APR 23 1985

Oral Roberts University
ATTN: Roger D. Hartman, Ph.D.
Chairman, Radiation Safety Commission
7777 South Lewis
Tulsa, Oklahoma 74171

Gentlemen:

Application Dated February 5, 1985, for an Amendment to Materials License 35-18282-01 and our Request for the License Fee Dated March 1, 1985. (Control No. 460534)

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This refers to the subject application and our letter (copy enclosed) in which we notified you that an amendment fee of $120 was required.

Please be advised that, unless we hear from you within 30 days from the date of this letter, we will consider your application as being abandoned.

The submission of any future applications with the prescribed fee would not be affected by this action.

Sincerely,

Glenda Jackson
License Fee Management Branch
Office of Administration

Enclosure:
Letter dated 3/1/85

cc: Region IV

DISTRIBUTION:
Pending Fee File
Weekly Reading File
Materials Reading File

8506120179 850425
REG4 LIC30
35-18282-01 PDR

Called Dr. Hartman 4/30/85 - told him fee is not received (not an emergency per RIV 4/30) to Jackson

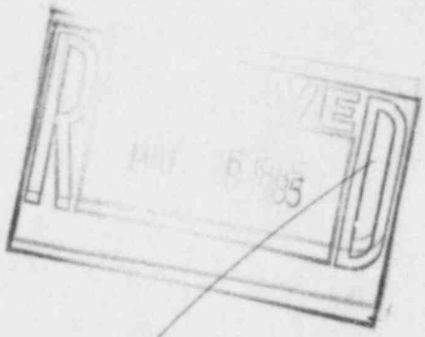
| OFFICE | LFMB:ADM | LFMB:ADM | | | | | |
|---|---|---|---|---|---|---|---|
| SURNAME | FBrown:pj | GJackson | | | | | |
| DATE | 4/22/85 | 4/22/85 | | | | | |

NRC FORM 318 (10-80) NRCM 0240 OFFICIAL RECORD COPY

UNITED STATES
NUCLEAR REGULATORY COMMISSION
REGION IV
611 RYAN PLAZA DRIVE, SUITE 1000
ARLINGTON, TEXAS 76011

BETWEEN: William O. Miller, Chief
License Fee Management Branch
Office of Administration

R. J. Everett, Chief
Material Radiation Protection Section, TPB,
DV&TP, RIV



LICENSEE FEE TRANSMITTAL

A. REGION IV

1. APPLICATION ATTACHED

Applicant/Licensee: Oral Roberts University

Application Dated: February 5, 1985

Control No.: 760534

License No.: 35-18282-01 (030-14781)

2. FEE ATTACHED

Amount: fee

Check No.: Voided not needed per RIV 4/30

3. COMMENTS

Amd needed per W. 11/30/88 TD

Signed Laura Murley

Date February 14, 1985

B. LICENSEE FEE MANAGEMENT BRANCH

1. Fee Category and Amount: _____

2. Correct Fee Paid. Application may be processed for:

Amendment _____

Renewal _____

License _____

Signed _____

Date _____

# Oral Roberts University

7777 South Lewis • Tulsa, Oklahoma 74171




February 5, 1985

Mr. Jack Whitten
Nuclear Regulatory Commission, Region 4
611 Ryan Plaza Drive, Suite 1000
Arlingtion, Texas 76011

Dear Mr. Whitten,

This letter is a follow-up to our telephone conversation on December 12, 1984 concerning the question of Lowery-type pipetting by mouth. An ad-hoc subcommittee was appointed to review this question. After gathering information (attached), interviewing the participants and viewing a demonstration of the actual procedure, the subcommittee recommended the procedure be allowed with the stipulations stated in their report (attached). The Radiation Safety Committee agreed with the recommendation of the subcommittee with the understanding there would be frequent random auditing and checking of the procedure and the personnel involved.

If there are any problems or questions concerning the decision of the committee to allow this procedure, please let me know immediately.

Sincerely,

Roger D. Hartman, Ph.D.
Chairman, Radiation Safety Committee

INTEROFFICE MEMO

TO: Dr. Don Godfrey, Dr. Dave Ross
FROM: Roger D. Hartman, Ph.D., Chairman, Radiation Safety Committee
DATE: January 29, 1985
SUBJ: Authorization to use Lowery System
CC: Drs. Bond, Gilmore, Anderson, and Ms. Pruitt

Earlier today, the Radiation Safety Committee considered the report of the Ad Hoc Subcommittee, which reviewed your request to pipette microcurie amounts of C-14, using a Lowery type system.

