contributes the same amount of toxicity at each dose of radiation, the regression lines may be parallel. Therefore, the intercepts could be different and the slopes could be the same. The null hypothesis was rejected for boric acid and BSH.

There was a large variation observed in experiments with boric acid and BSH. The variation may be due to my techniques related to plating efficiency, transporting the cells to the medical school with compound, or compound left in the flask after the media was removed and fresh media was added.

There was no significant interaction between γ-rays and BPA. The lack of interaction was probably due to the low toxicity of the amino acid derivative or the lack of uptake of BPA by the V-79 cell line. We accept the null hypothesis. There is no interaction between γ-rays and BPA.

C. 100 ppm ^{10}Boron Proton Irradiations

There was no statistically significant interaction observed between the proton beam and BPA due to boron neutron capture. However, boron neutron capture may have occurred.

The group with BPA and protons showed an insignificant decrease in cell survival. The decrease in cell survival was not statistically significant. The small decrease in cell survival could be caused by random variation or a contribution of cell death by neutron capture. Although neutron capture may have contributed to cell death, the influence of neutron capture could not be detected at a statistically significant level. We accept the null hypothesis that there is no interaction between the proton beam and BPA.

Results showed a) boric acid and BSH were toxic at high concentrations b) BPA was not toxic c) boric acid and BSH significantly interacted with γ-rays d) BPA did not interact with γ-rays or the proton beam. In this study, boron neutron capture of the neutrons produced by the proton beam did not significantly increase the biological effectiveness of the proton beam. However, the sampling size was small. In the future, more repetitions need to be done to increase the sampling size. An increase in sample size will more accurately determine the actual contribution boron neutron capture may have on the biological effectiveness of the proton beam.

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Appendix I

Cytotoxicity of Boric Acid

Experiments with Cytotoxicity of Boric Acid

Experiment 1

Flask	PE	Cells Plated	C**	S/So	Mean	s.d.	+-s.d
0*-200$-1	0.47	165	79	1.02			1.03
0-200-2	0.47	165	77	0.99	1.01	0.02	
0-200-3	0.47	165	79	1.02			0.99
0-2000-1	0.38	1805	651	0.99			1.03
0-2000-2	0.38	1805	701	1.02	0.99	0.04	
0-2000-3	0.38	1805	678	0.99			0.95
30-200-1	0.47	198	N/A				
30-200-2	0.47	198	N/A		0.75		
30-200-3	0.47	198	70	0.75			
30-2000-1	0.38	1981	590	0.78			0.79
30-2000-2	0.38	1981	511	0.68	0.74	0.05	
30-2000-3	0.38	1981	570	0.76			0.69
60-200-1	0.47	195	75	0.82			0.82
60-200-2	0.47	195	72	0.79	0.78	0.04	
60-200-3	0.47	195	68	0.74			0.74
60-2000-1	0.38	1946	603	0.82			0.94
60-2000-2	0.38	1946	657	0.89	0.88	0.06	
60-2000-3	0.38	1946	686	0.93			0.82
120-200-1	0.47	195	37	0.4			0.64
120-200-2	0.47	195	49	0.54	0.52	0.12	
120-200-3	0.47	195	58	0.63			0.40
120-2000-1	0.38	1945	482	0.65			0.78
120-2000-2	0.38	1945	568	0.77	0.72	0.06	
120-2000-3	0.38	1945	551	0.75			0.66

* = ppm ^{10}B. ** = Colonies observed. $ = Number of cells plated per flask.

Experiments with Cytotoxicity of Boric Acid

Experiment 2

Flask	PE	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
0*-1	0.6	294	171	0.97			1.025
0-2	0.6	294	187	1.06	1.0	0.025	
0-3	0.6	294	173	0.98			0.975
30-1	0.6	299	187	1.04			1.05
30-2	0.6	299	172	0.96	1.01	0.04	
30-3	0.6	299	184	1.03			0.97
60-1	0.6	299	205	1.14			1.16
60-2	0.6	299	191	1.07	1.05	0.11	
60-3	0.6	299	167	0.93			0.94
120-1	0.6	299	122	0.68			0.69
120-2	0.6	299	122	0.68	0.67	0.02	
120-3	0.6	299	117	0.65			0.65

* = ppm ^{10}B. ** = Colonies observed.

Experiments with Cytotoxicity of Boric Acid

Experiment 3

Flask	PE	Cells Plated	C**	S/So	Mean	s.d.	+-s.d
0-1	0.66	304	190	0.95			1.10
0-2	0.66	304	192	0.96	1.01	0.09	
0-3	0.66	304	222	1.11			0.92
30-1	0.66	307	215	1.06			1.06
30-2	0.66	307	205	1.01	1.01	0.05	
30-3	0.66	307	196	0.97			0.96
60-1	0.66	302	210	1.05			1.10
60-2	0.66	302	221	1.10	1.05	0.05	
60-3	0.66	302	202	1.01			1.00
120-1	0.66	309	166	0.81			0.83
120-2	0.66	309	161	0.79	0.79	0.03	
120-3	0.66	309	155	0.76			0.75

* = ppm ^{10}B. ** = Colonies observed.

Experiments with Cytotoxicity of Boric Acid

Experiment 4

Flask	PE	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
0*-1	0.76	295	226	1.01			1.02
0-2	0.76	295	220	0.98	1.0	0.02	
0-3	0.76	295	229	1.02			0.98
30-1	0.76	300	210	0.92			0.97
30-2	0.76	300	212	0.93	0.87	0.1	
30-3	0.76	300	173	0.76			0.77
60-1	0.76	298	214	0.95			0.95
60-2	0.76	298	191	0.84	0.85	0.1	
60-3	0.76	298	171	0.76			0.75
120-1	0.76	299	149	0.66			0.72
120-2	0.76	299	147	0.65	0.68	0.04	
120-3	0.76	299	165	0.73			0.64

* = ppm ^{10}B. ** = Colonies observed.

