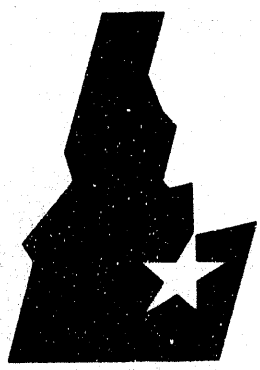

Idaho National Engineering Laboratory

Managed by the U.S. Department of Energy

EGG-BNCT-10016
October 1992

INEL BNCT RESEARCH PROGRAM
JULY/AUGUST 1992

J. R. Venhuizen

EG&G Idaho

Work performed under DOE Contract No. DE-AC07-76ID01570

EGG-BNCT--10016

DE93 005236

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Published October 1992

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Prepared for the
U.S. Department of Energy
Under DOE Field Office, Idaho
Contract DE-AC07-76ID01570

ABSTRACT

This report presents summaries for two months of current research of the Idaho National Engineering Laboratory (INEL) Boron Neutron Capture Therapy (BNCT) Program. Information is presented on development and murine screening experiments of low-density lipoprotein, carboranyl alanine, and liposome boron containing compounds. Pituitary tumor cell culture studies are described. Drug stability, pharmacology and toxicity evaluation of borocaptate sodium (BSH) and boronophenylalanine (BPA) are described. Treatment protocol development via the large animal (canine) model studies and physiological response evaluation in rats are discussed. Supporting technology development and technical support activities for boron drug biochemistry and purity, analytical and measurement dosimetry, and noninvasive boron quantification activities are included for the current time period. Current publications for the two months are listed.

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TABLES

ACRONYMS AND ABBREVIATIONS

BMRR	Brookhaven Medical Research Reactor
BNL	Brookhaven National Laboratory
BNCT	Boron Neutron Capture Therapy
BOPP	Water-soluble boronated porphyrin
BPA	Boronophenylalanine
BSH	Borocaptate Sodium ($Na_2B_{12}H_{11}SH$)
CE&A	Charles Evans & Associates
CHNS	Carbon nitrogen hydrogen-sulfur
CNS	Central nervous system
CT	Computed tomography
CTEM	Conventional transmission electron microscopy
DCP-AES	Direct coupled plasma-atomic emission spectroscopy
DORT	Two-dimensional S_n computer code
ECC	Elaidyl carborane carboxylate
EPXMA	Electron probe x-ray microanalysis
FTIR	Fourier transform infrared spectroscopy
H&E	Hematoxylin and eosin
HPLC	High performance liquid chromatography
ICP-AES	Inductively coupled plasma-atomic emission spectroscopy
INEL	Idaho National Engineering Laboratory
ISU	Idaho State University
IV	Intravenous
LDL	Low density lipoproteins
MCNP	Three-dimensional Monte Carlo computer code
MRI	Magnetic resonance imaging
NMR	Nuclear magnetic resonance
OHSU	Oregon Health Sciences University
ORSU	Oregon State University
OSU	Ohio State University
PAL	Phenylalanine ammonia lyase
PBS	Phosphate buffered saline
SIMS	Secondary ion mass spectrometry
SIRIS	Sputter-initiated resonance ionization spectroscopy
STEM	Scanning electron microscopy
UCLA	University of California, Los Angeles
UofR	University of Rochester
UofU	University of Utah
WSU	Washington State University

INEL BNCT RESEARCH PROGRAM
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INTRODUCTION

This report contains an overview of progress for the Idaho National Engineering Laboratory (INEL) Boron Neutron Capture Therapy (BNCT) Research Program for July/August 1992, including information on the Research Programs, Technical Support Details, and Miscellaneous Project Information.

The following sections are the reports from the various investigators about their current research.

BORON DRUG DEVELOPMENT

Carboranyl Alanine and Low-Density Lipoprotein (LDL) Development and Evaluation

The results of the first series of animal biolocalization studies utilizing boron-loaded LDL have been obtained. The LDL was isolated in the standard manner and loaded with ^{10}B-labeled elaidyl carborane carboxylate (ECC). Final dialysis against phosphate-buffered saline at pH 7.4 produced material suitable for animal injections. The protein content of the resulting phosphate buffered saline (PBS) solutions was found to be 1524 μg/ml. The boron content obtained by prompt gamma spectrometry at Brookhaven National Laboratory (BNL) was 237 μg/ ^{10}B/ml while the boron content obtained by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) at INEL was 180 μg/ml. The lack of concordance between the two values is of concern and suggests that we still have not optimized the ICP-AES sample preparation process. We will continue to monitor this problem by routinely sending samples of animal-destined material to both facilities for boron measurement. Also of some concern is the somewhat low ratio of boron-to-protein which is normally 0.22 ± .02.

The animal protocol was as follows. Mice bearing the B16BL6 murine melanoma on the dorsal thigh were administered a single 400 μl dose of ECC-LDL by tail vein injection. Using the BNL boron content of the sample, this converts to a dose of 95 μg boron per animal or approximately 4.75 mg/kg assuming a 20 g animal. This is a rather small dose of boron; by comparison our standard water-soluble boronated porphyrin (BOPP) dose is ~30 mg boron/kg. The dose is limited by the volume which can be injected in a single injection and by the protein content of the solution. Subsequent studies will examine multiple dosing schedules. Tissues were obtained from sacrifice of six animals each at 6, 12 and 24 hours following administration of the compounds. The results of boron analysis by ICP-AES are presented in Table 1; each number represents the average of six organ samples (in most cases) ± the standard deviation.

It is clear from these preliminary experiments that some partitioning of LDL amongst the organs takes place and does so on a fairly rapid time frame. Tumor and blood boron content are roughly parallel over time, while brain uptake is very small and characterized by large relative percentage errors (not shown). Liver and spleen uptake are large, as expected on the basis of normal LDL receptor populations in these organs. Muscle and skin are slightly lower than expected and kidney uptake is as anticipated. Considering the low boron dose, the difference in lipoprotein metabolism between mice and humans, and the preliminary nature of this "baseline" study, we are reasonably satisfied with these results.

Another batch of ^{10}B ECC-LDL was sent to researchers at Washington State University (WSU) in July and will be evaluated at a larger number and wider range of time points. Both the Principal Investigator and, especially, Dr. Radel have been preparing for and presenting at a series of meetings and review panels; there is thus no significant reportable progress on the amino acid project.

Table 1. Batch LW 1-33 organ boron content (μg/g).