After considerable discussion, the Committee approved your request, as follows:

1. Only three people, Dr. Don Godfrey, Dr. David Ross, and/or Katrina Baranek are approved to perform the procedures. Only these specified people will be allowed to use the apparatus. A written request for approval for someone new must be made with the Radiation Safety Committee and the new user must be interviewed by the RSO or his/her designee, and approved by the Committee.

2. Only C-14, in quantities not to exceed 20μCi/wk, will be utilized in the apparatus, in room(s) as specified in the "Regulations" attached hereto. Any change in isotope or quantity greater than 20μCi/wk must be requested in writing, and approved by the Radiation Safety Committee.

3. The apparatus and/or procedures, i.e., "regulations", will not be altered unless a written request to the Radiation Safety Committee has been approved. (Please note hand written comments on each page as dated 29 January 85 attached to this memo. These are the "official regulations".)

4. You agree to follow the current ORU/COF and NRC guidelines and regulations dealing with use of isotopes and hazardous materials, and to promptly report any incident to the Radiation Safety Office.

5. Permission for use of the apparatus and procedures on the ORU/COF premises can be rescinded at any time by a vote of the Radiation Safety Committee, if in the view of the Committee, violations are occuring or if it appears to be a health hazard.

6. Final authority to perform this technique rests in the hands of the NRC.

0104a

If you agree to these terms, please sign, date, and return one original set of papers to my office. Retain the others for your own records.

The Committee wishes to thank you for your assistance and cooperation during the site visit.

| | |
|---|---|
| Dr. Don Godfrey | Date |
| Dr. David Ross | Date |
| Ms. Katrina Baranek | Date |

nkv
0104a

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CITY OF FAITH
Medical and Research Center

# INTEROFFICE MEMO

TO: Roger D. Hartman, Ph.D.
Chairman, Radiation Safety Committee

FROM: David Anderson, Ph.D.
Ad Hoc Subcommittee on Use of Lowery-Type Pipette

DATE: 1-17-85

SUBJECT: Petition by Drs. Godfrey and Ross

C.C. David Jones, Ph.D., Phil Prosser, Delonda Pruitt

The subcommittee met in 2C22 GC, the Godfrey/Ross laboratory, on 1-9-85 and reviewed the procedures to be used. The data enclosed and the proposal recommendation was made available to the subcommittee for their perusal and by choice of the subcommittee a telephone vote was taken on 1-17-85. The recommendation passed 4 to 0. When possible please convene the entire committee to discuss this action.

PROPOSED RECOMMENDATION

We recommend approval of the procedures for micropipetting with the apparatus as outlined and described on the pages enclosed, with the stipulation that:

1. Apparatus and/or procedures will not be altered before submitting a written request to the ORU Radiation Safety Committee and receiving permission for such changes.

2. Only those people specifically approved by the Radiation Safety Committee will be allowed to use the apparatus in question with radioactive materials. A written request for approval for someone new must be made to the Radiation Safety Committee and the new person must be interviewed by the Radiation Safety Officer or his/her designee.

3. Permission for use of the apparatus and procedures on the ORU/COF premises can be rescinded at any time by a vote of the Radiation Safety Committee.

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REGULATIONS CONCERNING USE OF RADIOACTIVE MATERIALS IN GODFREY/ROSS LABORATORIES

I. Location

All radioactive materials for biochemical assay will be stored and used only in Room 2C22.

II. Personnel

The only people who will perform assays with radioactive materials will be those personnel who:

1. Are knowledgeable in general lab safety, proper micropipetting techniques, the assay procedure, and safety in handling radioactive materials.

2. Are certified as to the above by the senior technician responsible for training and the lab supervisor (Dr. Godfrey or Dr. Ross)

3. Are registered by the RSO office for a radiation badge and have attended the (or will attend the next) radiation safety refresher course.

4. Not pregnant (if female).

5. Have signed the "Agreement to Perform Radiometric Assays" consent form.

III. Safety procedures for using radioactive materials in assays

Personnel using radioactive materials will:

1. Perform radioactive assays only in Room 2C22.

2. Conform to standard safety procedures.

3. Wear protective clothing and a radiation badge.

4. Perform appropriate monitoring of mouthpieces and filters and wipe tests of work areas.

   Mouthpiece will be monitored each week that assays are done by placing it in 0.5 ml distilled water for 10 minutes, removing the mouthpiece, and placing the water in a scintillation vial for counting. Any counts above background will be reported to supervisor. Results of counting will be recorded in the log book and signed by user.

