BIOLOGICAL APPLICATIONS OF CALIFORNIUM - 252: UTILIZATION AND DOSIMETRY OF AN IRRADIATION FACILITY AND DESIGN OF AN IRRADIATION ASSEMBLY FOR USE WITH SMALL BIOLOGICAL TARGETS.

A THESIS

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by

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DEDICATED TO

My loving wife, Cathy

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ABSTRACT

The objective of this work was to design and construct an irradiation assembly that could be used in making a preliminary investigation of the potential of using californium-252 as a source for radiation effects on small organisms. Determinations of fast neutron, thermal neutron, and gamma ray dose rates were made, thereby permitting preliminary investigations with a biological target.

Male pupae of the greater wax moth, Galleria mellonella (L.) were used as representative example of a small animal. The effects of irradiation with ^{252}Cf were compared with those already known to result from gamma radiation on this insect species. Indications are that irradiation with ^{252}Cf causes less somatic damage for given degrees of induced sterility when compared to irradiation with gamma rays.

It was found that ^{252}Cf neutrons have a quality factor (QF) range of 3-20 in inducing F_1 sterility. The relatively low dose rate from irradiation with ^{252}Cf as presently designed and constructed made a more specific QF estimate difficult. Both the source size and the dimensions of the irradiation facility and assembly permitted only low dose rates. An increase in the source size and provisions to provide closer source to specimen placement would increase the dose rate and thus probably greatly improve further investigative capabilities.

Although this investigation is principally of an exploratory nature, it has provided a basis for more extensive investigations into the potential of ^{252}Cf as a tool for more advanced work in radiation biology.

CHAPTER I

Introduction

The use of X-ray and gamma radiation as providing a means of controlling harmful insect populations has received considerable attention in the past 15 years. The impetus for many researchers to undertake studies in insect radiation biology came from the dramatic success by the U. S. Department of Agriculture in eradicating the costly screw worm fly from the southern United States. This work, under the guidance and research findings of Dr. E. F. Knipling of the Entomology Research Division, proved that radiation-sterlized male flies when released in sufficient numbers to mate with natural population females, would eliminate the natural population within 6 generations (Knipling, 1955). The result was the saving of millions of dollars annually in the cattle industry, and the preservation of untold numbers of wildlife and game animals.

The ensuing literature in radiation biology reveals many attempts at various scientific laboratories to control a broad range of insect pests by radiation sterilization. With few exceptions, much of this work has been done with X-ray and gamma sources. Relatively little work has been done with neutron-emitting sources due to their scarcity, dispersement and high cost. These factors, coupled with the complexity of neutron radiation and its dosimetry, have been drawbacks in the study of neutron radiation effects in insects.

The problem of unavailability of neutron sources for radiation biology has been partially solved by the availability in milligram quantities of the man-made

radioelement, californium-252. This isotope, produced by intense neutron bombardment of ^{239}Pu, is an excellent and portable source of neutrons. It decays by both alpha emission and spontaneous fission in a ratio of about 31:1. Each fission also produces an average of 3.76 neutrons emitted in a fission spectrum having an energy of 2.3 million electron volts (MeV), and a most probable energy of 0.8 MeV. Californium-252 also emits 1.3×10^{13} gamma photons per second per gram.

Californium-252 offers many advantages and few disadvantages when compared with other neutron sources (such as nuclear reactors, Cockcoft-Walton generators and nuclear accelerators). These advantages are:

1. Readily available (Louisiana State University Californium Demonstration Center, Intelcom Rad Tech, Savannah River Lab of U.S.A. E. C., and several commercial ventors).

2. Reasonably long half life (2.646 years)

3. High neutron emission rate (2.34×10^{9}n/sec-mg) compared with other small and portable sources such as plutonium-beryllium (PuBe).

4. Low heat generation (38.5 milliwatts/mg)

5. Small volume and mass

6. Portability dependent only on shielding requirements

7. Low maintenance cost

The disadvantages of ^{252}Cf as a neutron source are few, but among them are:

1. Its mixed radiation field (namely neutron and gamma rays) causes difficulties in studying effects of neutrons only. However, as a radiation source for causing genetic damage, the mixed field may prove an asset.

2. The complexity and instrumentation costs for accurate neutron and secondary gamma dosimetry,

3. High expense in producing ^{252}Cf.

In spite of these drawbacks, the numerous advantages of using ^{252}Cf and the relative scarcity of work on neutron radiation biology of insects prompted this study. Additionally, neutrons offer a potential for causing greater biological effects in target tissues relative to X-ray and gamma ray effects (Morgan, 1968). This relative increase, ranging in estimates from 2.5 to 10 times that of photon radiation theoretically might produce significantly different kinds of radiation damage in target tissues.

Lamb et al. (1967), in working with Drosophila melanogaster showed that neutrons have roughly a two fold mutagenic effect on male germinal cells over X-rays. Neutrons have also damaged oogonia of Drosophila melanogaster more than X-rays (Dickerman, 1967).

Murakami (1966) found that neutrons were six times more effective than ^{137}Cs gamma rays in producing mutations in the silkworm.

Baldwin (1966) found that neutrons produced 30% higher mutations than ^{60}Co gamma rays in Dahlbrominus.

Thus, in view of these factors, there was a clear need to investigate the possibility of using ^{252}Cf as a radiation source in potential insect control programs.

Finally, because a large amount of work has been done at this University by Dr. Ross Nielsen, U. S. Department of Agriculture, and Dr. E. N. Lambermont, Nuclear Science Center, with gamma ray sterilization of the wax moth, Galleria mellonella (L.) (Nielsen, 1971), this insect was chosen as an experimental organism in this research. It was hoped to determine and contrast the possible effects of ^{252}Cf

neutrons with those already known to result from gamma radiation for this important economic and research insect.

CHAPTER II

Material and Methods

The ^{252}Cf Irradiation Facility

The insects were irradiated with a ^{252}Cf source in an existing irradiation facility at the Nuclear Science Center, Louisiana State University, Baton Rouge, Louisiana. The irradiation facility used in the experiments was designed and constructed in the summer of 1971 (Miller, 1973). The facility consists of a stainless steel tank with lead, water, boric acid and concrete as primary radiation shielding materials (See Appendix I for descriptive drawings of the existing irradiation facility).

The size of the ^{252}Cf source was 2.8 mg which emits 6.57×10^{9} n/sec. In this assembly, the source is raised from the storage position to the irradiation position and returned to the storage position at the end of the irradiation period by an elevator (Fig. A1-4). The elevator terminates in a control cable which connects with a stop that works in conjunction with the source stop assembly (Fig. A1-2) to place the source in exactly the same manner each time. The control cable also has an automatic release for ending irradiation at a precise time (Fig. A1-6). Total accumulated doses from the single source, therefore, are administered by controlling the irradiation time.

Descriptive drawings of the inner tank assembly, tank lid assembly, and tripod assembly are in Figures A1-1, A1-3, and A1-5, respectively.

The end of the control cable is approximately 30 feet from the source with about 4.25 feet of intervening concrete shielding between. Concrete was used as an effective neutron shield due to its high hydrogen concentration which provides a high degree of moderation (slowing down of fast neutrons emitted by the ^{252}Cf source).

The Irradiation Assembly

Due to its manageability, durability, and ease of fabrication, Plexiglass* was used in constructing the irradiation assembly. The major objectives in designing the assembly were: 1) to conform with the dimensions of the existing irradiation facility; 2) to minimize handling of the target specimens; 3) to allow rapid and geometrically consistent placement of the target specimens; and 4) to reduce dose due to primary, secondary, and capture gammas as much as possible with minimal reduction of neutron flux.

The external cylindrical dimensions of the irradiation assembly were fabricated to fit the previously existing facility. (Detailed descriptive drawings of the irradiation assembly can be found in Appendix II). The dimensions of the specimen chamber within the irradiation assembly (Fig. A2-1) allowed use of a 100 ml plastic container of a convenient shape and volume used in biological studies.

The interior of the irradiation assembly, excluding the specimen chamber, was used for gamma shielding material. Lead, a material of high atomic number, is a good attenuator of gamma rays (Stoddard, 1971) and has a relatively small effect on neutron flux (Iddings, 1969); therefore, it was used to reduce the gamma ray dose con-

* Registered trademark for acrylic sheet; Rohm and Haas Co.

tribution. Because a solid piece of lead, having dimensions necessary to fit the assembly was not available and would be somewhat difficult to cast to exact dimensions, lead shot was used. Thirty-seven pounds of #7½ lead shot were required to fill the shielding area of the chamber. The effective density of a shield made of spherical lead shot is 67% of that of solid lead.

Dosimetry, General Requirements

In this research, dosimetric determinations were necessary in areas occupied by operating personnel and in the area of the irradiation chamber occupied by specimens. Becuase ^{252}Cf emission results in both neutrons and gamma rays, three different types of dosimetry were required within the specimen chamber. These are: 1) determination of gamma dose rate, 2) determination of thermal neutron dose rate, and 3) determination of fast neutron dose rate. However, due to the distance and the amount of shielding between the source and areas occupied by operating personnel, it was assumed that the neutron contribution to the radiation dose would be negligible; thus, only gamma dosimetry was required.