	6 hr	12 hr	24 hr
tumor	5.37 ± 2.32	3.65 ± 1.78	4.11 ± 0.56
blood	5.92 ± 0.53	3.48 ± 1.61	3.07 ± 0.30
brain	1.02 ± 0.21	0.72 ± 0.22	0.75 ± 0.31
skin	3.39 ± 1.09	1.71 ± 0.73	2.46 ± 0.93
liver	28.09 ± 3.86	15.25 ± 9.37	11.18 ± 6.22
spleen	26.69 ± 4.30	19.89 ± 13.55	16.16 ± 8.72
kidney	5.27 ± 0.33	2.78 ± 1.26	2.79 ± 1.01
muscle	2.46 ± 0.69	1.48 ± 0.66	1.01 ± 0.33

Boronated Liposome Development and Evaluation

Liposome Encapsulation and Development: P1798 murine lymphosarcoma screenings of liposomes developed by researchers at the University of California, Los Angeles (UCLA) containing $Na_2(n\text{-}B_{20}H_{18})$ and $NaB_{10}H_9NH_3$, respectively, have been completed at WSU. The first liposomes doped with a lipophilic boron species embedded in the bilayer membrane have been sent to WSU for screening in P1798 murine lymphosarcoma. The species contained in the membrane is $K^+[C_2B_9H_{11}(CH_2)_{16}CH_3]^-$, a *nido*-carborane substituted by a hexadecyl moiety at one of the carbons. Three percent boron by weight was added to the 1:1 DSPC:cholesterol mixture. For this first experiment, phosphate buffered lactose was encapsulated in the aqueous core of the liposome. Following promising results of the buffer encapsulated liposomes, boron compounds known to be retained by the tumor will be encapsulated in the core of boron-doped liposomes to increase the overall boron delivered to the tumor.

Three liposomal suspensions have been sent to Vestar, Inc. for screenings utilizing EMT6 murine adenocarcinoma: $NaB_{10}H_9NH_3$, $Na[Co(C_2B_9H_{11}S)_2]$, and liposomes doped with $K^+[C_2B_9H_{11}(CH_2)_{16}CH_3]^-$. Analysis of the first compound, $NaB_{10}H_9NH_3$, should elucidate whether the tumor retention by $Na_3B_{20}H_{17}NH_3$ is due to the amine functionality or the ability to oxidize to the more reactive $NaB_{20}H_{17}NH_3$ *in vivo*. Analysis of the second compound, the first sulfur functionalized metallocarborane to be investigated, should indicate whether the sulfur moiety in this compound and similar compounds (i.e. $Na[Co(C_2B_9H_{11}SH)_2]$ and $Na[Co(C_2B_9H_{11}S)(C_2B_9H_{11}S(0))]$) will form disulfide bonds *in vivo*, and therefore, be retained by the tumor cells.

Encapsulation of $Na_3B_{20}H_{17}NH_3$ for screening at WSU and for double injection experiments at Vestar, Inc. is imminent.

Compound Development: Fluorescent borane species are currently under development for liposomal encapsulation and subsequent fluorescent analysis. This experiment is critical because it will establish whether the liposomes are entering the tumor cells, as expected from biodistribution results and from Vestar, Inc. results, or merely attaching themselves to the outer cell membrane. Attempts to make a dansyl derivative of $B_{20}H_{18}^{2-}$ have been thwarted by the presence of multiple isomers of the desired product in the reaction mixture. Performing the reaction at lower temperatures and under conditions which should minimize isomerization yield no product formation. A new reaction scheme has been designed. UCLA researchers have established that $B_{20}H_{18}^{2-}$ will react with ethylenediamine, $H_2NCH_2CH_2NH_2$, to form a species analogous to our $B_{20}H_{17}NH_3^{3-}$ compound. The free amine group in the ethylenediamine product allows reaction with known terminal amino acid identification reagents, such as dansyl chloride or more likely, fluorescamine. These reactions are in progress.

Additional reactions to derivatize $B_{20}H_{18}^{2-}$ are in progress. A thiol derivative of $B_{20}H_{18}^{2-}$ has been synthesized by allowing $B_{20}H_{18}^{2-}$ to react with anhydrous NaSH. Isolation of this product is imminent. Attempts to make a cyanide derivative of $B_{20}H_{18}^{2-}$ have not been successful, although UCLA researchers believe the product $B_{20}H_{17}CN^{4-}$ can be synthesized and would be a useful synthon to other $B_{20}H_{18}^{2-}$ derivatives. Alternative routes of synthesis are being planned.

Pituitary Tumor Evaluation

Oregon State University (ORSU) researchers are continuing AtT-20 cell irradiation experiments for Oregon Health Sciences University (OHSU) researchers with the ORSU reactor operating at full power (1 MW) for four minutes. Thermal neutron fluences are approaching 10^{13} n_{th}/cm^2. However, previous contaminating gamma doses appear to have been overestimated by as much as a factor of 2 to 3. New calculations are being made to determine the exact contaminating gamma dose to the pituitary tumor cells. Preliminary studies have indicated that AtT-20 cells are susceptible to BNCT by the fact that cells pretreated with corticotrophin conjugated carborane die at a significantly higher rate than control cells. Further studies are being carried out to verify this finding, and establish the exact parameters for pituitary cell death from BNCT. Additional new controls will include cells that do not have corticotropin receptors.

Reactor dosimetry experiments have been carried out at ORSU to establish the position within the thermal column at a given reactor power and time that cells just begin to show the effects of radiation. At full power for four minutes, this appears to be approximately 50 cm behind an 8 inch bismuth shielding array. This is the position at which an additive BNCT effect will be most easily distinguished. These studies will be repeated before any further BNCT experiments are carried out.

Measurement Dosimetry: Bismuth shielding appears to be eliminating the background and contaminating gamma dose to pituitary tumor cells. Bismuth is now configured around four of the total six sides of the cell containment area. Currently, all cell irradiations are being carried out in the new 4 inch by 12 inch port. With this additional shielding, the anticipated gamma dose should approach 1.5×10^{-11} cGy gamma/n_{th}/ cm^2.

Gamma sensitivity of the pituitary tumor cells is being established separately in a Cobalt-60 source. These experiments are currently in progress.

New smaller 5 ml *in vitro* cell sample holders have been tested in the thermal column to assess possible complications during irradiation. Irradiations lasted for four minutes at full power using distilled water as the contained medium. This encapsulation test proved successful with no spills, leaks, or other complications. These smaller cell vessels will enable more samples to be irradiated simultaneously and enable potentially more bismuth shielding to be used to further reduce the contaminating gamma dose to the cells.