5. Mouthpiece will be kept in a place remote to Room 2C22.

6. Keep records of radioactivity used with amounts and date signed by user.

7. Report all radiation spills and other accidents immediately to the supervisor.

IV. Amount of radioactivity

At present, the type of radioactive materials involves only $^{14}C$. The total amount of $^{14}C$ radioactivity in a 60-tube assay is about 1.5 microcurie.

The total amount of $^{14}C$ radioactivity that will be pipetted by all persons will not exceed 20 microcuries per week (although the average amount per week will be less than this).

The total amount of $^{14}C$ radioactivity that will be pipetted by any one person will not exceed 10 microcuries per week (although the average amount per week will be less than this).

V. Risk

Personnel will understand and agree to the extremely small, albeit present, risk involved in pipetting small amounts of radioactivity materials with a remote mouthpiece and agree to conform to these lab regulations.

VI. In Case Liquid Containing Radioactivity Enters the Mouth

1. Do not swallow. Spit out mouth contents into sink. Rinse mouth with water at least 10 times.

2. Monitor inside of mouth by rubbing with cotton swab and counting the swab in scintillation counter.

3. Report incident to supervisor.

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# Pipetting System for Radiometric Assays

1. Description of system

The system consists of rubber tubing (3 millimeter inside diameter) with cellulose filters interposed. The glass micropipet is inserted into one end of the tubing. The filters are inserted into plastic holders to which the tubing is attached. There is about 0.1 meter of tubing between the filters and the pipet and about 1 meter of tubing between the filters and the mouthpiece.

The system is used for pipetting volumes of fluid less than about 100 microliters, often less than 1 microliter. Movement of these very small amounts of fluid is accomplished to a high degree of accuracy (less than 1% variation) by use of specially constructed glass or quartz micropipets and suction or expulsion under mouth control. Use of mouth control provides very fine control of small fluid movements and leaves hands free for other manipulations.

2. Use of the system

This pipetting system has been used for over 10 years by the investigators, first at Washington University Medical School in St. Louis, Missouri, then at Oral Roberts University in Tulsa, Oklahoma.

There has never been a single incident of fluid reaching the mouth of a person using the system. In fact, it is extremely rare that fluid ever enters the rubber tubing from the glass micropipet because the part of the pipet into and from which fluid moves is a relatively very small compartment near its tip. The main purpose of the filters is to protect the user from volatile solvents.

3. Application for radiometric assays

The system has been used extensively for 2 radiometric assays. Both involve carbon-14-labelled organic chemicals. Both involve addition of 5 or 10 microliters of aqueous incubation medium; one involves further addition of 1 microliter of substrate and 1 microliter of acid. Both involve addition of 40 or 70 microliters of an organic solvent, then removal of 30 or 60 microliters of the solvent. The assay is carried out in 400 microliter-capacity tapered polyethylene tubes, 4 millimeters diameter at the mouth and 2 millimeters diameter near the tip. The carbon-14 label is on acetyl coenzyme A, acetylcholine, or acetate during the assays. These are all non-volatile compounds.

The amount of carbon-14-labelled material added to each tube during the assay is about 0.025 microcurie. The amount removed in the last step of each assay is routinely less than 0.002 microcurie. Approximately 60 tubes are usually involved in a single total assay procedure.

4. Monitoring of the system

Exposure of a user's mouth to radioactivity may be monitored by determining any counts on the mouthpiece in a scintillation vial, since no more radioactivity can reach the person's mouth than reaches the mouthpiece. As further checks, the filters or pieces of the rubber tubing may also be put into vials for counting. When this was most recently done, the following results were obtained for 5 such pipetting systems.

| item | counts per minute | picocuries (= microcuries x 0.000001) |
|---|---|---|
| mouthpiece | 0.5, 0.3, 1, 1, 0.4 (average 0.7) | 0.2, 0.1, 0.5, 0.5, 0.2 (average 0.3) |
| filter | 18, 107, 2, 0, 0 (average 25) | 8, 48, 1, 0, 0 (average 7) |
| part of tubing where pipet is inserted | 3, 70, 1299, 1980, 3 (average 671) | 1, 31, 585, 892, 1 (average 302) |

5. Alternative systems

Recently many mechanical pipetting systems have become available. Some of these are suitable for certain steps of assay procedures where high accuracy or very small volumes or use in confined spaces is not involved. So far, no system has proven adequate for all aspects of the radiometric assays.