There were two places in which dose rates had to be determined for protection of operating personnel. These were: 1) at the top of the source facility with the source in the storage position and 2) at the operating elevator with the source in the irradiation position. Dose rate was determined in both areas using an Eberline Instrument Corporation Geiger Counter, model E-120, equipped with an HP-190 probe. The range scale of this instrument was from 0.1-50 millirads/hour. The survey meter was calibrated with a standardized ^{60}Co source.

Gamma Dosimetry

Gamma exposure rate contribution to the specimen chamber was determined with a Victoreen Instrument Company R-Meter, model #70, and its associated ionization chamber. The ionization chamber had a gamma sensitive range of 0.4-1.3 MeV (Victoreen, 1969). The reading range of this chamber was from 0-25 roentgens. The hairline on the scale in the chamber was brought to read zero using the R-meter. The sensitive volume (black tip) of the ionization chamber was then centrally placed in the bottom of the specimen chamber. The source was raised for a predetermined irradiation period (15 minutes). At the end of irradiation, the ionization chamber was removed and change in hairline deflection was determined using the condenser roentgen meter. The difference in initial and final meter readings divided by the time of irradiation gave the gamma exposure rate in roentgens/unit time. Dose rate units expressed as rads/unit time were then calculated from exposure rate units by multiplying them by the factor 0.867 as described by Gloyna (1969).

Thermal Neutron Dosimetry

The dose rate due to thermal neutrons (0.025 eV) was determined by indium (In) foil activation. Indium has a large cross-section (155 barns) for thermal neutrons. The foil activation reaction involved is as follows:

$$^{115}In\ (n,\gamma)\ ^{116m}In$$

The resulting ^{116m}In has a half-life of 54 minutes.

A bare pre-weighed indium foil (approximately one square centimeter with a thickness of 250 microns and weighing about 0.3 grams) was centrally placed in a bottom of the specimen chamber and activated for a measured length of time (2-3 minutes depending upon foil placement). The foil was removed at end of activation, and induced radioactivity was determined immediately using a 3" x 3" NaI (Tl) crystal and a multi-channel analyzer. The multi-channel analyzer was a 400 channel Technical Measurement Corporation, model 4010, and was used in conjunction with a Hewlett-Packard 6515A DC Power Supply, a Ortec model 483 amplifier, and Franklin Serial Printer, model #CDC 1270. Decay time (time from end of activation to start of detector counting) as well as detector counting time were measured and recorded. These values were used in neutron flux calculations.

To determine how much of the induced foil activity was due to thermal neutron absorption, another indium foil was activated. This foil was covered with 0.6 mm thick cadmium (Cd) sheet and activated in exactly the same place in the specimen chamber as its bare In counterpart. At the end of activation, the Cd cover was removed and the foil analyzed in the same manner as the preceding bare In foil. Fig. 2-1 illustrates how the cadmium cover nearly eliminated the thermal portion of the neutron spectrum to which indium is sensitive. (Price, 1964).

The total counts from counting an activated foil (C_t) were then corrected for decay during counting, by

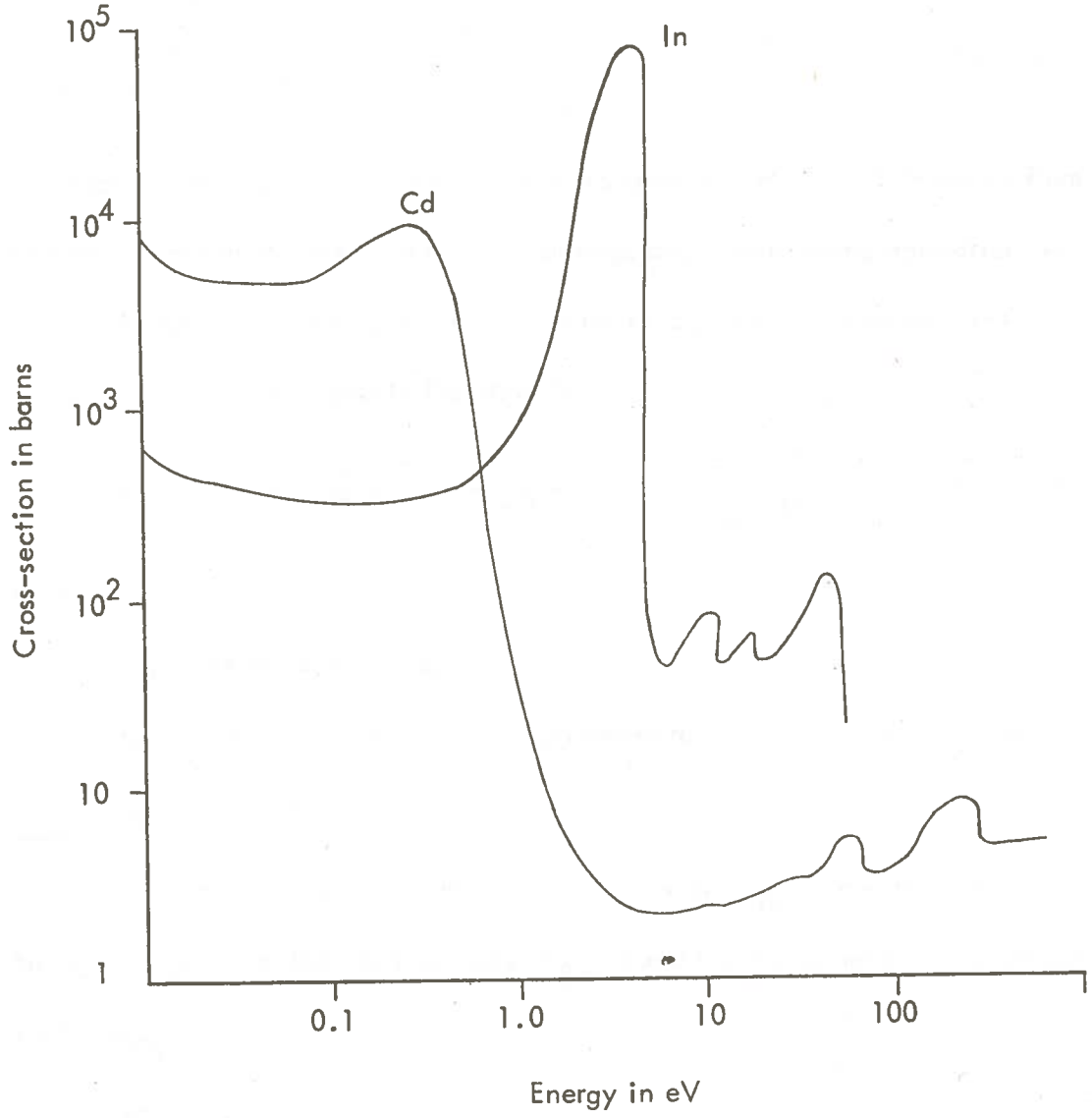
$$C_T = C_t \times \frac{\lambda}{1-e^{-\lambda t_c}} \qquad \text{(Eq. 2-1)}$$

where:

C_T = total counts corrected for decay during counting

Figure 2-1

Total cross-section of cadmium and indium
at various neutron energies.



λ = indium decay constant $(0.693/t_{\frac{1}{2}})$

$t_{\frac{1}{2}}$ = half-life of 116^{m}_{In} (54 min.)

t_c = counting time.

Converting C_T into a activity (A_i) by equation 2-2,

$$A_i = \frac{C_T}{t_c} \quad , \qquad \text{(Eq. 2-2)}$$

and then correcting A_i for: 1) detector efficiency (Morel, 1974); 2) decay from end of activation to start of detector counting; and decay during activation (saturation factor), the total corrected foil activity (A_c) was determined. The corrected activity is expressed in Equation 2-3.

$$A_c = A_i \times \frac{1}{\eta} \times \frac{1}{e^{-\lambda t_d}} \times \frac{1}{1-e^{-\lambda t_a}} \qquad \text{(Eq. 2-3)}$$

where:

η = detector efficiency

t_d = decay time from end of activation to start of detector counting

t_a = activation time

In Equation 2-4 the thermal induced activity (A_{th}) is the difference between the corrected bare foil activity (A_{Bare}) and the corrected Cd covered activity (A_{Cd}).

$$A_{th} = A_{Bare} - A_{Cd} \qquad \text{(Eq. 2-4)}$$

Equation 2-5 expresses the relationship between thermal neutron flux and A_{th}.

$$\phi_{th} = \frac{A_{th}}{N\bar{\sigma}_{In}} \qquad \text{(Eq. 2-5)}$$

where:

N = number of target atoms

ϕ_{th} = thermal neutron flux (n/cm^2-sec)

$\bar{\sigma}_{In}$ = average thermal neutron cross-section of indium ($1.55 \times 10^{-22} cm^2$)

The number of target atoms is given in the following equation:

$$N = \frac{\text{wt. of foil}}{\text{atomic wt.}} \times N_{A_v} \times P \qquad \text{(Eq. 2-6)}$$

where:

P = the fraction of ^{115}In in naturally occuring indium (0.9572).