Researchers are investigating the potential of using the new inductively coupled plasma-mass spectrometer located at ORSU College of Oceanography for quantitating intracellular levels of boron in our *in vitro* and future *in vivo* experiments. Anticipated levels of sensitivity of this unit for boron are in the part per billion range, thus quite applicable for research needs.

BORON LOCALIZATION SCREENING

Boronophenylalanine (BPA) Evaluation Studies

WSU College of Pharmacy researchers are continuing to investigate the effect of diet manipulation on BPA uptake.

Phenylalanine Ammonia Lyase (PAL)/BPA Toxicity: PAL (@ 0.025 units) was added to B16BL6 murine melanoma cells in culture. One hour later, BPA was added to cultures at a final concentration of 1 mg/ml. After six hour incubation period, medium was replaced and cell growth monitored. Cell proliferation was observed to be normal, indicating no toxic effects.

Three BDF mice were injected with 4 mg of BPA by intravenous (I.V.) injection (about 100

mg/kg) and observed for adverse reactions. Two mice were sacrificed at six hours post injection and tissue samples were collected and will be sent to INEL for analysis. No toxic effects were noted.

In Vitro BPA Dose Response Studies: Cellular BPA uptake by murine melanoma cells after treatment with PAL was investigated. Phenylalanine in cell culture medium was reduced using PAL for one hour. Then varying levels of BPA were added for six hours in culture. Cells were harvested, counted, pelleted, and sent to INEL for boron analysis. Results are pending.

In Vivo Enhancement of Boron Uptake by PAL: BPA biodistribution in BL6 melanoma bearing mice was screened following enzymatic depletion of plasma levels of tyrosine and phenylalanine by PAL. Mice were injected I/V with 0.2 units of PAL one hour prior to I/V injection of BPA. BPA concentration was administered at either a concentration of 1 mg/mouse or 4 mg/mouse. Time points used for screening were 6 and 24 hours. All samples have been sent to INEL. Results are pending.

Boron Screenings

Boron screenings are continuing at the WSU College of Pharmacy.

LDL Studies

Screening of Dr. Stephen Kahl's first and second compounds has been completed in the B16-BL6 murine model, and samples have been sent to INEL. Results are pending.

Liposome Evaluation Studies

The following four liposome preparations were screened in mice bearing P1798 lymphosarcoma tumors.

$Na_4B_{20}H_{17}OH$: Boron levels peaked at 12 hours for tumor at 11.02 μg/ml. Boron levels were highest at six hours for both blood and spleen (54.76 μg/ml and 656.96 μg/ml respectively). The maximum level for liver was at 6 and 12 hours (32.05 μg/ml). Tumor:organ ratios are shown in Table 2.

$Na_2B_{20}H_{18}$: Tumor and blood samples were sent to INEL for analysis. Remaining samples are in -20°C storage. Results are pending.

$NaB_{10}H_9NH_3$: Blood, tumor and brain samples were sent to INEL for analysis. Remaining samples are stored in 1-20°C storage. Results are pending.

$Na_2B_{10}H_9NCO$: Partial analysis of tumor and blood samples taken from this boron screening are currently underway.

Table 2. Tumor:organ ratios, $Na_4B_{20}H_{17}OH$ in liposomes.

Time (hours)	Tumor:Blood	Tumor:Liver	Tumor:Spleen
0	1.41:1	0.48:1	0.44:1
6	0.16:1	0.27:1	0.15:1
12	0.43:1	0.34:1	0.40:1
18	0.33:1	0.38:1	0.54:1
24	0.47:1	0.26:1	0.48:1
48	1.01:1	0.26:1	0.64:1
72	1.30:1	0.55:1	0.58:1

Miscellaneous: Liver and spleen sections were sent to Washington Animal Disease Diagnostic Laboratories at WSU for histopathologic analysis to test for lymphosarcoma metastasis. The histologic diagnosis showed acute, severe, necrosis of the liver. The spleen showed extramedullary hematopoieses. The final diagnosis is that lymphosarcoma was found both in the liver and spleen and was metastatic.

DRUG STABILITY, PHARMACOLOGY AND TOXICITY

Idaho State University (ISU) researchers are continuing their drug toxicity studies using small animal (rat) models.

Toxicological Evaluation of BPA

I.V. infusion of 2,822 mg/kg BPA over one hour is not lethal if rats are subject only to minimal stress. Four rats infused with this dose of BPA have been allowed to survive for seven days before sacrifice. Infusion of BPA at a rate of 1,487.5 mg/kg/mg/kghr for three hours (total dose: 4,462 mg/kg) is lethal (three of three rats died: two died before completion of the infusion, one died three hours after completion of the infusion). If rats are subject to additional surgical stress, or to the stress of additional physiological instrumentation, a lower dose of BPA will be lethal. For example, a dose of 2,125 mg/kg BPA is usually lethal in open chest rats whose cardiac output is being monitored. Doses of 2,822 mg/kg are usually lethal to instrumented rats. The lethal effects of the BPA formulation cannot be attributed to the volume delivered, the rate of volume delivery, or changes in system osmolarity. Control experiments demonstrated that infusion of equal or larger volumes of a control solution in which mannitol replaced the BPA, (formulation osmolarity equivalent to 4,800 mg/kg BPA) did not elicit BPA-type effects. Infusion of BPA resulted in an initial increase in blood pressure, cardiac output, stroke output and heart rate, followed by a slow but sustained reduction in these parameters. Within two hours of infusion, cardiac output was often reduced by 50%. There was little or no change in central venous pressure. Left ventricular pressure data following BPA infusion has been recorded, but dP/dt has not yet been calculated, so it cannot yet be determined if ventricular contractility has decreased. Collection of left ventricular pressure data from animals receiving control infusions are scheduled. Animals supplemented with artificial ventilation die about the same time post-BPA infusion as non-ventilated rats. This observation, coupled with the speed of death (at most a few hours post-infusion), and the apparent normalcy of rats (observation period: seven days) given high but sublethal doses, suggests that cardiovascular collapse is the major limiting toxicity of BPA. Although cardiovascular collapse only occurs after large doses of BPA, its apparent potentiation by stress needs to be further characterized, since most human candidates for BPA/BNCT will be under significant stress and thus may be more susceptible to the toxic effects of BPA.