Appendix II

Cytotoxicity of BSH

Experiments with Cytotoxicity of BSH

Experiment 1

Flask	PE	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
0-200-1	0.69	204	140	0.99			1.08
0-200-2	0.69	204	152	1.08	1.0	0.08	
0-200-3	0.69	204	131	0.93			0.92
0-2000-1	0.62	2035	1251	0.99			1.01
0-2000-2	0.62	2035	1278	1.01	0.99	0.02	
0-2000-3	0.62	2035	1226	0.97			0.97
30-200-1	0.69	201	65	0.47			0.55
30-200-2	0.69	201	67	0.48	0.50	0.05	
30-200-3	0.69	201	78	0.56			0.45
30-2000-1	0.62	2007	545	0.44			0.46
30-2000-2	0.62	2007	571	0.46	0.45	0.01	
30-2000-3	0.62	2007	N/A				0.44
60-200-1	0.69	201	78	0.56			0.60
60-200-2	0.69	201	83	0.60	0.58	0.02	
60-200-3	0.69	201	80	0.58			0.56
60-2000-1	0.62	2012	887	0.71			0.71
60-2000-2	0.62	2012	841	0.67	0.68	0.03	
60-2000-3	0.62	2012	816	0.65			0.65
120-200-1	0.69	227	28	0.18			0.20
120-200-2	0.69	227	32	0.2	0.17	0.03	
120-200-3	0.69	227	22	0.14			0.14
120-2000-1	0.62	2270	206	0.15			0.16
120-2000-2	0.62	2270	232	0.16	0.15	0.01	
120-2000-3	0.62	2270	195	0.14			0.14

* = ppm ^{10}B. ** = Colonies observed. $ = Number of cells plated per flask.

Experiments with Cytotoxicity of BSH

Experiment 2

Flask	PE	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
0*-1	0.76	301	226	0.99			1.03
0-2	0.76	301	224	0.98	1.0	0.03	
0-3	0.76	301	238	1.04			0.97
30-1	0.76	298	142	0.63			0.63
30-2	0.76	298	137	0.61	0.62	0.01	
30-3	0.76	298	138	0.61			0.61
60-1	0.76	303	165	0.72			0.72
60-2	0.76	303	154	0.67	0.68	0.04	
60-3	0.76	303	149	0.65			0.64
120-1	0.76	606	349	0.76			0.76
120-2	0.76	606	339	0.74	0.75	0.01	
120-3	0.76	606	345	0.75			0.74

* = ppm ^{10}B. ** = Colonies observed.

Experiments with Cytotoxicity of BSH

Experiment 3

Flask	PE	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
0*-1	0.49	303	156	1.03			1.09
0-2	0.49	303	162	1.07	1.01	0.08	
0-3	0.49	303	139	0.92			0.93
30-1	0.49	314	204	1.33			1.33
30-2	0.49	314	195	1.27	1.30	0.03	
30-3	0.49	314	199	1.29			1.27
60-1	0.49	304	175	1.17			1.21
60-2	0.49	304	179	1.20	1.15	0.06	
60-3	0.49	304	162	1.09			1.09
120-1	0.49	627	118	0.38			0.47
120-2	0.49	627	136	0.44	0.43	0.04	
120-3	0.49	627	142	0.46			0.39

* = ppm ^{10}B. ** = Colonies observed.

Experiments with Cytotoxicity of BSH

Experiment 4

Flask	PE	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
0*-1	0.52	307	149	0.93			1.07
0-2	0.52	307	165	1.03	1.01	0.06	
0-3	0.52	307	164	1.03			0.95
30-1	0.52	318	188	1.14			1.24
30-2	0.52	318	206	1.25	1.18	0.06	
30-3	0.52	318	189	1.14			1.12
60-1	0.52	304	204	1.29			1.27
60-2	0.52	304	171	1.08	1.11	0.16	
60-3	0.52	304	154	0.97			0.95
120-1	0.52	592	246	0.8			0.86
120-2	0.52	592	263	0.85	0.83	0.03	
120-3	0.52	592	263	0.85			0.80

* = ppm ^{10}B. ** = Colonies observed.

Appendix III

Cytotoxicity of BPA

Experiments with Cytotoxicity of BPA

Experiment 1

Flask	PE	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
0*-200$-1	0.66	209	134	0.97			1.06
0-200-2	0.66	209	147	1.07	1.0	0.06	
0-200-3	0.66	209	133	0.96			0.94
0-2000-1	0.56	2093	1126	0.96			1.03
0-2000-2	0.56	2093	1150	0.98	0.99	0.04	
0-2000-3	0.56	2093	1219	1.04			0.95
30-200-1	0.66	345	280	1.23			1.24
30-200-2	0.66	345	227	1.00	1.12	0.12	
30-200-3	0.66	345	255	1.12			1.00
30-2000-1	0.56	3021	1804	1.07			1.09
30-2000-2	0.56	3021	1814	1.07	1.04	0.05	
30-2000-3	0.56	3021	1670	0.99			0.99
60-200-1	0.66	345	252	1.11			1.12
60-200-2	0.66	345	223	0.98	1.06	0.06	
60-200-3	0.66	345	250	1.10			1.00
60-2000-1	0.56	3021	1585	0.94			0.94
60-2000-2	0.56	3021	1549	0.92	0.93	0.01	
60-2000-3	0.56	3021	1565	0.93			0.92
120-200-1	0.66	297	268	1.37			1.49
120-200-2	0.66	297	231	1.18	1.34	0.15	
120-200-3	0.66	297	291	1.48			1.19
120-2000-1 120-2000-2 120-2000-3	N/A	N/A	N/A		N/A	N/A	N/A

* = ppm ^{10}B. ** = Colonies observed. $ = Number of cells plated per flask.