N_{A_v} = Avogadro number (6.023×10^{23})

Solving for Equation 2-3 for ϕ_{th} gives the thermal neutron flux. By Equation 2-7 (Stoddard, 1971), ϕ_{th} can be converted into an equivalent thermal neutron dose rate (D_{th}) in mrad/hr.

$$D_{th} = (1.43 \times 10^{-2})\, \phi_{th} \qquad \text{(Eq. 2-7)}$$

Thus, the dose rate due to thermal neutron flux can be determined.

Fast Neutron Dosimetry

Dose rate due to fast neutrons (neutrons of energy greater than 0.4 eV) was determined by sulfur activation. The activation reaction,

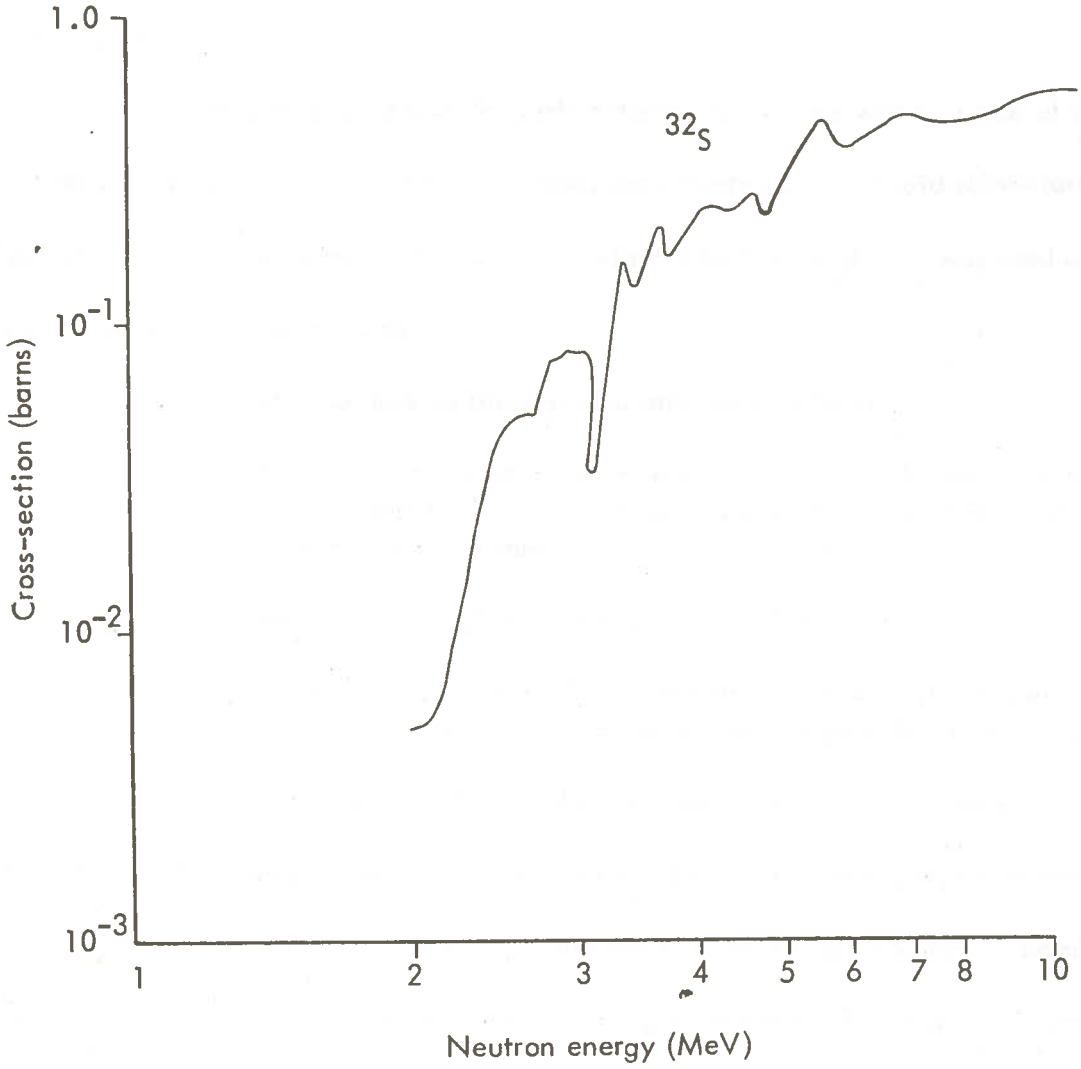
$$^{32}S(n,p)^{32}P$$

results in a pure beta emitter, ^{23}P, which has a half-life of 14.3 days.

The reaction cross-section as a function of energy is given on Figure 2-2. The reaction effective threshold energy has been computed with the Air Force

Figure 2-2

Total cross-section of ^{32}S at various neutron energies



Weapons Laboratory CDC-6600 digital computer, and found to be 3.0 MeV (Murphy, 1967). A measure of the ^{32}P activity following activation is a measure of the fast neutron flux. Because of its virtually 100% detection efficiency for ^{32}P beta emission, it was decided to assay the induced ^{32}P by a liquid scintillation spectrometer.

An important requirement for such a dosimetric system was a source of sulfur that could be used with both the irradiation chamber and a liquid scintillation system. Due to the following advantages, ethyl disulfide $(C_2H_5S)_2$ was used as the source of sulfur to be activated.

1. It is soluble in the liquid scintillation solution
2. Since it is a liquid at room temperature, it conformed exactly to the interior dimensions and occupied the area of interest with constant geometry
3. High percent sulfur by weight (52.47%)
4. High boiling point (153°C), and thus relatively lower volatility, than other liquid organosulfur compounds, such as CS_2

The ability of ethyl disulfide to dissolve most plastics was its only disadvantage. After trying several types of plastic, it was found that polypropylene was impervious to the ethyl disulfide. Therefore, a 50 ml polypropylene container was used to hold the ethyl disulfide while being irradiated. Polypropylene was chosen over glass because of the silicon in glass which has a high thermal neutron cross-section.

A liquid scintillation system, Beckman LS-250 Spectrophotometer, was used to measure the induced ^{32}P activity. The ^{32}P betas have an energy distribution which ranges from 0 to 1.71 MeV; consequently, the use of solid phase

detector systems would have resulted in a relatively high degree of self-absorption with resulting lower detection efficiency. However, self-absorption is eliminated through the use of the liquid scintillation system in which the ^{32}P is dissolved in the liquid scintillation solution. The solution is made up of PPO (6 g/L); and POPOP (0.05 g/L) in toluene. Also, essentially 4π geometry is obtained, since the ^{32}P atom is completely surrounded by molecules of scintillator. Both of these factors make the liquid scintillation system highly efficient and sensitive in detecting and counting the ^{32}P beta activity.

Since ethyl disulfide contained not only the mass 32 sulfur isotope but another stable isotope of sulfur, ^{34}S, which constitutes 4.2% of natural sulfur, there were two reactions that interfered with fast neutron sulfur dosimetry. The first reaction, $^{34}S\ (n,\gamma)\ ^{35}S$, is a thermal activation with a cross-section of 260 mb, and hence, can seriously affect sulfur measurements made in the ^{252}Cf spectrum (which has a large thermal component). To avoid this unwanted reaction, the ethyl disulfide was shielded with 600 microns of cadmium. The cadmium cladding was fabricated to conform to the interior dimensions of the 50 ml polyproplene container.

The second reaction, $^{34}S\ (n,\alpha)\ ^{31}Si$, also occurs producing thereby a radiosilicon isotope which decays with a half-life of 2.6 hours by beta and gamma emission. This (n,α) reaction has a threshold of approximately 4.8 MeV and a relatively low cross-section (70 mb at 7 MeV). Due to the short half-life of the ^{31}S, a 24 hour delay in counting permitted its radioactivity to decrease to a negligible level.

Because an organic solution of calibrated ^{32}P was not available, the ^{32}P induced activity from the ethyl disulfide activation was used to determine the quenching effect of ethyl disulfide. Quenching, whether it be chemical, dilution, of color, results in a decreased light output per beta particle and, consequently, a possible failure to yield a detectable radioactive event. The net effect of quenching is, therefore, to reduce detection efficiency. Determination of the quenching due to ethyl disulfide was accomplished by first activating a cadmium shielded 6 ml solution of ethyl disulfide to obtain a uniform ^{32}P content in a known amount of ethyl disulfide. A series of samples with compositions as prescribed in Table 1 were then prepared for liquid scintillation radioassay. The weight of each sample was recorded and then 0.5 ml of activated ethyl disulfide was added to each so that all sample vials had the same ^{32}P content but variable amounts of quencher. Each sample was weighed again and the net increase in weight recorded to get a more precise determination of the amount of weight of radioactive ethyl disulfide added to each sample. After 24 hours, the samples were counted with the Beckman LS-250 liquid scintillation system. A plot of the activity in counts per minute versus quantity of ethyl disulfide in ml gives the quenching curve for ethyl disulfide (Figure 2-3). Thus, the reduction in detector efficiency can be obtained from the quench plot for a given amount of activated ethyl disulfide being counted, and appropriate corrections can be made.