Toxicological Evaluation of Borocaptate Sodium (BSH)

It was previously reported that i.v. infusion of 625 mg/kg BSH in distilled water (100 mg/ml) was lethal to Long Evans rats. However, review of that data indicated that while the doses of BSH were intended to be 625 mg/kg, they were actually closer to 750 mg/kg doses. These experiments have been repeated and have shown that i.v. infusion of 625 mg/kg BSH is also lethal to Long Evans rats.

It has been previously reported that Long Evans rats infused with 500 mg/kg BSH, but not instrumented for recording of physiological parameters, survived longer than rats infused with the same BSH and instrumented for recording of physiological parameters. However, these rats sill died about 24 hours after the BSH infusion. These rats were anesthetized with urethane, an anesthetic that is known to cause liver damage. Thus, the delayed deaths may have been secondary to anesthetic-induced hepatotoxicity rather than BSH related. To investigate this possibility, rats were anesthetized with pentobarbital, BSH (500 mg/kg) was infused and the rats were allowed to recover. One rat died within 24 hours of infusion, while a second rat died six days post-infusion. The current data suggest that BSH, not urethane, was responsible for the delayed deaths. Additional pentobarbital-anesthetized rats will be treated with BSH to determine if

urethane administration exacerbated BSH toxicity.

It was previously reported that osmotic controls did not mimic the toxic effects of BSH infusions. These studies have been expanded by using mannitol as an osmotic replacement for BSH. Three animals infused with a mannitol solution osmotically equivalent to 625 mg/kg BSH (same volume, same rate of infusion as BSH experiments) maintained good health until sacrificed 7+ days after the infusion. This data is consistent with the previous suggestion that the toxic effects of BSH cannot be ascribed to volume loading or changes in systemic osmolarity.

BSH is typically infused at a concentration of 100 mg/ml, which is about four times the iso-osmotic concentration of BSH. To determine if the concentration of BSH affects the expression of toxicity, a series of experiments was begun by infusing BSH at the same volume rate (17 ml/kg/hr), but at a different dose rate and concentration. A concentration of 25 mg/ml was chosen because it is approximately iso-osmotic. Infusion of 625 mg/kg BSH at a concentration of 25 mg/ml (N = 2) is also lethal. Additional experiments are underway to expand the N value and so confirm this observation.

It is hypothesized that the delayed death following 500 mg/kg BSH might reflect shock-induced renal damage secondary to inadequate tissue perfusion. To test this hypothesis, evaluation of the antidotal value of two forms of anti-shock therapy; dobutamine infusion and dextran-40 infusion is underway. Control experiments to establish infusion rates for dobutamine and dextran have been completed and the effectiveness of these therapies in protecting BSH treated rats will soon be evaluated.

TREATMENT PROTOCOL DEVELOPMENT

Large Animal Model Studies

Pharmacokinetics: "Lynny Bob" Elliott (#35447-165), six year old castrated male Standard Poodle, was referred to WSU on August 4, 1992 for circling to the right, lethargic, disorientation and aggressive behavior. The computed tomography (CT) and magnetic resonance imaging (MRI) scans reveled an enhancing mass in the left frontal region with other areas of enhancement in the right hemisphere. Since irradiation treatment is not currently being offered; "Lynny Bob" became a candidate for a terminal 100 mg/kg split dose boron pharmacokinetics study. "Lynny Bob" was infused with two 50 mg/kg infusions 24 hours apart. At the end of the blood sampling on the second day, "Lynny Bob" was euthanized, and perfusion and a necropsy were performed. Histological results are pending.

Normal Tissue Tolerance (Neutron Irradiation): All the dogs that have been irradiated are clinically normal.

The last dog in the 25 μg ^{10}B/gm (BSH) and 19 Gy group was irradiated at the BNL on July 16, 1992. This dog was scheduled to be irradiated in April but problems with the reactor prevented irradiation of this dog.

Three dogs, all at 25 μg ^{10}B/gm and 23 Gy, had their six month post irradiation checkups at the end of July.

All four dogs in the 0 μg ^{10}B/gm and 12.5 Gy group, one dog from 100 μg ^{10}B/gm and 27 Gy, and two dogs from 50 μg ^{10}B/gm and 27 Gy had their 12 month post irradiation checkups. At this time the dogs were euthanized and necropsied. Histological results are pending.

Spontaneously-Occurring Brain Tumor Dogs: "Dudley" Fiset (#35447-151), a seven year old castrated male Golden Retriever, is doing great. "Dudley" had his 18 month checkup in Berkeley, CA. Physical exams and blood work were clinically normal. CT and MRI results will be sent to WSU.

"Brandy" Hoff (#35447-94), a seven year old spayed female Golden Retriever, is doing great.

"Muffy" Lower (#35447-140), a five year old female spayed Terrier cross, was returned to WSU the first week of July. Her neurological signs, circling, head tilt, and disorientation were increasing, despite steroid therapy. Muffy was euthanized after MRI and CT scans were performed. Histological results are pending.

Physiological Response Evaluation and Interdiction

Cellular Basis of Central Nervous System (CNS) Injury: BNCT vs. Photon: University of Rochester (UofR) researchers have exposed Long Evans rats to a total gamma dose of 60 Gy utilizing a Picker C8 cobalt source. A Lexan stereotaxic frame was used in conjunction with a collimator to restrict exposure to a region involving the cortex, striatum, fimbria, corpus callosum, and hippocampus. Before the sacrifice, locomotor tests and MRI studies were serially obtained. At the time of sacrifice, cerebrospinal fluid and serum samples were collected for myelin basic protein studies. Horse radish peroxidase perfusion was done on some rats just prior to sacrifice and all of the rat brains were prepared for electron microscopy analysis as well as hematoxylin and eosin (H&E) histopathology.

Animals are now being sacrificed at their longest time points of 30 and 32 weeks. Earlier time points of 2, 6, 12 and 24 weeks are completed. Compared to control animals, 5 to 10 fold increase in blood-brain-barrier leakage has been observed and is most evident at the 12th week post-irradiation time point. Progression is clearly evident at 24 weeks. At that time (24 weeks) there is clear evidence of demyelination. The first expression of vascular injury is a visible lesion seen with MRI *in vivo* and its earliest detection is at 2 to 6 week time intervals.

Therapeutic Ratio in RAT 9L Gliosarcoma: BNCT vs. Photons: Fisher 344 rats were implanted with 9L gliosarcoma, 14 days later they received irradiation utilizing BNCT only, x-rays only, or BNCT and x-rays. This work was done at BNL (Jeff Coderre). The methods used in treating these animals was described in *Radiation Research* (Coderre, et. al., 129: 290-296, 1992). Twelve animals surviving one year and appearing clinically well but not subjected to locomotor analysis were brought to the UofR and sacrificed for histopathologic, horse radish peroxidase and electron microscopy studies. The twelve animals consisted of three control rats, four BNCT only, two x-ray only, and three BNCT and x-ray.