Experiments with Cytotoxicity of BPA

Experiment 2

Flask	PE	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
0*-1	0.76	314	251	1.05			1.11
0-2	0.76	314	209	0.87	1.00	0.11	
0-3	0.76	314	258	1.08			0.89
30-1	0.76	298	162	0.71			0.75
30-2	0.76	298	172	0.76	0.72	0.03	
30-3	0.76	298	159	0.70			0.69
60-1	0.76	301	212	0.92			0.95
60-2	0.76	301	184	0.08	0.88	0.07	
60-3	0.76	301	214	0.93			0.81
120-1	0.76	307	184	0.79			0.86
120-2	0.76	307	205	0.87	0.81	0.05	
120-3	0.76	307	182	0.78			0.76

* = ppm ^{10}B. ** = Colonies observed.

Experiments with Cytotoxicity of BPA

Experiment 3

Flask	PE	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
0*-1	0.58	314	176	0.95			1.05
0-2	0.58	314	185	1.00	1.00	0.05	
0-3	0.58	314	193	1.05			0.95
30-1	0.58	298	246	1.4			1.41
30-2	0.58	298	232	1.32	1.32	0.09	
30-3	0.58	298	216	1.23			1.23
60-1	0.58	298	209	1.19			1.24
60-2	0.58	298	215	1.23	1.22	0.02	
60-3	0.58	298	215	1.23			1.20
120-1	0.58	302	179	1.01			1.02
120-2	0.58	302	171	0.96	0.99	0.03	
120-3	0.58	302	180	1.01			0.96

* = ppm ^{10}B. ** = Colonies observed.

Experiments with Cytotoxicity of BPA

Experiment 4

Flask	PE	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
0*-1	0.62	345	198	0.91			1.07
0-2	0.62	345	222	1.02	0.99	0.08	
0-3	0.62	345	231	1.06			0.91
30-1	0.62	310	228	1.17			1.23
30-2	0.62	310	203	1.04	1.14	0.09	
30-3	0.62	310	235	1.21			1.05
60-1	0.62	323	183	0.9			0.93
60-2	0.62	323	184	0.91	0.87	0.06	
60-3	0.62	323	162	0.80			0.81
120-1	0.62	320	234	1.16			1.21
120-2	0.62	320	246	1.22	1.18	0.03	
120-3	0.62	320	233	1.16			1.15

* = ppm ^{10}B. ** = Colonies observed.

Appendix IV

Boric Acid with γ Irradiations

RBE of γ-ray only for Boric Acid

Experiment 1

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
0*-1		305	189	1.02			1.04
0-2	N/A	305	185	1.03	1.01	0.03	
0-3		305	193	0.97			0.98
200-1		305	155	0.79			0.84
200-2	1.18×10^{-3}	305	149	0.75	0.79	0.05	
200-3		305	144	0.84			0.74
400-1		525	141	0.52			0.52
400-2	1.73×10^{-3}	525	136	0.49	0.50	0.02	
400-3		525	162	0.48			0.48
600-1		1010	162	0.22			0.26
600-2	2.48×10^{-3}	1010	132	0.25	0.24	0.02	
600-3		1010	112	0.24			0.22
800-1		3277	138	0.089			0.091
800-2	3.07×10^{-3}	3277	149	0.089	0.086	0.005	
800-3		3277	124	0.081			0.081
1000-1		8095	147	0.038			0.038
1000-2	3.41×10^{-3}	8095	122	0.029	0.033	0.005	
1000-3		8095	116	0.032			0.028

PE=0.61. * = RADS. ** = colonies observed.

RBE of γ-ray with 100 ppm ^{10}B-Boric Acid

Experiment 1

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
***B-^{8}0-1		525	166	0.86			1.14
B-0-2	N/A	525	221	1.14	1.00	0.14	
B-0-3		525	191	0.99			0.86
B-200-1		525	92	0.48			0.55
B-200-2	3.47×10^{-3}	525	108	0.56	0.50	0.05	
B-200-3		525	90	0.47			0.45
B-400-1		1015	89	0.24			0.24
B-400-2	3.79×10^{-3}	1015	85	0.23	0.22	0.02	
B-400-3		1015	72	0.19			0.20
B-600-1		2035	47	0.063			0.061
B-600-2	5.13×10^{-3}	2035	31	0.042	0.047	0.014	
B-600-3		2035	27	0.036			0.033
B-800-1		6105	10	0.0045			0.0097
B-800-2	6.22×10^{-3}	6105	15	0.0067	0.007	0.0027	
B-800-3		6105	22	0.0098			0.0043
B-1000-1		20345	13	0.0017			0.0017
B-1000-2	6.65×10^{-3}	20345	9	0.0012	0.0013	0.0004	
B-1000-3		20345	7	0.0009			0.0009

PE= 0.37. * = RADS. ** = colonies observed. *** = Boric Acid.