(Sketch approximates actual size of pipetting system)

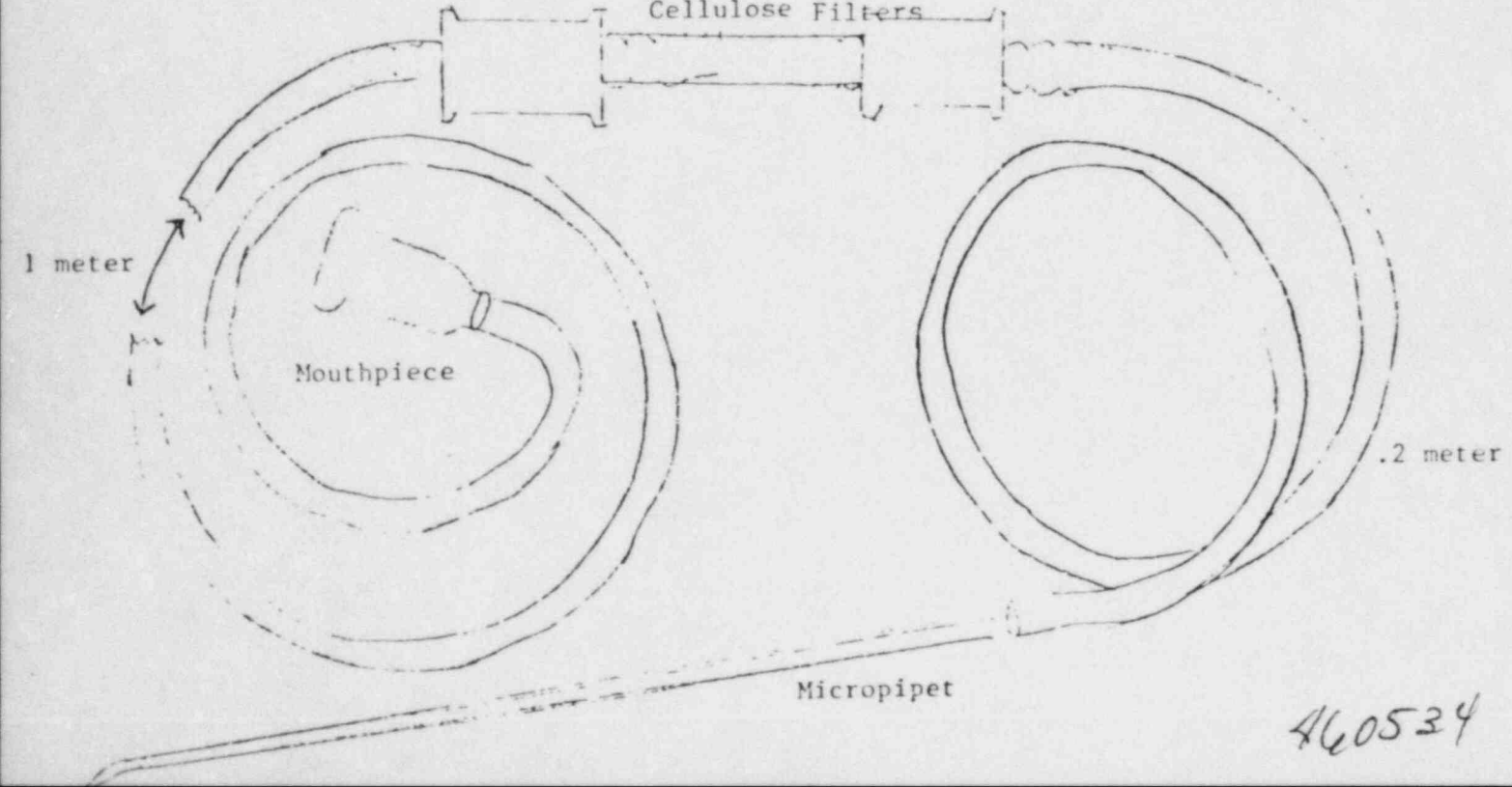


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Table 2.10 Maximum Permissible Levels of Radionuclides