Preliminary analysis of the H&E histopathology, horse radish peroxidase studies and electron microscopy analysis dramatically show a very selective effect of boron neutron capture as compared to x-ray photon or combined photons and BNCT irradiated animals. Animals receiving photon irradiation survived but with significant injury; this injury consisted of severe brain atrophy, hydrocephalus, demyelination and white matter necrosis, loss of normal neuronal cellular populations, very severe disruption of the blood-brain-barrier and considerable leakage of horse radish peroxidase, whiting out the fine microcirculation of the brain in the striatum and hippocampus in particular. By contrast, the BNCT only brains were comparable to the normal brain a few high power fields removed from the tumor bed scar. The microcirculation was intact, all sections particularly the contralateral brain, in the hippocampal area and the striatum, were quite normal in cellularity. The study of the other organs have thus far revealed cataracts in only the BNCT animals (but the retina appears to be intact).

This histopathologic evidence for the first time dramatically demonstrates the selective affect of BNCT. It fulfills the predictions of the microdosimetry, analytic and stochastic models that there may be significant sparing of the microcirculation of the normal brain. That is, even with no differential in boron salt concentration in the tumor as compared to normal brain, the calculated microdosimetry sparing of the vascular wall could be producing a more favorable therapeutic ratio than is possible with photon irradiation.

Future Planned Studies: In progress are collaborative efforts with BNL (Jeff Coderre) to increase the boron loading doses with BSH and BPA, as well as increasing the exposure times to the equivalent dose of 30 to 60 Gy radiation to the capillary tree microvasculature of the normal brain in anticipation of producing injury with BNCT. BSH compound is now available and such work is now in progress.

SUPPORTING TECHNOLOGY DEVELOPMENT

Task 1: Biochemistry of BSH and Its Oxidation Products

No activity to report.

Task 2: Noninvasive Boron Quantification

Research on high field nuclear magnetic resonance (NMR) studies of the interaction between BSH and serum albumin is continuing at the University of Utah (UofU).

To understand the saturation of the longitudinal relaxation rates of BSH at high concentration of serum albumin the data were further analyzed mathematically using a saturation function

$$Y=Y_o-ae^{-bx},$$

where x is the concentration of albumin, Y is the observed longitudinal relaxation rate at different concentration of albumin, Y_o is that when x approaches infinity. In other words, Y_o is the longitudinal relaxation rate of bound boron, because all BSH is supposed to be bound at such a high concentration of albumin according to the binding equilibrium. The rate of bound BSH with three types of albumin at 295°k and 310°k are obtained, and will be reported later.

According to the equation

$$R_1^o=R_1^b f+R_1^u(1-f),$$

where R_1^b is the relaxation rate of bound BSH from the above analysis and R_1^u is that of unbound BSH, which can be measured directly, the concentration of bound and unbound BSH can be evaluated from the binding fraction (f). Assume there is only one kind of binding in the interaction, the binding constant (k) and number of binding sites (n) can be calculated from the linear regression:

$$\frac{[B]^b}{[B]^u[SA]_o}=kn-k\frac{[B]^b}{[SA]_o}$$

where $[B]^b$ is the concentration of bound BSH, $[B]^u$ is the concentration of unbound BSH, and $[SA]_o$ is the original concentration of serum albumin.

The k (in $mole^{-1}$) and n at 295°k and 310°k were calculated and will be reported later.

Task 3: Real-Time Measurement Dosimetry Research

An experimental arrangement for measuring the angular and energy distribution of source neutrons arising from 2 MeV protons incident on a "thick" lithium target has been assembled at ISU van de Graaf accelerator by an Associated Western Universities student. Preliminary neutron data from this target have been obtained using a Helium-3 detector for the angular distribution, and a photon recoil counter for the energy distribution. Measurements have been made to characterize the detector sensitivity.

Task 4: Analytical Dosimetry

Task 4A: Macrodosimetric Model Development

No activity to report.

Task 4B: Microdosimetric Model Development

Calculations for the planned rat irradiations in the Brookhaven Medical Research Reactor (BMRR) thermal neutron beam were completed and the results were tabulated for use. An engineering design file report is in preparation.

Task 4C: Microdosimetric Cellular Response Study

No activity to report.

TECHNICAL SUPPORT CORE ACTIVITIES

Task 1: ICP-AES Analyses of Boron in Biological Samples

Both INEL ICP-AES instruments are fully operational. The new samples received this period were from ISU and WSU.

Samples Received	993
Samples Prepared for Analysis	~1545
Samples Analyzed	~1000
Backlog	
Awaiting Preparation	~2207
Prepared, Awaiting Analysis	780

Task 2: Boron Compound Purity Determinations

One batch of unenriched $Na_2B_{12}H_{11}SH$ (Na_2BSH) was received in the month of June from Callery Chemical Company. The Na_2BSH shipment contained a total of 30 grams packaged in 5 g quantities. One vial was opened for the purity investigations, this vial represented the Callery Chemical Lot number (2351-82-1(91-238)). Two grams of the opened vial were transferred under nitrogen to a crimp top vial for archive purposes, one gram was used for the analyses and the remainder was stored for other uses. A laboratory specific number (BNCT 439) was assigned for tracking purposes. The purity of BNCT 439 was determined by high performance liquid chromatography (HPLC), NMR, carbon-hydrogen-nitrogen-sulfur (CHNS) analysis and ICP-AES.

HPLC Analyses: The chromatography was performed on a 250 x 2 mm column packed with 5 μm particles of Nucleosil® C_{18}. The mobile phase was approximately a 50% methanol - 50% water solution with 5 mM tetra bulyl ammonium sulfate ion pairing reagent flowing at 0.4 ml/min. The described conditions are ideal for evaluating the BSS^{-4} and $BSSO^{-4}$ component concentrations and overall sample integrity. A slower flow rate and changes in the methanol concentration enhance the separation of the $B_{12}H_{12}^{2-}$ from the BSH parent peak.The HPLC analysis results for the new BSH sample are listed in Table 3. A sample chromatogram of BNCT 439 is displayed with the reference sample, BNCT 202, in Figure 1.