RBE of γ-ray only for Boric Acid

Experiment 2

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
*0-1		300	199	1.14			1.12
0-2	N/A	300	165	0.94	1.00	0.12	
0-3		300	162	0.92			0.88
200-1		300	171	0.98			1.00
200-2	4.17×10^{-4}	300	164	0.94	0.92	0.08	
200-3		300	146	0.83			0.84
400-1		522	141	0.46			0.54
400-2	1.73×10^{-3}	522	166	0.54	0.50	0.04	
400-3		522	154	0.51			0.46
600-1		1005	148	0.25			0.27
600-2	2.31×10^{-3}	1005	143	0.24	0.25	0.02	
600-3		1005	160	0.27			0.23
800-1		3254	223	0.12			0.13
800-2	2.76×10^{-3}	3254	211	0.11	0.11	0.02	
800-3		3254	177	0.09			0.09
1000-1		8040	223	0.047			0.047
1000-2	3.08×10^{-3}	8040	214	0.046	0.046	0.001	
1000-3		8040	212	0.045			0.045

PE=0.58. * = RADS. ** = colonies observed.

RBE of γ-ray with 100 ppm ^{10}B-Boric Acid

Experiment 2

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
***B-*0-1		520	124	0.89			1.13
B-0-2	N/A	520	133	0.96	1.00	0.13	
B-0-3		520	160	1.15			0.87
B-200-1		520	52	0.37			0.67
B-200-2	3.68×10^{-3}	520	51	0.37	0.48	0.19	
B-200-3		520	97	0.70			0.29
B-400-1		1010	121	0.45			0.47
B-400-2	2.60×10^{-3}	1010	61	0.23	0.35	0.12	
B-400-3		1010	104	0.39			0.23
B-600-1		2020	60	0.11			0.13
B-600-2	3.79×10^{-3}	2020	70	0.13	0.10	0.03	
B-600-3		2020	36	0.067			0.07
B-800-1		6060	46	0.028			0.029
B-800-2	4.68×10^{-3}	6060	29	0.018	0.024	0.005	
B-800-3		6060	40	0.025			0.019
B-1000-1		20195	6	0.0011			0.0056
B-1000-2	5.65×10^{-3}	20195	26	0.0048	0.0035	0.0021	
B-1000-3		20195	25	0.0046			0.0014

PE= 0.27. * = RADS. ** = colonies observed. *** = Boric acid.

RBE of γ-ray only for Boric Acid

Experiment 3

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
0*-1		313	180	0.99			1.04
0-2	N/A	313	189	1.04	1.00	0.04	
0-3		313	177	0.97			0.96
200-1		313	146	0.80			0.84
200-2	9.92×10^{-4}	313	150	0.83	0.82	0.02	
200-3		313	148	0.82			0.80
400-1		542	166	0.53			0.56
400-2	1.59×10^{-3}	542	157	0.50	0.53	0.03	
400-3		542	177	0.56			0.50
600-1		1043	151	0.25			0.26
600-2	2.31×10^{-3}	1043	155	0.26	0.25	0.01	
600-3		1043	145	0.24			0.24
800-1		3128	153	0.084			0.094
800-2	3.02×10^{-3}	3128	167	0.092	0.089	0.005	
800-3		3128	164	0.09			0.84
1000-1		8342	174	0.036			0.035
1000-2	3.54×10^{-3}	8342	106	0.022	0.029	0.007	
1000-3		8342	144	0.030			0.022

PE=0.58. * = RADS. ** = colonies observed..

RBE of γ-ray with 100 ppm ^{10}B-Boric Acid

Experiment 3

Flask	-lnS/So Dose	Cells Plated	C	S/So	Mean	s.d.	+-s.d.
B***-0*-1		590	214	1.18			1.22
B-0-2	N/A	590	136	0.75	1.00	0.22	
B-0-3		590	192	1.06			0.78
B-200-1		590	76	0.42			0.79
B-200-2	2.46×10^{-3}	590	116	0.64	0.61	0.18	
B-200-3		590	139	0.77			0.43
B-400-1		2060	241	0.38			0.54
B-400-2	2.16×10^{-3}	2060	208	0.33	0.42	0.12	
B-400-3		2060	350	0.56			0.30
B-600-1		4120	158	0.13			0.13
B-600-2	3.49×10^{-3}	4120	164	0.13	0.12	0.01	
B-600-3		4120	143	0.11			0.11
B-800-1		12360	103	0.027			0.029
B-800-2	4.55×10^{-3}	12360	106	0.028	0.026	0.003	
B-800-3		12360	88	0.023			0.023
B-1000-1		41200	69	0.0055			0.0073
B-1000-2	5.11×10^{-3}	41200	95	0.0075	0.0060	0.0013	
B-1000-3		41200	64	0.0051			0.0047

PE=0.31. * = RADS. ** = colonies observed. *** = Boric acid.

Appendix V

BSH with γ Irradiations

RBE of γ-ray only for BSH

Experiment 1

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
0*-1		301	101	0.76			1.20
0-2	N/A	301	154	1.16	0.99	0.21	
0-3		301	141	1.06			0.78
200-1		301	109	0.82			0.95
200-2	8.13×10^{-4}	301	129	0.97	0.85	0.10	
200-3		301	102	0.77			0.75
400-1		522	131	0.57			0.59
400-2	1.49×10^{-3}	522	132	0.57	0.55	0.04	
400-3		522	114	0.50			0.51
600-1		1004	157	0.36			0.36
600-2	1.85×10^{-3}	1004	135	0.31	0.33	0.03	
600-3		1004	137	0.31			0.30
800-1		3012	154	0.12			0.11
800-2	2.88×10^{-3}	3012	125	0.094	0.10	0.01	
800-3		3012	137	0.1			0.09
1000-1		8033	119	0.034			0.036
1000-2	3.51×10^{-3}	8033	120	0.034	0.03	0.006	
1000-3		8033	81	0.023			0.024

PE=0.44. * = RADS. ** = colonies observed.