As with the indium foil, the detected activity from the activated ^{32}S was corrected for decay during counting using Equation 2-1, where $t_{\frac{1}{2}}$ is the half-life of ^{32}S. Also corrections for: 1) detector efficiency (quench factor); 2) decay

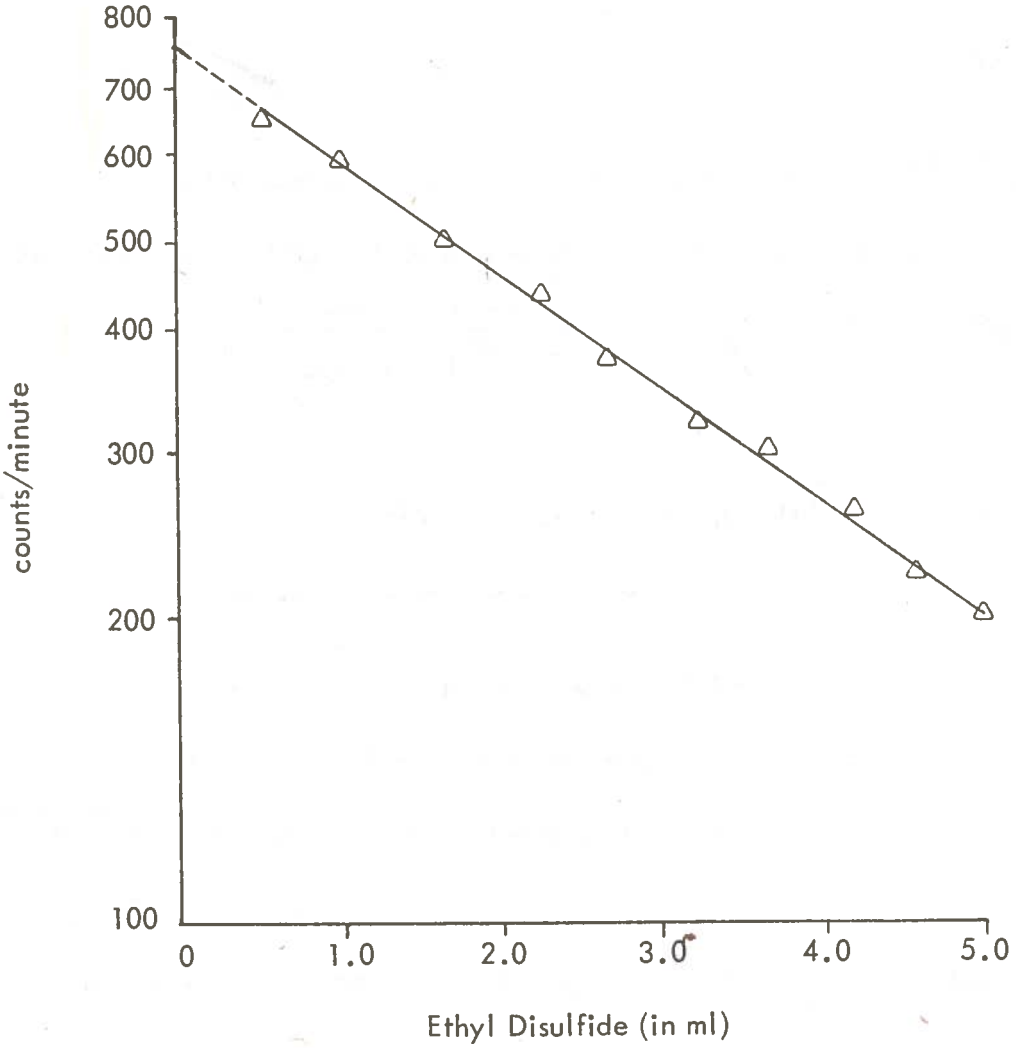
Table I. Compositions of samples used in determination of ethyl disulfide quenching in the liquid scintillation detector system.

SAMPLE NO.	LIQUID SCINTILLATOR SOLUTION* (ml)	TOLUENE (ml)	UNACTIVATED ETHYL DISULFIDE (ml)
1	10	4.5	0.0
2	10	4.0	0.5
3	10	3.5	1.0
4	10	3.0	1.5
5	10	2.5	2.0
6	10	2.0	2.5
7	10	1.5	3.0
8	10	1.0	3.5
9	10	0.5	4.0
10	10	0.0	4.5

* PPO (6 g/L); and POPOP (0.05 g/L) in toluene

Figure 2-3

Quench Curve for Ethyl Disulfide



from end of activation to start of counting; and 3) decay during activation were accomplished using Equation 2-2; where the detector efficiency will be equal to the quench factor.

The relationship between fast neutron flux above 3 MeV (ϕ_f) and corrected activity (A_c) is:

$$\phi_f = \frac{A_c}{N\bar{\sigma}_S} \qquad \text{(Eq. 2-8)}$$

where: $\bar{\sigma}_S$ is the average sulfur reaction cross-section (0.3×10^{-24} cm^2) and N is the number of ^{32}S target atoms expressed in the following equation:

$$N = \frac{\text{wt. of ethyl disulfide}}{\text{atomic wt. of } ^{32}S} \times f \times N_{A_v} \times P \qquad \text{(Eq. 2-9)}$$

where:

f = fraction of sulfur in ethyl disulfide (0.5247)

N_{A_v} = Avogadro number (6.023×10^{23})

P = fraction of ^{32}S in natural sulfur (0.95)

Solving Equation 2-8 for ϕ_f gives the fast neutron flux above 3 MeV. ϕ_f is 28.17% of the total fast neutron flux ϕ_F (Stoddard, 1971); therefore,

$$\phi_F = \phi_f / 0.2817 \qquad \text{(Eq. 2-10)}$$

By Equation 2-9 (Stoddard, 1971), ϕ_F can be converted into fast neutron dose rate (D_F) in mrad/hr.

$$D_F = (1.43 \times 10^{-2})\,\phi_F \qquad \text{(Eq. 2-11)}$$

Thus, the dose rate due to fast neutron flux can be determined.

Insect Material

The greater wax moths (Galleria mellonella (L.)) used in the experiments were obtained from the Bee Breeding Research Laboratory, U. S. Department of Agriculture, Baton Rouge, Louisiana. Three to four-day-old male pupae were used for irradiation at varying time intervals in each experiment. A control group for each experimental group was separated and established which would receive no radiation. Each experimental group was composed of four replicates, with ten pupae per replicate. Pupae were irradiated in a 100 ml plastic container. Within a twenty-four hour period following transformation into adults, the males from both the control and irradiated groups were mated with normal females by placing the insect pairs in wide-mouth gallon jars where mating took place. All glassware was previously sterlized in the autoclave before use. Eggs, which were deposited between the folds of accordion-pleated wax paper, were gathered at 2 five-day intervals. Eggs were weighed on a Roller-Smith torsion balance to determine the number deposited (500 eggs per 15.7 mg).

Approximately 500 eggs were placed within a 3-dram cotton stoppered glass vial which were filled two-thirds full with a food medium. The food medium consisted of Gerber's Mixed Cereal® and a liquid solution of water, 100 ml, and Poly-Vi-Sol®, 1.0 ml, (Nielsen, 1971). The vials were placed in an incubator maintained at 32-33 degrees C.

Larvae from the eggs placed in the food vials developed to the second or third instar (approximately 10 days). At this development stage they required larger amounts of food for further growth. Therefore, the cotton stoppers were removed,

and the vials were placed in one-gallon jars containing one liter of the food medium. A small amount of food from the gallon jar was pushed into the vial to make contact with the food and the larvae in the vial. The larvae then crawl out of the vial and feed ad libitum on the larger food mass in the gallon jar. The jar was covered tightly with a lid and sealed with one-inch crepe masking tape to prevent small larvae from entering or leaving. The jars were placed at 32-33 degrees C for continuation of the insects' development.

The larvae were left in the jars until pupation occurred. The pupae were removed and each was placed in an individual 3-dram cotton-stoppered vial. Upon emergence, they were separated by sex. Ten F_1 irradiated males were mated with ten non-irradiated females, likewise, ten F_1 irradiated females were mated with ten non-irradiated males. Then ten F_1 irradiated males were mated with ten irradiated females. All of the matings occurred in one-gallon glass jars. The eggs were once again gathered from the wax paper, as done with the parental generation. The eggs were weighed and analyzed for percent hatch.

To determine percent hatch, various batches of eggs were allowed sufficient time at 32-33 degrees C for the embryos to develop. When hatching took place, counts were made under a light dissecting microscope from which percentages could easily be calculated.

Chemicals and Other Materials

The PPO (2, 5- diphenyloxazole) and POPOP (1, 4-bis 2- (5-phenyloxozolyl benzene) used in the liquid scintillation solution were of scintillation grade and were

purchased from Packard Instrument Company, Inc., La Grange, Illinois. The toluene ($C_6H_5CH_3$) solvent used in the liquid scintillation solution was reagent grade and was obtained from J. T. Baker Chemical Co., Phillipsburg, New Jersey.