The chromatographic analysis scheme included preparation of the new sample (BNCT 439) in triplicate, an individual preparation of the reference sample (BNCT 202), and duplicate chromatograms of all samples. The chromatogram of BNCT 202 in Figure 1 shows a slight retention time shift due to equilibration. The sample chromatograms of BNCT 439 and 202 indicate the presence of the late eluting peaks, although at a somewhat lower level in BNCT 439. At this time the identity of these products is still unknown. The HPLC analyses were unclear regarding the presence of the $B_{12}H_{12}^{2-}$ component in the BNCT 439 sample. The NMR spectrum was unremarkable but may hint at the presence of a limited quantity of $B_{12}H_{12}^{2-}$ in the sample.

ICP-AES Analysis: Elemental Analysis for B, S, and Na by ICP-AES were performed on aliquots of all samples and standards prepared for HPLC. The results of these analyses are presented in Table 4. The wt % of B for many of the samples indicated a slight over recovery, possibly pointing to the presence of $B_{12}H_{12}^{2-}$.

CHNS Combustion Analysis: A nitrogen atmosphere was utilized as much as possible for preparation of the samples used in the combustion analyses. The results of these analyses are presented in Table 5. Sulfur results were once again not reported since they are irreproducible and usually low.

Table 3. Oxidation products in BNCT 439 (new BSH) and the reference sample (BNCT 202) as determined by HPLC.

Sample	Compound	BSS^{4-} (wt%)	$BSSO^{4-}$ (wt%)
202	$Na_2B_{12}H_{11}SH$	1.04 ± 0.14	0.13 ± 0.02
439	$Na_2B_{12}H_{11}SH$	0.95 ± 0.18	0.58 ± 0.05

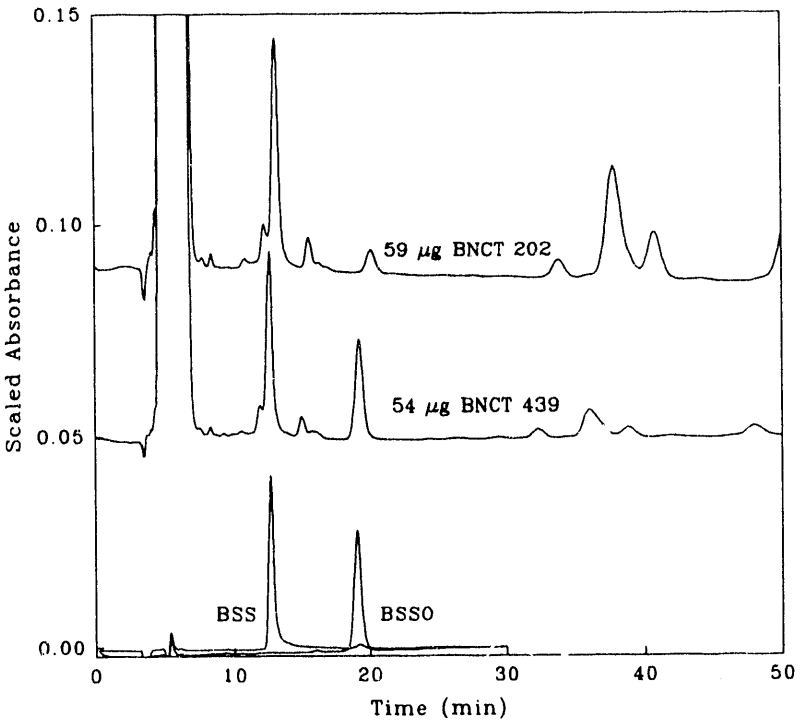

Figure 1. Reversed phase, ion pair HPLC chromatograms of the new compound (BNCT 439), the reference sample (BNCT 202), and standards of BSS^{4-} and $BSSO^{4-}$.

Table 4. ICP-AES analysis of BSH compounds.

					Mole Ratios		
Sample	Compound	wt% S	wt% B[a]	wt% Na	B/S	B/Na	Na/S
202	$Na_2B_{12}H_{11}SH$	11.7	55.7	17.8	14.2	6.7	2.1
210	$Cs_4{}^{10}B_{24}H_{22}S_2$	7.5	29.7	0	12.4		
212	$Cs_4{}^{10}B_{24}H_{22}S_2$	6.8	31.5	0	13.4		
439A	$Na_2B_{12}H_{11}SH$	12.9	61.1	19.7	14.1	6.7	2.1
439B	$Na_2B_{12}H_{11}SH$	12.7	61.5	19.1	14.4	6.9	2.1
439C	$Na_2B_{12}H_{11}SH$	14.7	62.6	20.0	12.8	6.7	1.9

[a] All samples have been corrected for isotopic abundance.

Table 5. CHN combustion analysis results.

Sample	wt% C	wt% H	wt% N
313	0.26 ± 0.20	7.11 ± 0.24	0
439	0.06 ± 0.09	7.38 ± 0.26	0
Expected		5.5	

The weight % for H may provide evidence that the older sample (BNCT 202) may be "wet", most likely enhanced by it's frequent handling. Although carbon is present, the concentration appears to be slightly reduced when compared with previous batches.

NMR Spectroscopy: The natural abundance B NMR of the new BSH batch indicates that BSH is the primary constituent. However, there was some possibility that $B_{12}H_{12}^{2-}$ may be present at low levels. The $B_{12}H_{12}^{2-}$ component was not confirmed by HPLC analysis probably due to it's low molar absorptivity.

Fourier Transform Infrared Spectroscopy (FTIR) Spectroscopy: The new and reference samples were prepared for FTIR analysis entirely under nitrogen. The actual quantity of sample in each pellet was not accurately determined, consequently, some pellets had more sample material in them than others thus, the spectra in Figure 2 have been normalized for clarity and to offset the sample mass differences. All single-beam infrared spectra were ratioed to a single beam spectrum of a blank KBr pellet. The spectrum the BNCT 313 sample used as a reference sample and indicates a significant presence of water represented by the peaks at ~3500 cm^{-1} and ~1640 cm^{-1}. Organic contamination is also notable in the BNCT 313 spectrum because of the peaks between 2900 and 3000 cm^{-1}. By comparison, the new sample, BNCT 439, appears to be quite dry and free from significant organic contamination.