RBE of γ-ray with 100 ppm ^{10}B-BSH

Experiment 1

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
B***-0*-1		585	205	1.02			1.03
B-0-2	N/A	585	202	1.01	1.00	0.03	
B-0-3		585	194	0.97			0.97
B-200-1		585	152	0.76			0.76
B-200-2	1.59×10^{-3}	585	136	0.68	0.73	0.03	
B-200-3		585	149	0.74			0.70
B-400-1		2048	243	0.35			0.45
B-400-2	2.32×10^{-3}	2048	259	0.37	0.40	0.05	
B-400-3		2048	329	0.47			0.35
B-600-1		4097	175	0.13			0.106
B-600-2	4.48×10^{-3}	4097	60	0.042	0.068	0.038	
B-600-3		4097	51	0.036			0.30
B-800-1		12291	102	0.024			0.027
B-800-2	4.59×10^{-3}	12291	110	0.026	0.026	0.0011	
B-800-3		12291	110	0.026			0.025
B-1000-1		40972	130	0.0093			0.0093
B-1000-2	4.84×10^{-3}	40972	91	0.0065	0.0079	0.0014	
B-1000-3		40972	111	0.0079			0.0065

PE=0.34. * = RADS. ** = colonies observed. *** = BSH.

RBE of γ-ray only for BSH

Experiment 2

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
0*-1		302	181	1.05			1.09
0-2	N/A	302	182	1.06	1.00	0.09	
0-3		302	153	0.89			0.91
200-1		302	131	0.76			0.83
200-2	1.31×10^{-3}	302	143	0.83	0.77	0.06	
200-3		302	123	0.71			0.71
400-1		523	135	0.45			0.52
400-2	1.94×10^{-3}	523	154	0.52	0.46	0.06	
400-3		523	123	0.41			0.40
600-1		1006	138	0.24			0.24
600-2	2.6×10^{-3}	1006	108	0.19	0.21	0.03	
600-3		1006	118	0.21			0.18
800-1		3019	150	0.087			0.109
800-2	3.02×10^{-3}	3019	187	0.11	0.089	0.02	
800-3		3019	121	0.07			0.069
1000-1		8050	144	0.031			0.031
1000-2	3.54×10^{-3}	8050	124	0.027	0.029	0.002	
1000-3		8050	135	0.029			0.027

PE=0.57. * = RADS. ** = colonies observed.

RBE of γ-ray with 100 ppm ^{10}B-BSH

Experiment 2

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
B***-0*-1		603	152	0.67			1.29
B-0-2	N/A	603	270	1.19	1.00	0.29	
B-0-3		603	258	1.14			0.71
B-200-1		603	115	0.51			0.82
B-200-2	2.33×10^{-3}	603	119	0.53	0.63	0.19	
B-200-3		603	193	0.85			0.44
B-400-1		2110	452	0.57			0.63
B-400-2	1.29×10^{-3}	2110	490	0.62	0.60	0.03	
B-400-3		2110	480	0.61			0.57
B-600-1		4221	422	0.27			0.276
B-600-2	2.17×10^{-3}	4221	441	0.28	0.27	0.006	
B-600-3		4221	431	0.27			0.264
B-800-1		12664	577	0.12			0.125
B-800-2	2.69×10^{-3}	12664	535	0.11	0.12	0.005	
B-800-3		12664	549	0.12			0.115
B-1000-1		40214	1084	0.072			0.070
B-1000-2	2.88×10^{-3}	40214	780	0.052	0.056	0.014	
B-1000-3		40214	684	0.045			0.042

PE=0.38. * = RADS. ** = colonies observed. *** = BSH.

RBE of γ-ray only for BSH

Experiment 3

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
0*-1		303	158	1.02			1.11
0-2	N/A	303	170	1.10	1.01	0.1	
0-3		303	139	0.9			0.91
200-1		303	133	0.86			0.89
200-2	7.54×10^{-4}	303	152	0.98	0.86	0.12	
200-3		303	116	0.75			0.65
400-1		524	133	0.50			0.48
400-2	1.63×10^{-3}	524	141	0.53	0.52	0.02	
400-3		524	140	0.52			0.44
600-1		1008	149	0.29			0.24
600-2	2.18×10^{-3}	1008	123	0.24	0.27	0.03	
600-3		1008	146	0.28			0.18
800-1		3025	244	0.16			0.17
800-2	2.46×10^{-3}	3025	244	0.16	0.14	0.03	
800-3		3025	172	0.11			0.11
1000-1		8067	185	0.045			0.045
1000-2	3.12×10^{-3}	8067	184	0.045	0.044	0.001	
1000-3		8067	175	0.043			0.043

PE=0.51. * = RADS. ** = colonies observed.