The reagent grade ethyl disulfide used was purchased from Eastman Organic Chemicals, Rochester 3, New York. Indium and cadmium materials used were purchased from Reactor Experiments Inc., San Carlos, California. The ^{252}Cf source was on loan to the Louisiana State University Californium Demonstration Center from U. S. A., E. C. Savannah River Laboratory.

CHAPTER III

Results and Discussion

Gamma Dosimetry

Accurate and complete gamma dose determination in a mixed field, one having both neutrons and gammas, is very difficult. Not only are there primary gammas from the ^{252}Cf but also capture gammas which are a result of neutron interaction with chemical elements in surrounding media.

Primary gammas from ^{252}Cf have an energy range of 0.12-10 MeV, with approximately 90% having an energy within the peak response of the detector, 0.4-1.3 MeV (Stoddard, 1971).

Capture gammas can be emitted from a number of potential sources from the typical (n, γ) reaction. Many are known to have a wide range of energies. Possible sources of capture gammas and their energies are:

1. Hydrogen in the water, concrete, and air (2.23 MeV)
2. Boron in the boric acid (0.48 MeV)
3. Lead in the irradiation chamber (7.2 MeV)
4. Antimony, a hardener in the lead shot (3-7 MeV)
5. Calcium and silicon in the concrete (1.8 - 8 MeV)
6. Iron, nickel, and chromium in the stainless steel (0.8 - 9 MeV), (Groshev, 1959).

The majority of capture gammas are of energies beyond the peak response of the detector; therefore, any measurement underestimates the dose due to gamma rays.

Additionally, due to their high energy, it is assumed that relatively little energy would be imparted to most biological targets which have a rather low density.

Gamma dose rate at the bottom of the specimen chamber (Fig. A2-1) was found to be 7.45 rads/hour, compared to a dose rate of 64.25 rads/hour on the support shelf (Fig. A1-2). The difference in distance between the two points of measurement was 8.9 cm with the support shelf being approximately 10 cm from the source. The irradiation assembly's lead shielding theoretically reduced the primary gammas and capture gammas from hydrogen (2.23 MeV) by a factor of 60 (Stoddard, 1971). However, the 2.5 - 5 cm of lead shielding would have less shielding effect on the higher energy gammas from other capture gamma sources.

Neutron Dosimetry

Dose rates due to thermal neutrons at the bottom of the specimen chamber and on the support shelf were found to be 10.8 rads/hour and 10.3 rads/hour, respectively. These measurements are nearly equal because fast neutrons are being thermalized throughout the facility. Most thermalization occurs in the pool of water, from which the ^{252}Cf source is raised to the irradiate position, and from the surrounding concrete, both of which have relatively high concentration of hydrogen. Since thermalization of fast neutrons is occuring throughout the irradiation facility, these two measurements represent dose rates due to thermal neutrons at two points within a volume source of thermal neutrons, rather than measurements made at two points of given distances from a point source.

At the same two points as mentioned above, the bottom of the specimen chamber and on the support shelf, the dose rates due to fast neutrons were found to

be 6.1 rads/hour and 37.2 rads/hour, respectively. Unlike the situation with gamma and thermal neutrons, fast neutrons are emitted only from the ^{252}Cf, which can be assumed to be a point source of fast neutrons. The theoretical unshielded neutron dose rate in air at a point 10 cm from a 2.8 mg ^{252}Cf source is 76.8 rads/hour. A combination of 3.2 cm of lucite, which is located between the source and point of detection, and 1.8 cm of water (which could very possibly be retained in the source holder when raising the source from the water to the irradiate position) would account for the difference in theoretical and measured fast neutron dose rate (Stoddard, 1971).

Radiation Effects on the Greater Wax Moth (Galleria mellonella (L.))

Effects of irradiation on 3-6 day-old pupae and their F_1 progeny are reported in Table 2. Total dose is expressed in rads and is made up of the following components: gamma (31%), thermal neutrons (44%), and fast neutrons (25%).

Emergence. Irradiated male pupae usually emerged within 6-36 hours upon completion of irradiation. Irradiations of 132 - 1468 rads did not adversely affect pupae emergence into the adult stage. However, in comparing the time of emergence of the irradiated pupae with a control group established at the same temperature in the Nuclear Science Center, it was noted that the irradiated pupae emerged from 12-24 hours earlier than the control group.

Evidence of Mating. Females from the 1468 rad group were selected for mating as follows: 1) normal, i.e. non-irradiated, females mated with irratiated (P_1) males; 2) normal females with F_1 males from irradiated P_1 parent, and 3) nor-

mal males with F_1 females from an irradiated P_1 parent. Dissection of females from these 3 mating groups showed that all contained one or more transferred spermatophores. This is acceptable as evidence that they had therefore mated. These dissections were carried out by Dr. Ross Nielsen of the Bee Breeding Research Laboratory, U. S. Department of Agriculture.

Eggs Produced. Oviposition by females of both the parental and F_1 generations was not significantly affected at the range of dosages indicated in Table 2.

Eggs Hatched. The percent of egg hatch decreased consistently as the irradiation increased from 264 rads to 1468 rads. The decrease was most prominent in eggs from F_1 females mated with F_1 males with both being from an irradiated parent. Eggs from normal females mated with irradiated males were least affected.

F_1 Survival. Little mortality was observed in the F_1 larvae and pupae developing from male parents when irradiated from 132 to 396 rads. The number of emerging F_1 males and females began to decrease slightly but steadily as the irradiation increased from 396 to 1468 rads.

Mortality in F_2 Larvae. By placing F_2 larvae from the last experimental group (1468 rads) on the food medium, it was found that approximately 40% did not develop to the third instar as compared to non-irradiated controls.

The above results indicate that the irradiated moths were biologically affected: namely, a reduction in the percent hatch from F_1 generation eggs and a high incidence of mortality of F_2 larvae. The much lower percent egg hatch from F_1 females crossed with F_1 males is supporting evidence that the irradiation did induce significant chromosomal damage (Snieder, 1965).

Table 2. Effects of irradiation on 3-6 day-old male pupae of Greater Wax Moth (Galleria mellonella (L.)) and their F_1 progeny. All figures are reported as percent of controls.

	Irradiated Parent (P_1), Their Offspring (F_1), x Normal Moths and F_1 x F_1										
	Adults Emerged			Eggs Produced				Eggs Hatched			
Rads	P_1 ♂	F_1 ♂	F_1 ♀	P_1 ♂	F_1 ♂	F_1 ♀	$F_1 \times F_1$	P_1 ♂	F_1 ♂	F_1 ♀	$F_1 \times F_1$
132	100	102	105	98	116	111	104	98	101	92	89
265	100	117	109	117	120	131	104	96	95	94	89
400	100	97	101	101	127	127	89	95	90	95	94
760	100	95	95	80	87	88	88	94	90	90	82
1140	100	97	91	104	87	116	138	92	84	79	68
1470	100	64	63	92	96	107	119	87	68	58	33

In comparing the above effects with effects resulting from pure gamma irradiation by a ^{60}Co source (Nielsen, 1971), neutron irradiation produced comparable levels of induced sterility with 1) an apparently lower degree of somatic damage and 2) lower values of dose, expressed in rads. Reduction of somatic damage was indicated by increased survival of the parental (irradiated) progeny and no apparent decrease in emergence of adults from irradiated pupae. Further evidence for a relatively lower somatic effect comes from the mating studies which showed normal transfer of spermatophores for various crossings of insects given 1468 rads. Low somatic damage with high genetic effects in irradiated insects is very desirable in producing vigorous, competitive P_1 stock to give a high yield of affected F_1 under field conditions. Such effects are very important in designing programs to control dynamic insect populations.

Based on Nielsen's (1971) findings, an estimate of the quality factor (QF) of fast neutrons to gamma rays for induced sterility was determined. Assuming:

1. That thermal neutrons have approximately the same QF as gamma rays (Morgan, 1968)

2. That approximately 25% of the total dose in rads, from irradiation with ^{252}Cf was due to fast neutrons,

the ranges of QF for fast neutrons are: 13-20 relative to 6-7 day-old male pupae and less than 3 relative to 3-4 day-old male pupae. Pupae irradiated using ^{252}Cf were normally $4\frac{1}{2}$-7 days old; therefore, an QF of 13-20 is assumed to be the more probable. This high range of QF for fast neutrons (Snieder, 1973), could be related to the relatively low dose rate which dictated long exposure times. Because most ir-

radiation damage occurs during cell division, a longer exposure time to achieve a given dose would theoretically be biologically more damaging (Morgan, 1968). Therefore, to determine a true QF for fast neutrons to gamma rays, the dose rates from both must be approximately the same.

A study of this latter aspect of comparative QF would require a ^{252}Cf irradiation facility quite different from that used here. It is suggested that future studies along this line be made with increased amounts of ^{252}Cf.