Task 3: Intra- and Intercellular Boron Analyses

Secondary Ion Mass Spectrometry (SIMS): Tissue preparation procedures specific for this project are being developed by Charles Evans & Associates (CE&A) and WSU researchers. The methodology is based on cryotechniques to stabilize and "fix" ultrastructures of the specimen cells *as it exists under physiological conditions*. Tissue preparation by chemical fixation is avoided to prevent possible leaching of endogenous diffusible elements. Under ideal experimental conditions, cryofixation will preserve the constituents of the biological system

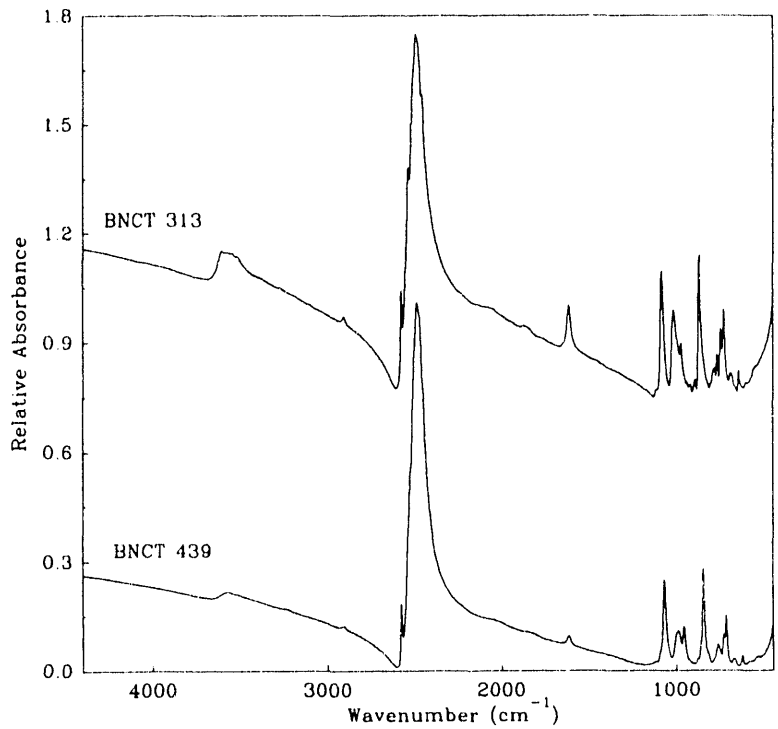

Figure 2. FTIR spectra of the new BSH compound (BNCT 439) and a representative reference (BNCT 313, $Na_2{}^{10}BSH$).

in their viable position and minimize ice crystal formation to within a range which is smaller than the spatial resolution of the observation technique.

We are currently evaluating the cold metal block freezing technique with liquid nitrogen using a Reichert-Jung (distributed by Leica) MM 80E Metal Mirror Cryofixation System for ultrarapid freezing. This method provides cooling rates in the vicinity of 50,000°K/sec and good freezing (vitrification) to a depth of about 10-15 μm. With liquid helium a freezing front of about 30 μm may be achieved. A test of tissue viability will be developed by WSU researchers to determine the physiological state of the tissue at the time of freezing. An *in-situ* ultrarapid cryofixation method is also being considered as an alternative technique. A flow chart outlining the general sample preparation procedures from chemical fixation and ultrarapid freezing to SIMS analysis is shown in Figure 3. Examples of different tests of viability and rapid freezing methods are illustrated in Figure 4.

In addition to preparing freeze-dried samples for SIMS analyses, freeze substituted and embedded samples will also be prepared in parallel to evaluate tissue quality during ultrarapid freezing. This is an important control to establish that no loss in tissue integrity occurs during sample transfer and freeze-drying. The flow chart in Figure 3 indicates the final analysis technique to be SIMS. However, ultrarapid freezing and freeze-drying of tissues is also appropriate for conventional transmission electron microscopy (CTEM), scanning electron microscopy (STEM), electron probe x-ray microanalysis (EPXMA) and sputter-initiated resonance ionization spectroscopy (SIRIS) analyses.

Preliminary sample preparation experiments with rats infused with boron were performed at WSU in June/July using CE&A's cryofixation unit. WSU (Dr. Swartz's group) prepared the rats while CE&A (Dr. Chia) cryofixed the brain and liver tissues. Each member of the WSU group practiced with the unit to familiarize with the technique. The samples are currently

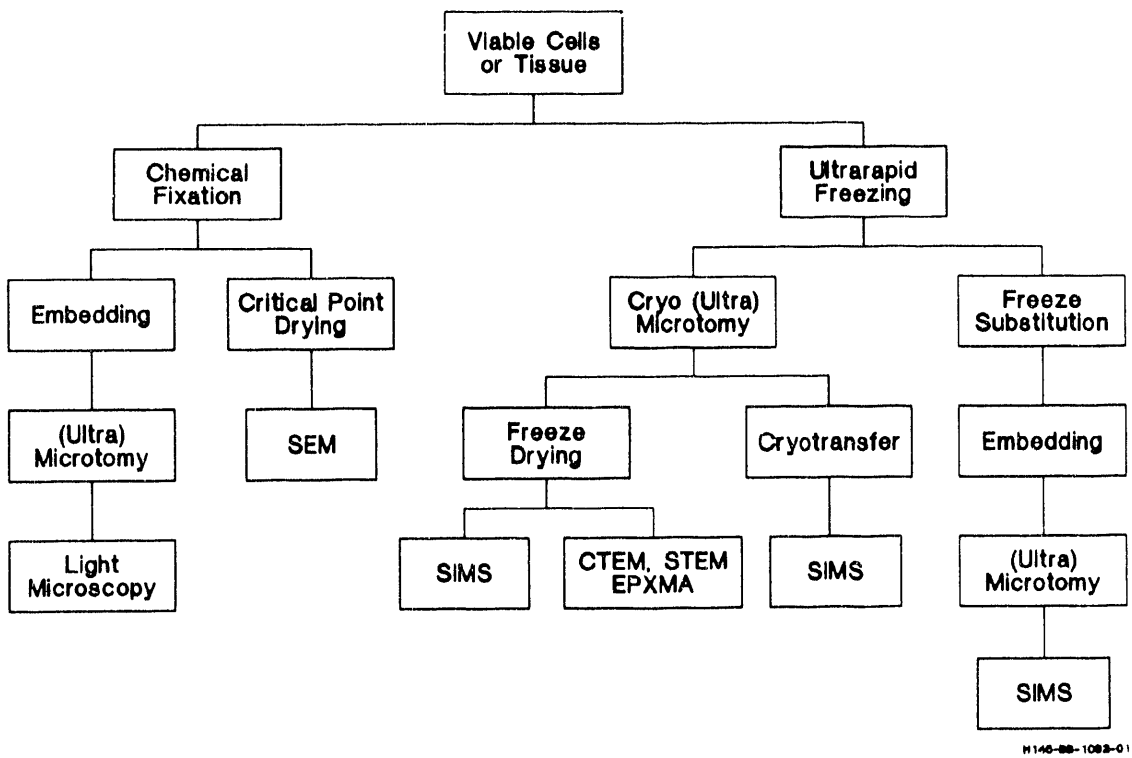

Figure 3. Biological tissue preparation flow chart.