RBE of γ-ray with 100 ppm ^{10}B-BSH

Experiment 3

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
B***-0*-1		602	672	1.02			1.02
B-0-2	N/A	602	639	0.97	1.00	0.02	
B-0-3		602	657	1.00			0.98
B-200-1		602	292	0.45			0.52
B-200-2	N/A	602	333	0.52	0.48	0.04	
B-200-3		602	321	0.49			0.44
B-400-1		2108	699	0.30			0.313
B-400-2	9.28×10^{-4}	2108	710	0.31	0.31	0.003	
B-400-3		2108	707	0.31			0.307
B-600-1		4215	781	0.17			0.173
B-600-2	1.75×10^{-3}	4215	762	0.17	0.17	0.003	
B-600-3		4215	753	0.16			0.167
B-800-1		12646	991	0.072			0.072
B-800-2	2.46×10^{-3}	12646	882	0.064	0.065	0.007	
B-800-3		12646	812	0.059			0.058
B-1000-1		40153	1272	0.029			0.029
B-1000-2	2.9×10^{-3}	40153	1115	0.026	0.027	0.002	
B-1000-3		40153	1147	0.026			0.025

PE= 1.09. * = RADS. ** = colonies observed. *** = BSH.

Appendix VI

BPA with γ Irradiations

RBE of γ-ray only for BPA

Experiment 1

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
0*-1		298	121	1.02			1.04
0-2	N/A	298	123	1.03	1.00	0.04	
0-3		298	113	0.95			0.96
200-1		298	89	0.75			0.97
200-2	7.54×10^{-4}	298	101	0.85	0.86	0.11	
200-3		298	115	0.97			0.75
400-1		516	106	0.51			0.78
400-2	1.08×10^{-3}	516	154	0.75	0.65	0.13	
400-3		516	144	0.70			0.52
600-1		993	132	0.33			0.376
600-2	1.75×10^{-3}	993	135	0.34	0.35	0.026	
600-3		993	151	0.38			0.324
800-1		2268	148	0.16			0.185
800-2	2.21×10^{-3}	2268	168	0.19	0.17	0.015	
800-3		2268	152	0.17			0.155
1000-1		7941	208	0.065			0.097
1000-2	2.5×10^{-3}	7941	305	0.096	0.082	0.015	
1000-3		7941	263	0.083			0.067

PE=0.4. * = RADS. ** = colonies observed.

RBE of γ-ray with 100 ppm ^{10}B-BPA

Experiment 1

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
B***-0*-1		168	89	1.13			1.15
B-0-2	N/A	168	66	0.84	1.00	0.15	
B-0-3		168	81	1.03			0.85
B-200-1		168	32	0.41			0.77
B-200-2	1.78×10^{-3}	168	51	0.65	0.60	0.17	
B-200-3		168	58	0.74			0.43
B-400-1		590	130	0.47			0.50
B-400-2	1.68×10^{-3}	590	132	0.48	0.43	0.07	
B-400-3		590	96	0.35			0.36
B-600-1		1770	169	0.20			0.37
B-600-2	1.8×10^{-3}	1770	277	0.33	0.29	0.08	
B-600-3		1770	276	0.33			0.21
B-800-1		5900	310	0.11			0.132
B-800-2	2.46×10^{-3}	5900	321	0.12	0.12	0.012	
B-800-3		5900	370	0.13			0.108
B-1000-1		11800	307	0.056			0.061
B-1000-2	2.86×10^{-3}	11800	195	0.035	0.049	0.012	
B-1000-3		11800	305	0.055			0.037

PE=0.47. * = RADS. ** = colonies observed. *** = BPA.

RBE of γ-ray only for BPA

Experiment 2

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
0*-1		281	127	0.87			1.14
0-2	N/A	281	145	0.99	1.00	0.14	
0-3		281	167	1.14			0.86
200-1		281	109	0.75			0.77
200-2	1.51×10^{-3}	281	104	0.71	0.74	0.03	
200-3		281	111	0.76			0.71
400-1		488	139	0.55			0.60
400-2	1.41×10^{-3}	488	139	0.55	0.57	0.03	
400-3		488	153	0.60			0.54
600-1		939	158	0.32			0.34
600-2	2.06×10^{-3}	939	154	0.32	0.29	0.05	
600-3		939	112	0.23			0.24
800-1		2817	239	0.16			0.17
800-2	2.29×10^{-3}	2817	230	0.16	0.16	0.01	
800-3		2817	220	0.15			0.15
1000-1		7511	251	0.064			0.065
1000-2	2.83×10^{-3}	7511	240	0.061	0.059	0.006	
1000-3		7511	205	0.052			0.053

PE=0.52. * = RADS. ** = colonies observed.

RBE of γ-ray with 100 ppm ^{10}B-BPA

Experiment 2

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
B***-0*-1		322	151	0.92			1.07
B-0-2	N/A	322	166	1.02	1.00	0.07	
B-0-3		322	173	1.06			0.93
B-200-1		322	123	0.75			0.78
B-200-2	2.08×10^{-3}	322	92	0.56	0.68	0.1	
B-200-3		322	118	0.72			0.58
B-400-1		571	162	0.56			0.65
B-400-2	1.36×10^{-3}	571	167	0.58	0.60	0.05	
B-400-3		571	189	0.65			0.55
B-600-1		1714	343	0.40			0.39
B-600-2	1.7×10^{-3}	1714	286	0.33	0.36	0.03	
B-600-3		1714	300	0.35			0.39
B-800-1		5712	560	0.19			0.20
B-800-2	2.29×10^{-3}	5712	351	0.12	0.16	0.04	
B-800-3		5712	492	0.17			0.12
B-1000-1		11424	497	0.086			0.085
B-1000-2	2.60×10^{-3}	11424	421	0.073	0.076	0.009	
B-1000-3		11424	399	0.069			0.067

PE=0.51. * = RADS. ** = colonies observed. *** = BPA.