| Radionuclide | In body, occupational (microcuries) | In air, environmental[a] (picocuries/cc) | In water, environmental (picocuries/cc) |
|---|---|---|---|
| HTO | 1000 | 0.2 | 3000 |
| $^{14}C$ | 300 | .1 | 800 |
| $^{32}P$ | 6 | .002 | 20 |
| $^{131}I$ | 0.7 | .0003 | 2 |
| $^{226}Ra$ | .1 | $1 \times 10^{-6}$ | 0.02 |
| $^{90}Sr$ | 2 | $1 \times 10^{-5}$ | .1 |
| $^{228}Th$ | 0.009 | $3 \times 10^{-7}$ | 7 |
| $^{239}Pu$ | .004 | $6 \times 10^{-8}$ | 5 |

[a]Maximum concentration in air for continuous exposure of members of the public in unrestricted areas. Maximum concentrations for exposure of radiation workers in restricted areas are approximately 30 times as high, based upon exposure for 40 hours in any period of 7 consecutive days. The occupational limits may be adjusted when exposure times differ from 40 hours, provided the total weekly exposure does not exceed that incurred in 40 hours at the tabulated values.

ing the radiation to permissible levels. When we deal with radioactive contamination, the problem is to prevent the contamination from entering the body. This can occur through inhalation, ingestion, absorption through the unbroken skin, or penetration through abrasions, cuts, and punctures. The protective measures needed are similar to those used with any other contaminant that presents an internal hazard and are probably familiar to any person trained to work in the laboratory.

It is general practice to handle significant quantities of radioactive material in a hood to prevent release of these materials to the working environment. The velocity of the air flowing into the hood should be between 100 and 150 linear feet per minute. Gloves should be worn and forceps used to handle sources. Hands should be washed and monitored for contamination after working with these materials. A generally cautious attitude with common sense precautions should provide adequate protection in most working situations. Of course, as the amount of the active material handled increases, the measures must become more stringent. A detailed discussion of protective measures for individuals working with radioactive materials is given in Part V.

Questions always arise as to what levels of radioactivity require use of a hood, or a glove box, or gloves, etc. We shall not attempt to specify protection measures for various levels of activity. However, every person beginning work with radioactive materials should take strict pre-

AGREEMENT TO PERFORM RADIOMETRIC ASSAYS

I will be carrying out radiometric assays using a Lang-Levy (Lowry type) pipetting system, wherein movement of fluid is controlled by my mouth. I understand that this constitutes using glass or quartz micropipets attached by rubber tubing, through cellulose filters, to a mouthpiece which is held in my mouth. Although the procedures involve very small amounts (about 0.025 microcurie per tube) of carbon 14- labelled radioisotopes, which are known to be hazardous only if taken into my body, I understand that there is a finite possibility that this could occur in my use of the system. I will therefore make every effort to follow safe procedures in doing these assays.

C. David Rose
Name

1/7/85
Date

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Donald A. Godfrey
Name

1/7/85
Date

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[illegible]
Name

[illegible]
Date

## Summary of Results

CPM / μCi

| | JAP | KLG | LIN | JSK | DAG | AVG. ± S.D. |
|---|---|---|---|---|---|---|
| Mouthpiece | 0.5 | 0.3 | 1.2 | 1.2 | 0.4 | 0.7 ± 0.4 |
| | 0.2 | 0.1 | 0.5 | 0.5 | 0.2 | 0.3 ± 0.2 |
| Tubing at Mouthpiece | 1.1 | 16.1 | 4.3 | 53.0 | 2.3 | 15.4 ± 21.9 |
| | 0.5 | 7.3 | 1.9 | 23.9 | 1.0 | 6.9 ± 9.9 |
| Filter (a) | 18.3 | 107.2 | 1.9 | 0.0 | 0.0 | 25.5[θ] ± 46.3 |
| | 8.2 | 48.2 | 0.9 | 0.0 | 0.0 | 11.5[θ] ± 20.8 |
| Tubing at gigget | 2.9 | 69.9 | 1299.1 ←? | 1980.4 | 3.3 | 671.1 ± 916.7 |
| | 1.3 | 31.5[θ] | 585.2 | 892.1 | 1.5[θ] | 302.3 ± 413.0 |
| Total | 22.8 | 193.5 | 1306.5 | 2034.6 | 6.0 | 712.7 ± 914.5 |
| | 10.2 | 87.1 | 588.5 | 916.5 | 2.7 | 321.0 ± 411.9 |