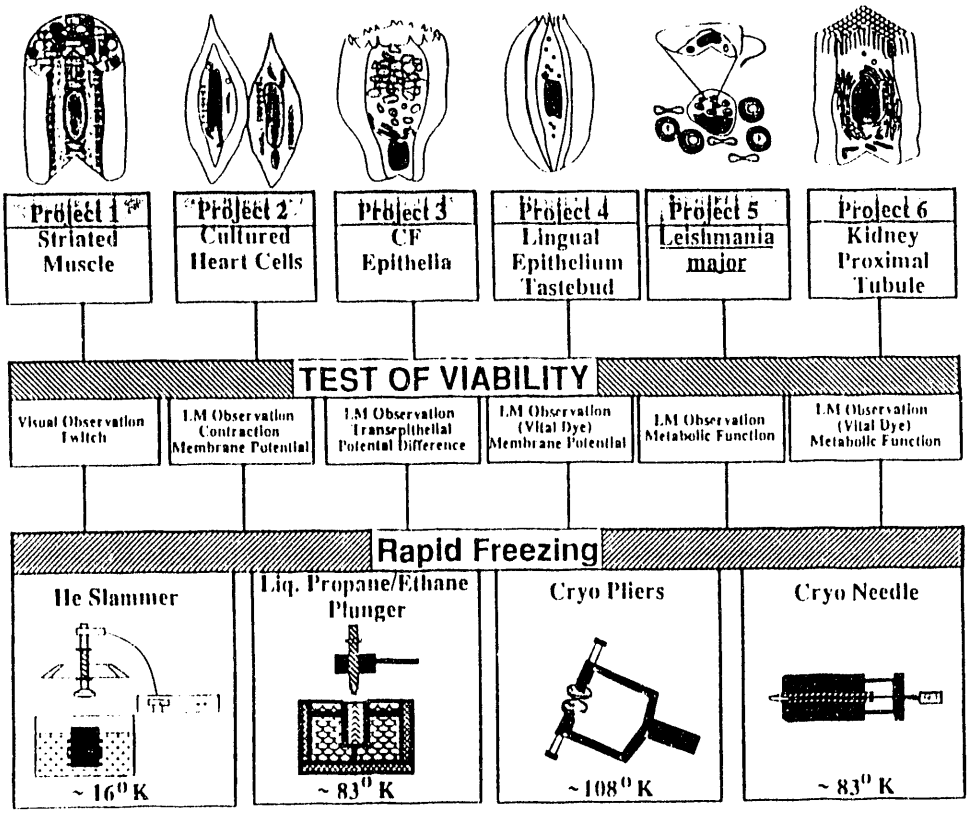

Figure 4. Tests of viability and cryofixation methods.

stored in liquid nitrogen at WSU awaiting cryoultramicrotomy. The present emphasis is to develop preparation protocols for viable, freeze-dried specimens. However, the best sample preparation protocol is to maintain the tissue in a frozen hydrated state at a temperature below the ice crystallization point of about 130°K and to cryotransfer the sample to a cold stage in the SIMS instrument for analysis.

Once the sample preparation protocols are established there are two possible sites for sample preparation. The first possibility is to perform all sample preparations at WSU where the animal studies are carried out. However, WSU does not possess a cryofixation unit, a cryoultramicrotome or a freeze-dryer - all of which are available at CE&A. A primary goal of CE&A, therefore, is to demonstrate the importance of correct sample preparation by obtaining quality SIMS images. CE&A researchers are actively pursuing this in conjunction with other collaborators. Once it has been demonstrated that quantitative boron distributions can be located in cellular structures by SIMS, a decision can be made to establish WSU or WSU and CE&A as the center for sample preparation for this research project.

Task 4: Neutron Beam Measurement Dosimetry

The last dog of the current dose tolerance series (left over from the April group) was irradiated July 16 at the BMRR. The scheduled dose was 15 Gy at a 25 ppm boron loading. This was done as a split irradiation, with a blood sample drawn between irradiation segments. Direct coupled plasma atomic emission spectroscopy (DCP-AES) was used for all blood analyses, no capture gamma analysis was done.

A new beam delimiter for use at the thermal port at the BMRR was fabricated and sent to Dr. Jeff Coderre at BNL. It is for use for rat irradiations using the existing BNL rat holder. The delimiter is made from the same kind of Li_2CO_3-loaded polyethylene material (93% enriched in Li-6) that was installed for additional shielding at the epithermal neutron filtered beam port. The delimiter is a 25.4 x 25.4 cm piece 2.5 cm thick designed to fit in the bismuth gamma shield at the thermal port. The aperture is conical, tapering from 6 cm diameter on the reactor side to 2 cm at the beam exit.

A neck collar of the same material was also fabricated and sent to BNL. This collar is a piece 1.27 cm thick, 5.0 cm OD with a 2.2 cm hole in it. It's purpose is to go around the rat's neck and fit into the BNL rat holder. The collar is split into two semicircular pieces to facilitate placing it around the rat's neck.

Task 5: Canine Dosimetry Calculations

No activity to report.

Task 6: BNCT Database Management System

Database development and data entry continue at WSU.

Task 7: Georgia Tech Research Reactor Physics Support

No activity to report.

Task 8: Research Reactor/Accelerator Physics Support

The calculated results for an accelerator-based neutron source for epithermal BNCT that were published recently by the group at Ohio State University (OSU) were independently confirmed at INEL using the three-dimensional Monte Carlo computer code (MCNP) code. The intensity and spectral quality of the existing BMRR epithermal beam, operating at 1 MW of reactor power, can be roughly duplicated using the OSU accelerator source design operating at 30 mA and 2.5 MeV. A deterministic model of the OSU design has been developed using the two-dimensional S_n computer code (DORT) discrete ordinates code. This will provide a baseline for planned INEL work in this area. INEL personnel intend to look at beryllium targets (as opposed to lithium) and to examine the effects of certain changes to the OSU neutron filter design.

Miscellaneous:

Program principal investigators participated August 11 in the Department of Energy Office of Health and Environmental Research Nuclear Medicine Research Review of the INEL BNCT Program held in Chicago, IL.

END

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