RBE of γ-ray only for BPA

Experiment 3

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
0*-1		274	176	1.21			1.19
0-2	N/A	274	140	0.96	1.00	0.19	
0-3		274	120	0.83			0.81
200-1		274	100	0.69			0.79
200-2	1.93×10^{-3}	274	82	0.56	0.68	0.11	
200-3		274	113	0.78			0.57
400-1		475	114	0.45			0.52
400-2	1.83×10^{-3}	475	133	0.53	0.48	0.04	
400-3		475	115	0.46			0.44
600-1		915	153	0.32			0.32
600-2	2.12×10^{-3}	915	137	0.28	0.28	0.04	
600-3		915	116	0.24			0.24
800-1		2744	173	0.12			0.14
800-2	2.65×10^{-3}	2744	202	0.14	0.12	0.02	
800-3		2744	161	0.11			0.10
1000-1		7318	223	0.057			0.07
1000-2	2.90×10^{-3}	7318	269	0.069	0.055	0.015	
1000-3		7318	154	0.040			0.04

PE=0.53. * = RADS. ** = Colonies observed.

RBE of γ-ray with 100 ppm ^{10}B-BPA

Experiment 3

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
B***-0*-1		305	183	1.08			1.07
B-0-2	N/A	305	168	0.99	1.00	0.07	
B-0-3		305	159	0.94			0.93
B-200-1		305	158	0.93			0.91
B-200-2	1.23×10^{-4}	305	116	0.68	0.78	0.13	
B-200-3		305	125	0.74			0.65
B-400-1		542	171	0.57			0.58
B-400-2	1.47×10^{-3}	542	162	0.54	0.56	0.02	
B-400-3		542	170	0.56			0.54
B-600-1		1627	302	0.33			0.34
B-600-2	2.05×10^{-3}	1627	275	0.30	0.29	0.05	
B-600-3		1627	221	0.24			0.24
B-800-1		5423	414	0.14			0.14
B-800-2	2.62×10^{-3}	5423	386	0.13	0.12	0.02	
B-800-3		5423	317	0.11			0.10
B-1000-1		10847	331	0.055			0.056
B-1000-2	2.91×10^{-3}	10847	316	0.052	0.054	0.002	
B-1000-3		10847	340	0.056			0.52

PE=0.56. * = RADS. ** = colonies observed. *** = BPA

Appendix VII

BPA with Proton and γ Irradiations

RBE of γ-rays only for proton + 100 ppm ^{10}B-BPA

Experiment 1

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
0**-1		293	237	1.00			1.03
0-2	N/A	293	226	0.96	0.99	0.04	
0-3		293	246	1.05			0.95
0-4		293	228	0.97			
200-0		293	172	0.73			0.743
200-1	1.56×10^{-3}	293	174	0.74	0.73	0.013	
200-3		293	167	0.71			0.717
200-4		293	173	0.73			
400-1		489	182	0.47			0.51
400-2	2.05×10^{-3}	489	135	0.35	0.44	0.07	
400-3		489	176	0.45			0.37
400-4		489	196	0.50			
600-1		979	162	0.21			0.25
600-2	2.52×10^{-3}	979	156	0.20	0.22	0.03	
600-3		979	163	0.21			0.19
600-4		979	211	0.27			
800-1		2938	206	0.088			0.113
800-2	2.85×10^{-3}	2938	257	0.109	0.102	0.011	
800-3		2938	231	0.098			0.091
800-4		2938	263	0.112			
1000-1		8814	303	0.043			0.054
1000-2	3.05×10^{-3}	8814	284	0.040	0.047	0.007	
1000-3		8814	342	0.049			0.040
1000-4		8814	401	0.057			

P.E.= 0.80. * = RADS. ** = colonies observed.

RBE of protons only for proton + 100 ppm ^{10}B-BPA

Experiment 1

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
P$-0**-1		293	206	0.94			1.05
P-0-2	N/A	293	216	0.99	1.00	0.05	
P-0-3		293	219	1.00			0.95
P-0-4		293	233	1.07			
P-200-0		293	139	0.64			0.74
P-200-1	1.93×10^{-3}	293	155	0.71	0.68	0.06	
P-200-3		293	165	0.76			0.62
P-200-4		293	135	0.72			
P-400-1		489	139	0.38			0.38
P-400-2	4.30×10^{-3}	489	114	0.31	0.18	0.20	
P-400-3		489	7	0.019			-0.02
P-400-4		489	1	0.0027			
P-600-1		979	125	0.17			0.129
P-600-2	5.18×10^{-3}	979	6	0.008	0.045	0.084	
P-600-3		979	0	0.00			-0.039
P-600-4		979	0	0.00			
P-800-1		2938	0	0.00			0.055
P-800-2	4.99×10^{-3}	2938	161	0.074	0.018	0.037	
P-800-3		2938	0	0.00			-0.019
P-800-4		2938	0	0.00			
P-1000-1		8814	2	0.0003			0.024
P-1000-2	4.81×10^{-3}	8814	0	0.00	0.008	0.016	
P-1000-3		8814	0	0.00			-0.008
P-1000-4		8814	212	0.032			

P.E.= 0.75. * = RADS. ** = colonies observed. $ = Proton.