Results from
Check of Radiation Contamination
of pipetting tubing, filters,
mouthpieces
(experiment done 12/31/64)

# ROUTINE RADIATION SAFETY WIPES

INVESTIGATOR Gallagher / Rust DATE 12/4/84

DEPARTMENT Physiology ROOM 2C22

PRINCIPLE ISOTOPES USED $C^{14}$

KEY:

C - COUNTER TOP

H - HOOD

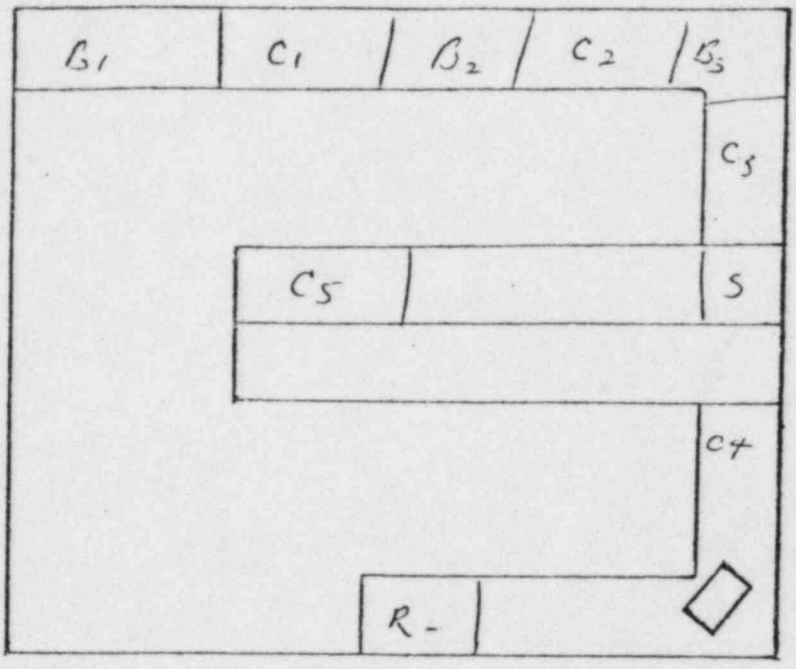
F - FLOOR

R - REFRIGERATOR

S - SINK

/ - DOOR

OTHER -




LABORATORY DIAGRAM

RESULTS:

| | COUNT | BACKGROUND | TOTAL(cpm) | COUNT | BACKGROUND | TOTAL(cpm) |
|---|---|---|---|---|---|---|
| C1 | 38.55 | 28.37 | 10.18 | | | |
| C2 | 63.45 | 28.37 | 35.08 | | | |
| C3 | 32.60 | 28.37 | 4.23 | | | |
| C5 | 60.05 | 28.37 | 31.68 | | | |
| F1 | 46.90 | 28.37 | 18.53 | | | |
| F2 | 32.65 | 28.37 | 4.28 | | | |
| B3 | 29.55 | 28.37 | 1.18 | | | |
| C4 | 32.10 | 28.37 | 3.73 | | | |
| | | 28.37 | — | | | |
| R | 29.00 | 28.37 | 0.63 | | | |
| S | 48.60 | 28.37 | 20.23 | | | |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | 236 | 1 | 38.55 | 7.20 | 20.00 | 21.00 | .474 |
| | | 2 | 18.30 | 10.45 | | | |
| | | 3 | 55.70 | 5.99 | | | |
| 2 | 237 | 1 | 63.45 | 5.61 | 20.00 | 41.24 | .498 |
| | | 2 | 31.65 | 7.94 | | | |
| | | 3 | 81.65 | 4.94 | | | |
| 3 | 238 | 1 | 32.60 | 7.83 | 20.00 | 61.48 | .547 |
| | | 2 | 17.85 | 10.58 | | | |
| | | 3 | 47.90 | 6.46 | | | |
| 4 | 239 | 1 | 60.05 | 5.77 | 20.00 | 81.72 | .526 |
| | | 2 | 31.60 | 7.95 | | | |
| | | 3 | 78.35 | 5.05 | | | |
| 5 | 240 | 1 | 46.90 | 6.52 | 20.00 | 101.96 | .524 |
| | | 2 | 24.60 | 9.01 | | | |
| | | 3 | 63.05 | 5.63 | | | |
| 6 | 241 | 1 | 32.65 | 7.82 | 20.00 | 122.20 | .537 |
| | | 2 | 17.55 | 10.67 | | | |
| | | 3 | 49.95 | 6.32 | | | |
| 7 | 242 | 1 | 29.55 | 8.22 | 20.00 | 142.44 | .565 |
| | | 2 | 16.70 | 10.94 | | | |
| | | 3 | 46.55 | 6.55 | | | |