Usefulness of the ^{252}Cf Irradiation Facility: Further Considerations for Future Designs

The existing facility was both safe and easy to operate. Concise specimen and detector placement could be quickly accomplished (10 seconds maximum time required), thus minimizing exposure time of the operator. Approximate operator dose from a 10 second exposure during specimen placement into the specimen chamber was found to be 0.4 millirad. The elevator assembly provided exact placement of the source for each irradiation. Dose rate at the elevator station was 0.1 rad/hour.

The combination of too small a source and too great a distance from source to specimen resulted in very long irradiation times to achieve desired accumulated doses. The maximum was 62 hours to deliver 1468 estimated rads. The facility was designed to house a 5 mg source of ^{252}Cf. Replacing the existing 2.8 mg source with a 5 mg source coupled with altering the facility to reduce the distance from source to specimens would reduce irradiation times.

The irradiation facility was composed of variety of elements that resulted

in a large range of high energy capture gammas. These high energy gammas were difficult both to detect and remove from the neutron field.

Usefulness of the Irradiation Assembly: Suggested Changes for Future Designs

Sizing of the assembly's specimen chamber to allow use of the 100 ml plastic container used to transport the pupae from the Bee Breeding Research Laboratory to the Nuclear Science Center accomplished two objectives. First, it minimized handling of the pupae and, secondly, it provided reproducible placement of the pupae for each experiment. Minimizing handling of the pupae reduced the possibility of harmful tissue damage resulting from factors than radiation.

The lead shot used for gamma shielding reduced the dose due to primary gammas and capture gammas from hydrogenous materials by a factor of about 60, thus increasing the fraction of the total dose that was associated with neutrons.

Replacing the lead shot with an equivalent amount of pure, solid lead, molded to fit the assembly, would have a two-fold effect in improving the irradiation assembly design. First, it would reduce the distance from source to specimen by 4.5 cm and thus increase the dose rate. Secondly, it would decrease the capture gamma contribution by removing the antimony hardener in lead shot which is a strong source of capture gammas and by providing more effective lead shielding (1.3 cm) around the sides of the specimen chamber.

CHAPTER IV

Conclusions

1. Irradiation of the greater wax moth, Galleria mellonella (L.), with mixed radiation does produce a biological effect, but one that is different from that obtained by gamma irradiation. The QF of fast neutrons to gamma rays for the induction of sterility is greater than 3 and could be as high as 20, with an apparently lower degree of somatic damage as judged by several biological parameters.

2. When working with mixed irradiation sources (neutron and gamma), there is a need for more accurate dosimetry. Without an accurate determination of the portion of dose due to fast neutrons, an accurate estimate of the RBE of fast neutrons to gamma rays is greatly limited.

3. An increase in dose rate is needed to reduce irradiation times. A reduction in irradiation times would allow exposure during more specific developmental stages occuring during short time intervals and thus would provide better data to compare to other published reports involving gamma irradiation. To determine most accurately the RBE of induced sterility from fast neutrons to gamma irradiation, the dose rates (in rads) should be equal. The dose rate from fast neutron could be increased by: 1) replacing the 2.8 mg ^{252}Cf source with a larger ^{252}Cf source; 2) reducing the distance from source to specimens. Replacing the lead shot with solid lead and altering the design of the irradiation facility to provide closer placement of the source to the

specimens would increase the fast neutron dose rate.

4. This present investigation has shown that ^{252}Cf might be a better source than ^{60}Co for use in irradiating the greater wax moth to induce sterility. Although this investigation has been of an exploratory nature, it has provided a good basis for more detailed studies.