RBE for proton + 100 ppm ^{10}B-BPA

Experiment 1

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
@BP$-0**-1		436	270	1.07			1.05
BP-0-2	N/A	436	243	0.96	1.00	0.05	
BP-0-3		436	247	0.97			0.95
BP-0-4		436	254	1.00			
BP-200-0		436	177	0.70			0.70
BP-200-1	2.22×10^{-3}	436	149	0.59	0.64	0.06	
BP-200-3		436	176	0.70			0.58
BP-200-4		436	149	0.59			
BP-400-1		784	99	0.22			0.281
BP-400-2	6.28×10^{-3}	784	1	0.0022	0.081	0.20	
BP-400-3		784	43	0.094			-0.119
BP-400-4		784	5	0.011			
BP-600-1		1962	0	0.00			0.089
BP-600-2	8.67×10^{-3}	1962	2	0.0018	0.005	0.084	
BP-600-3		1962	23	0.021			-0.079
BP-600-4		1962	0	0.00			
BP-800-1		5886	0	0.00			0.0016
BP-800-2	9.30×10^{-3}	5886	0	0.00	0.0006	0.001	
BP-800-3		5886	0	0.00			-0.0004
BP-800-4		5886	8	0.0023			
BP-1000-1		9814	0	0.00			0.0028
BP-1000-2	7.14×10^{-3}	9814	18	0.0032	0.0008	0.002	
BP-1000-3		9814	0	0.00			-0.0012
BP-1000-4		9814	0	0.00			

P.E.= 0.58. * = RADS. ** = colonies observed. @ = BPA. $ = Proton.

RBE of γ-rays only for proton + 100 ppm ^{10}B-BPA

Experiment 2

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
0*-1		255	233	1.04			1.08
0-2	N/A	255	241	1.07	1.00	0.08	
0-3		255	225	1.00			0.92
0-4		255	201	0.89			
200-0		255	177	0.79			0.857
200-1	9.62×10^{-4}	255	189	0.84	0.83	0.027	
200-3		255	185	0.82			0.803
200-4		255	191	0.85			
400-1		425	166	0.44			0.44
400-2	2.18×10^{-3}	425	149	0.40	0.42	0.02	
400-3		425	157	0.42			0.40
400-4		425	154	0.41			
600-1		850	170	0.23			0.23
600-2	2.67×10^{-3}	850	160	0.21	0.20	0.03	
600-3		850	119	0.16			0.17
600-4		850	154	0.21			
800-1		2550	230	0.102			0.106
800-2	2.87×10^{-3}	2550	241	0.107	0.10	0.006	
800-3		2550	232	0.103			0.094
800-4		2550	207	0.092			
1000-1		7651	202	0.030			0.040
1000-2	3.34×10^{-3}	7651	245	0.036	0.036	0.004	
1000-3		7651	245	0.036			0.032
1000-4		7651	265	0.039			

P.E.= 0.88. * = RADS. ** = colonies observed..

RBE of γ-rays only for proton + 100 ppm ^{10}B-BPA

Experiment 2

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
P$-0*-1		255	233	1.22			1.32
P-0-2	N/A	255	216	1.13	1.00	0.32	
P-0-3		255	215	1.13			0.68
P-0-4		255	100	0.52			
P-200-0		255	155	0.81			0.82
P-200-1	1.53×10^{-3}	255	121	0.63	0.74	0.08	
P-200-3		255	146	0.76			0.66
P-200-4		255	140	0.73			
P-400-1		425	112	0.35			0.37
P-400-2	2.7×10^{-3}	425	112	0.35	0.34	0.03	
P-400-3		425	111	0.35			0.31
P-400-4		425	96	0.30			
P-600-1		850	97	0.15			0.17
P-600-2	3.3×10^{-3}	850	97	0.15	0.14	0.03	
P-600-3		850	60	0.09			0.11
P-600-4		850	99	0.16			
P-800-1		2550	140	0.073			0.074
P-800-2	3.33×10^{-3}	2550	126	0.066	0.070	0.004	
P-800-3		2550	139	0.073			0.066
P-800-4		2550	129	0.068			
P-1000-1		7651	110	0.019			0.026
P-1000-2	3.8×10^{-3}	7651	123	0.022	0.022	0.004	
P-1000-3		7651	117	0.02			0.018
P-1000-4		7651	160	0.028			

P.E.= 0.75. * = RADS. ** = colonies observed. $ = Proton.

RBE for proton + 100 ppm ^{10}B-BPA

Experiment 2

Flask	-lnS/So Dose	Cells Plated	C**	S/So	Mean	s.d.	+-s.d.
@BP$-0*-1		417	171	1.05			1.03
BP-0-2	N/A	417	161	0.99	1.00	0.3	
BP-0-3		417	159	0.97			0.97
BP-0-4		417	163	0.97			
BP-200-0		417	105	0.64			0.76
BP-200-1	1.83×10^{-3}	417	113	0.69	0.69	0.07	
BP-200-3		417	130	0.80			0.62
BP-200-4		417	105	0.64			
BP-400-1		752	91	0.31			0.40
BP-400-2	2.77×10^{-3}	752	126	0.43	0.33	0.07	
BP-400-3		752	84	0.29			0.26
BP-400-4		752	88	0.30			
BP-600-1		1880	104	0.14			0.19
BP-600-2	3.17×10^{-3}	1880	74	0.10	0.15	0.04	
BP-600-3		1880	139	0.19			0.11
BP-600-4		1880	121	0.16			
BP-800-1		5641	157	0.071			0.077
BP-800-2	3.36×10^{-3}	5641	122	0.055	0.068	0.009	
BP-800-3		5641	170	0.077			0.059
BP-800-4		5641	151	0.068			
BP-1000-1		9402	68	0.018			0.017
BP-1000-2	4.17×10^{-3}	9402	59	0.016	0.015	0.002	
BP-1000-3		9402	50	0.014			0.013
BP-1000-4		9402	50	0.014			

P.E.= 0.39. * = RADS. ** = colonies observed. @ = BPA. $ = Proton.