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U0466

| SAMP | POS | CH | CPM | 2S% | TIME | ELTIME | SCR |
|---|---|---|---|---|---|---|---|
| 8 | 243 | 1 | 32.10 | 7.89 | 20.00 | 162.80 | .562 |
| | | 2 | 18.05 | 10.52 | | | |
| | | 3 | 48.05 | 6.45 | | | |
| 9 | 244 | 1 | 29.00 | 8.30 | 20.00 | 183.05 | .582 |
| | | 2 | 16.90 | 10.87 | | | |
| | | 3 | 45.25 | 6.64 | | | |
| 10 | 245 | 1 | 48.60 | 6.41 | 20.00 | 203.29 | .552 |
| | | 2 | 26.85 | 8.62 | | | |
| | | 3 | 65.05 | 5.54 | | | |
| 11 | 246 | 1 | 27.90 | 8.46 | 20.00 | 223.53 | .530 |
| | | 2 | 14.80 | 11.62 | | | |
| | | 3 | 44.35 | 6.71 | | | |
| 12 | 247 | 1 | 28.85 | 8.32 | 20.00 | 243.77 | .547 |
| | | 2 | 15.80 | 11.24 | | | |
| | | 3 | 45.90 | 6.59 | | | |

$2\overline{)\dfrac{28.32}{56.75}}$

4

16

Xerox copies of 3 most recent pages in our Radioactive storage log book. Total μCi ³²P on this log is kept in room 2022 and filled out by each technician at the time the assay is done. Total ³²P μCi recorded on the right in Red column of each page.

| DATE | BOOK / PAGE | ASSAY | INCUBATION AMT. USED | INCUBATION BOOK / PAGE | RETRIEVERY AMT. USED | RETRIEVERY BOOK / PAGE | RECORDED |
|---|---|---|---|---|---|---|---|
| 8-31-84 | 56/62 LJ | ChAT | 67.2 | 45/15 | 1.44 | 39/101 | 1.357 ✓ |
| 8-31-84 | 56/64 LJ | AChE | 10.62 | 45/17 | 4.20 | 26/63 | 2650 ✓ |
| 9-1-84 | 53/258 JK | ChAT | 33.6 | 45/15 | 1.44 | 39/101 | ✓ |
| 9-5-84 | 40/195 | | | 121/215 | | | 1.961 ✓ |
| 9/11/84 | 49/207 | ChAT | 39.90 | 45/15 | 1.44 | 39/101 | 1.356 ✓ |
| 9/12/84 | 56/78 LJ | ChAT | 67.2 | 45/15 | 1.44 | 39/101 | 0.789 ✓ |
| 9/12/84 | 49/208 | ChAT | 58.90 | 45/15 | 1.35 | 39/101 | 1684 ✓ |
| 9/12/84 | 53/282 JK | | | | | | 696 ✓ |
| 9/13/84 | 53/284 JK | ChAT | 33.60 | 45/15 | 2.88 | 39/101 | 2.650 ✓ |
| 9/18/84 | 56/90 LJ | AChE | 10.62 | 45/17 | 4.20 | 26/63 | 4413 ✓ |
| 9/19/84 | 49/210 | AChE | 19.04 | 45/17 | 4.44 | 26/63 | 1524 ✓ |
| 9/21/84 | 56/93 LJ | ChAT | 75.6 | 45/15 | 1.44 | 39/101 | 696 ✓ |
| 9/21/84 | 53/291 JK | ChAT | 33.60 | 45/15 | 2.88 | 39/101 | 1.032 ✓ |
| 9/25/84 | 53/296 JK | ChAT | 50.40 | 45/15 | 2.88 | 39/101 | 1.567 ✓ |
| 9/26/84 | 49/211 | ChAT | 97.25 | 45/15 | 1.38 | 39/101 | 1.032 ✓ |
| 9/26/84 | 53/299 JK | AChE | 10.62 | 45/17 | 5.60 | 26/63 | 1956 ✓ |
| 9/27/84 | 49/212 | ChAT | 97.25 | 45/15 | 1.38 | 39/101 | 1.032 ✓ |
| 9/28/84 | 58/12 JK | ChAT | 50.40 | 45/15 | 2.88 | 39/101 | 4460 ✓ |
| 10/1/84 | 58/16 JK | AChE | 21.24 | 45/17 | 5.60 | 26/63 | 5.07 ✓ |
| 10/1/84 | 58/17 JK | AChE | 21.24 | 45/17 | 5.60 | 26/63 | 1.032 ✓ |
| 10/3/84 | 58/18 JK | ChAT | 50.40 | 45/15 | 2.88 | 39/101 | 7.251 ✓ |
| 10/3/84 | 58/19 JK | AChE | 31.86 | 45/17 | 5.60 | 26/63 | 1.032 ✓ |
| 10/5/84 | 58/ JK | ChAT | 50.40 | 45/15 | 2.88 | 39/101 | 1.032 ✓ |
| 10/5/84 | 58/ JK | ChAT | 50.40 | 45/15 | 2.88 | 39/101 | ✓ |
| 10/2/84 | 49/213 | AChE | [illegible] | 45/17 | 4.44 | 26/63 | 6.068 |