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APPENDIX I

Descriptive Drawings of the Existing ^{252}Cf Irradiation Facility

Figure A1-1

Inner Tank Assembly

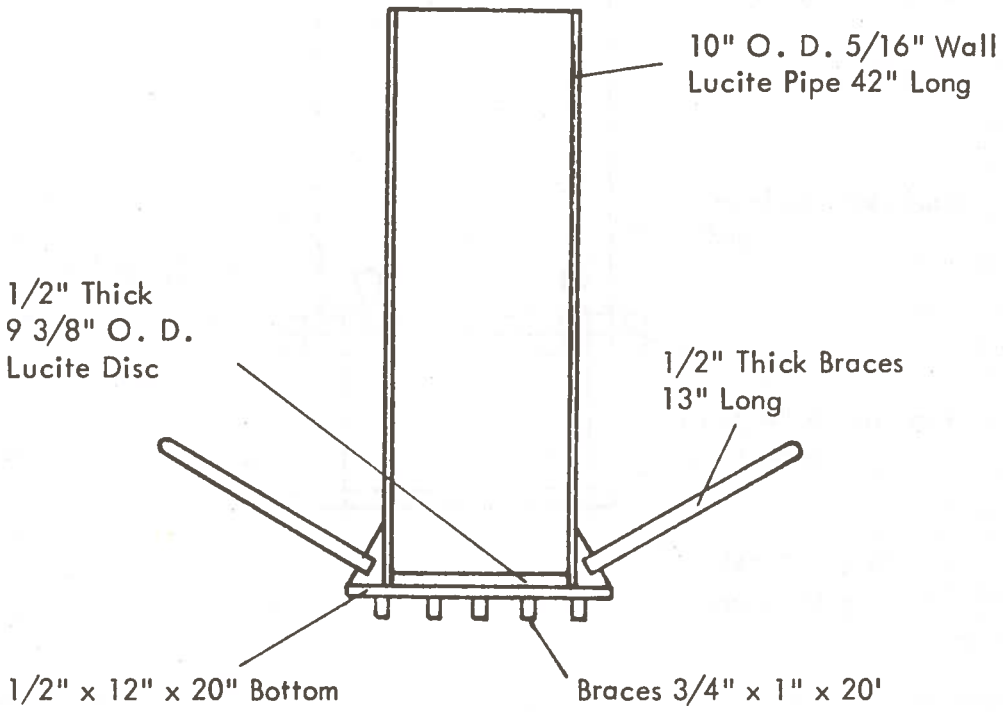


Figure A1-2

Sample Holder And Source Stop Assembly

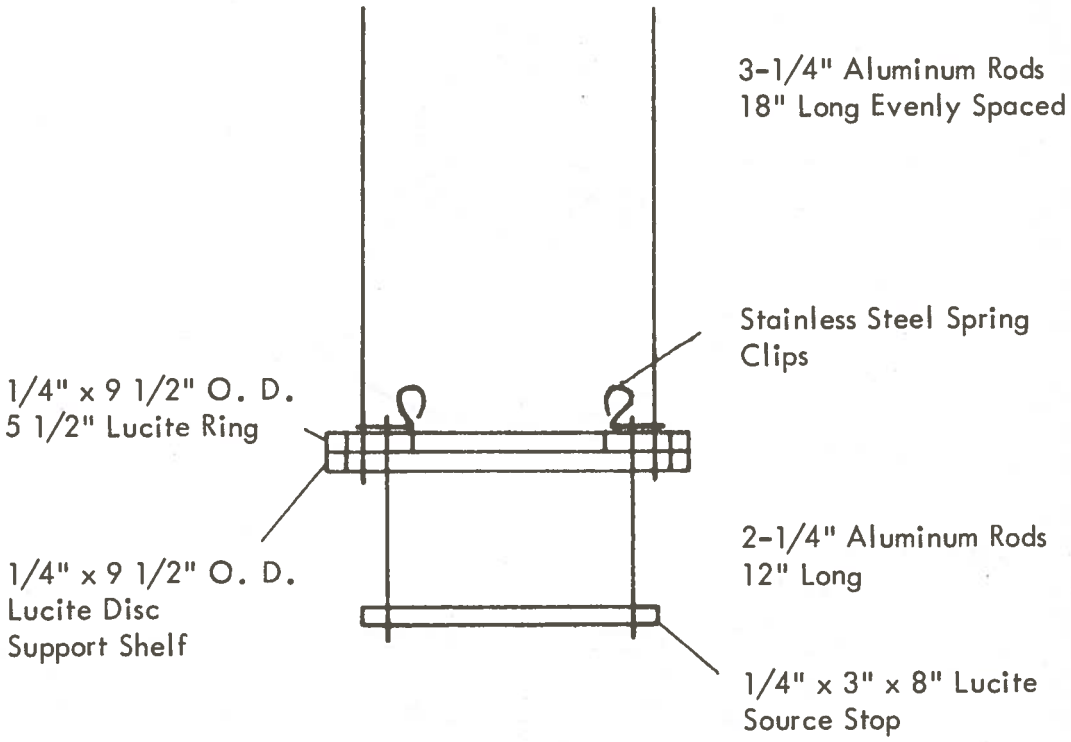


Figure A1-3

Tank Lid Assembly

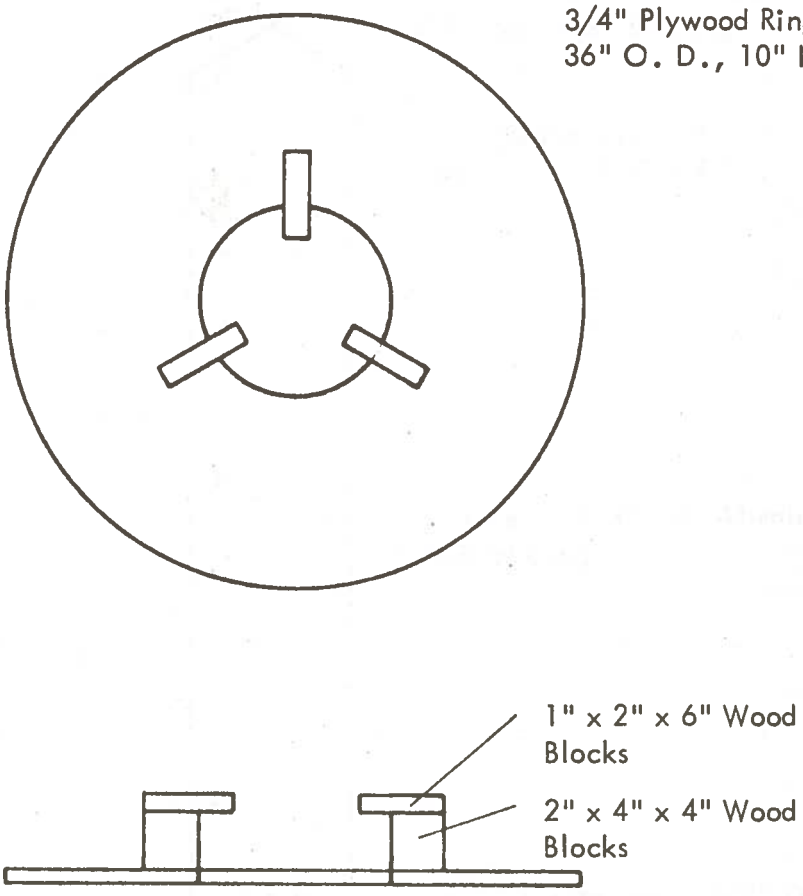


Figure A1-4

Elevator Assembly

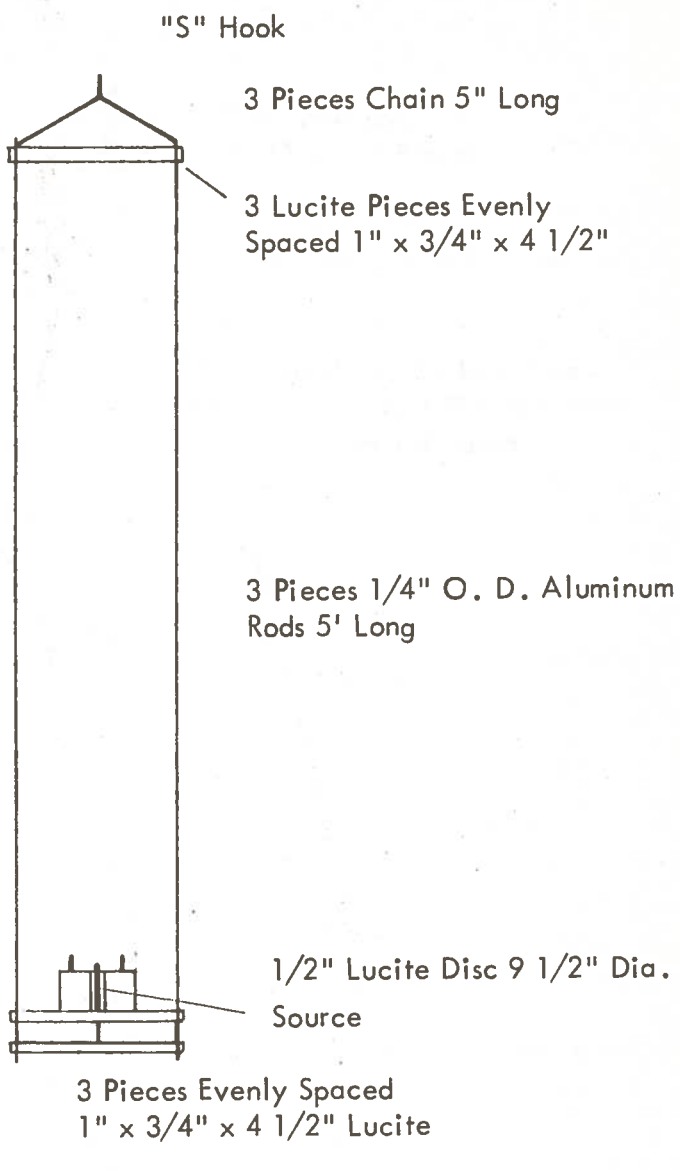


Figure A1-5

Tripod Assembly

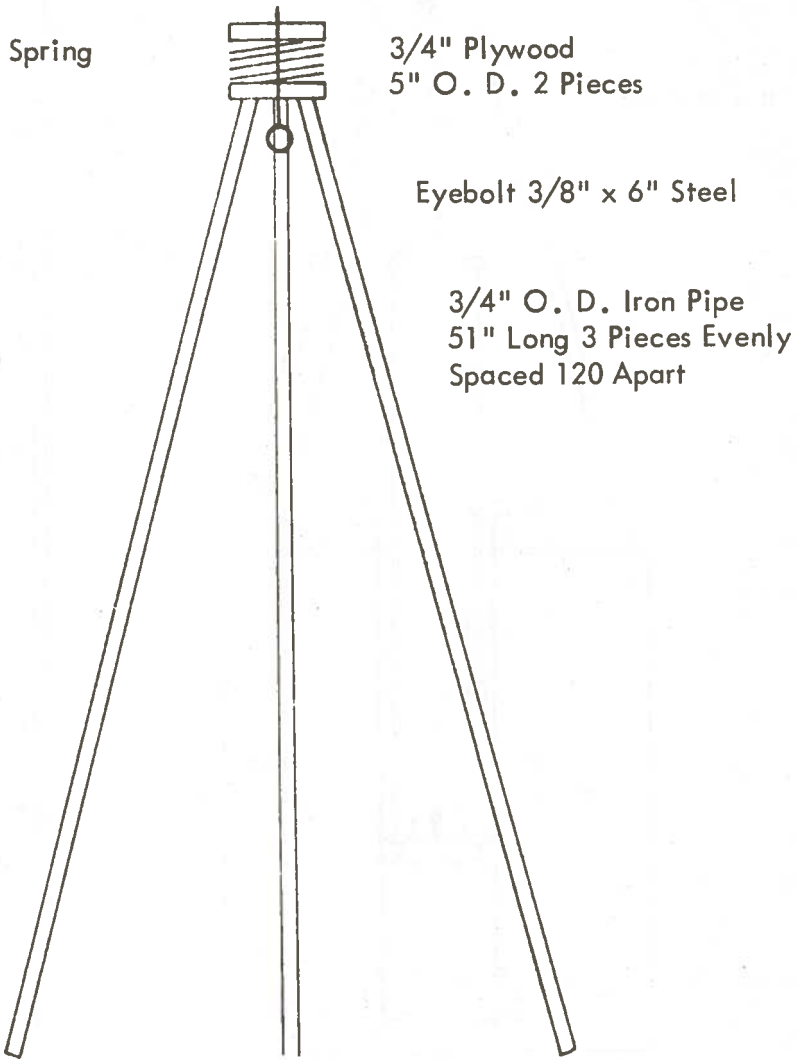
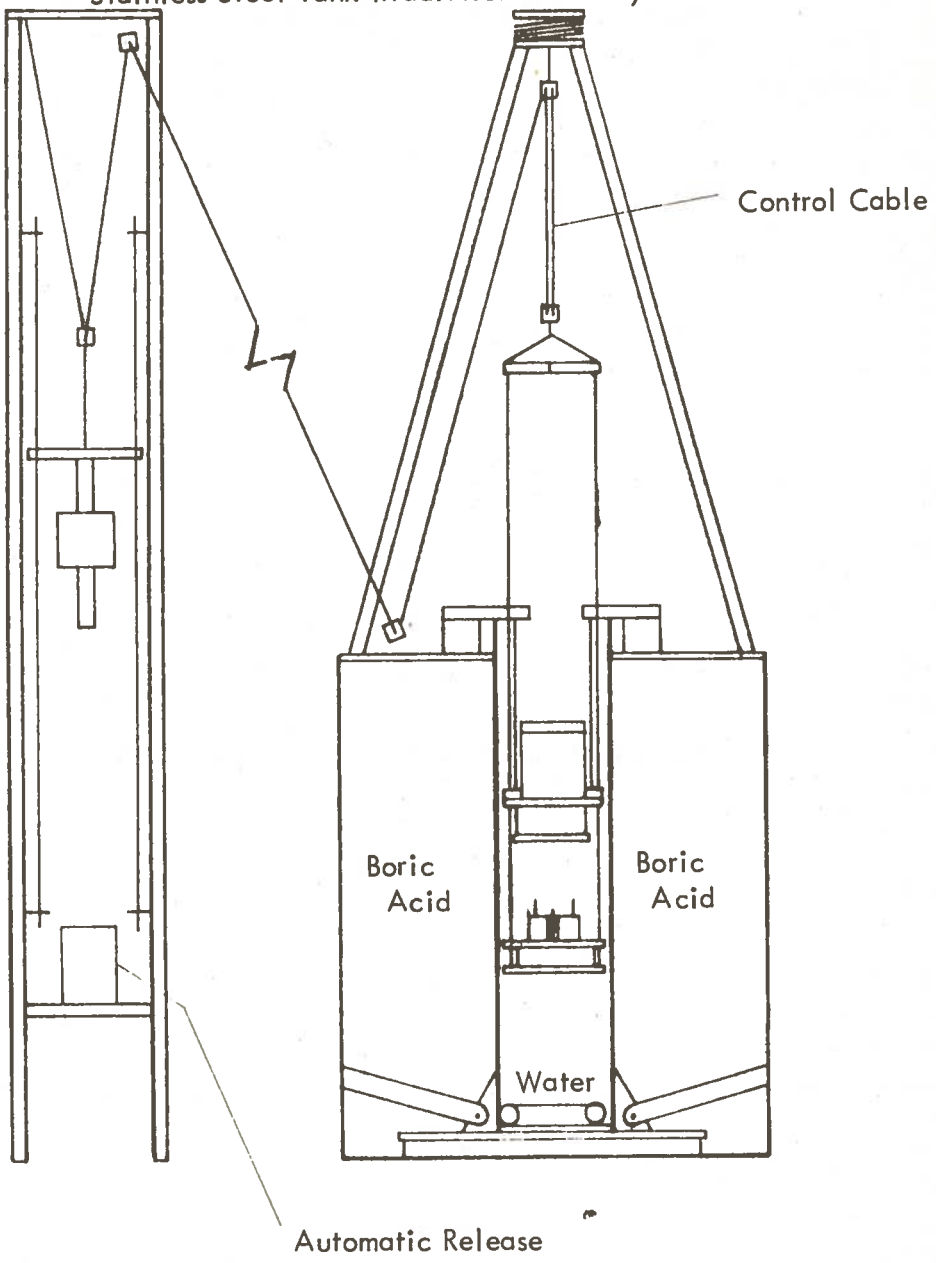


Figure A1-6

Stainless Steel Tank Irradiation Assembly



APPENDIX II

Descriptive Drawings of the Irradiation Assembly

Figure A2-1

Side View of Irradiation Assembly

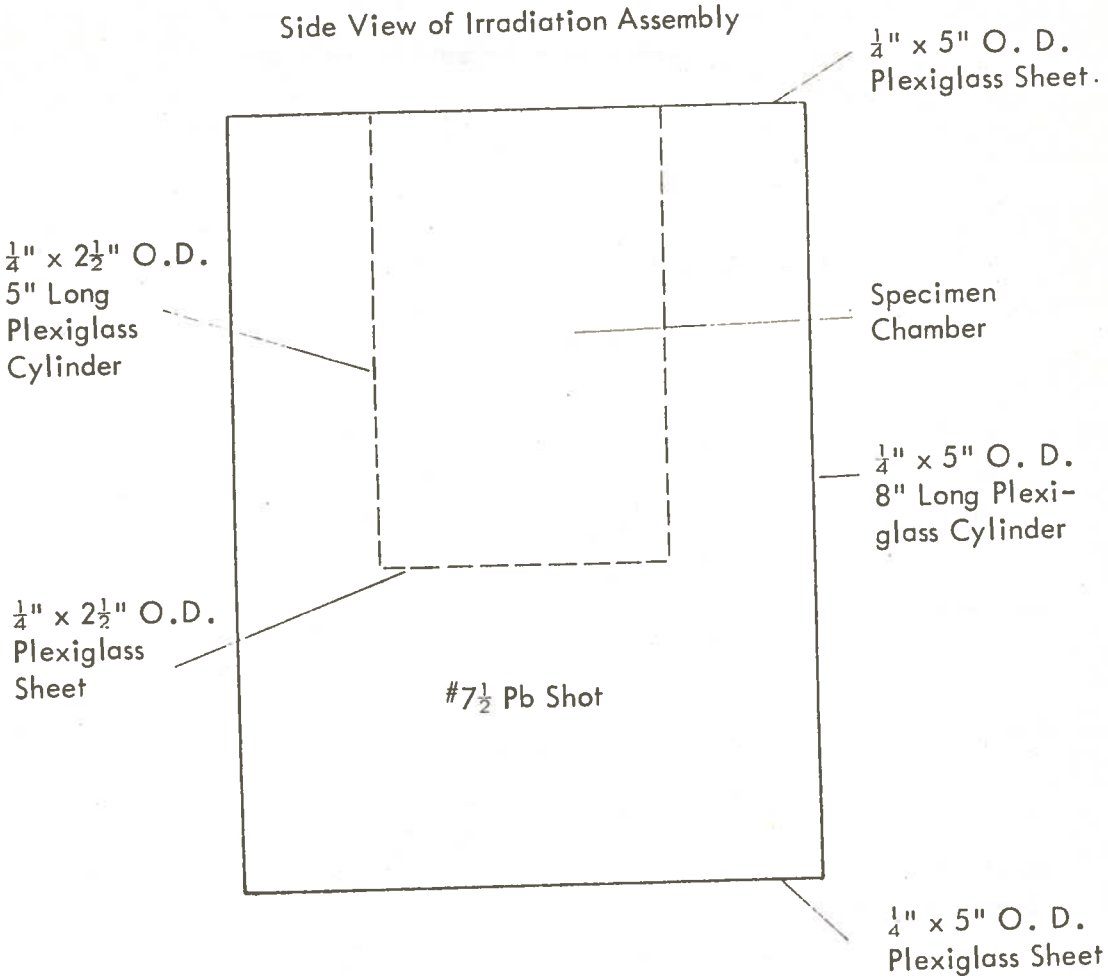
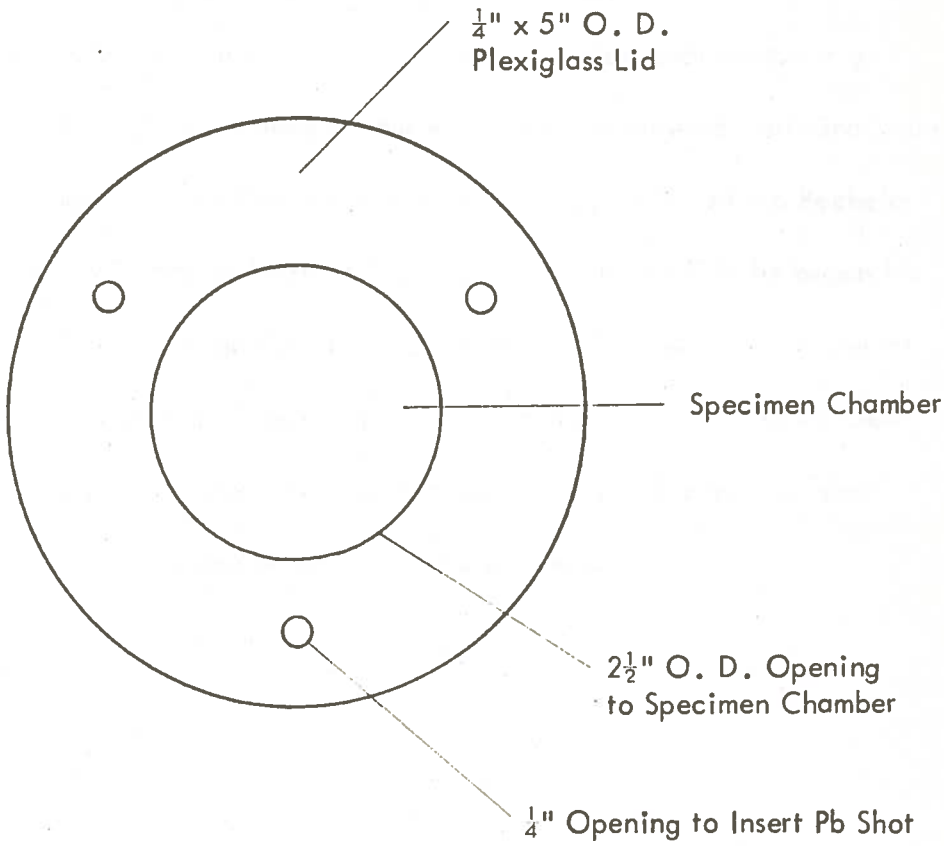


Figure A2-2

Top View of Irradiation Assembly



VITA

Thomas Alan Greene, son of Mr. and Mrs. George H. Greene, was born on September 7, 1948, in Fenton, Louisiana. He graduated from Fenton High School in May, 1966. In the summer of the same year, he entered Louisiana State University and graduated from that institution in January, 1971, with a Bachelor of Science degree in Electrical Engineering. In the spring of 1972, he began his graduate study in Nuclear Engineering at Louisiana State University. At present, he is a condiate for a degree of Master of Science in the Department of Nuclear Engineering. On December 23, 1972, he married the former Cathy Castleman of Dallas, Texas. They have one daughter, Holland Greene.