# Protocol for Acetycholinesterase (AChE) Assay

1. Turn on water bath and fill with deionized water until it reaches the top level of micro tube rack when sitting on top of metal rack. The water bath needs to warm up to 37.5°C.

2. Remove necessary chemicals from Forma freezer.
   These include:
   - Incubation Substrate - $^{14}C$ AcChI (white label)
   - Recovery Standard - $^{14}C$ Acetate (blue label)
   - Preincubation Medium
   - Rat Brain Homogenate
   - 1% Buffered Bovine Serum Albumin (BSA)
3. Get styrofoam ice container full of ice.
4. When chemicals from step 2 have thawed, place immediately on ice.
5. If samples are in desiccator, place a piece of filter paper over opening and open to slowly release vacuum. This takes about 5 minutes, during which time the following dilutions can be made.
6. Wash mouthpiece of pipet tubing well with warm water and soap. Once you have started the assay if you remove the mouthpiece from your mouth, do not let it hang or touch any surfaces. If it accidentally does, be sure to wash again before using.
7. Take two small glass test tubes and label I (incubation medium), and H (homogenate dilution).
8. Mix up the incubation medium in the above labeled tube I by diluting ~10µl $^{14}C$ ACh with ~15µl 100mµ $KPO_4$ Buffer. Mix up as much as needed for the number of tubes in the assay, keeping the same proportions. Always mix up at least 5-10µl over what will actually be used in assay. Be sure to mix well with vortex. Place on ice.
9. Mix up the Homogenate Dilution in the above labeled tube H. Vortex the homogenate tube very well before pipeting. Use a ~3.3 µl pipet with a fairly large constriction and tip, or the homogenate will clog up the pipet. Dilute with ~130µl 1% Buffered BSA. Vortex until well mixed. Place on ice. Each homogenate tube can be thawed three times, so be sure to label the tube with the thaw number. After the third thaw dispose of the tube.
10. Pipet ~1 µl of the homogenate dilution into each of three plastic micro tubes labeled H1, H2, H3. The homogenate dilution should be pipetted into the bottom of the tubes and be in one droplet. Place the tubes immediately on ice. The homogenate dilution should be vortexed between each pipet delivery.
11. Label three plastic micro tubes for blanks (B1, B2, B3) and three for the recoveries (R1, R2, R3).
12. If samples are in desiccator, carefully removed them once the vacuum has been released. To remove desiccator lid, carefully slide lid off bowl. If lid will not slide, place a razor blade between the lid and bowl and tap with the gray plastic cap until the seal breaks.
13. Check all tubes under the microscope at 6X to make sure that samples are still at bottom of tubes.
14. Place the tubes in a rack in the following order: 3 Blanks, Tissue Samples, 3 Homogenates (leave these 3 on ice for now, skip 3 spaces in rack), 3 recoveries.

15. Stir the preincubation medium and place tube in a small plastic beaker with no ice. Add ~10µl preincubation medium to all tubes at room temperature. Do not place on ice.
16. Check water bath to make sure temperature is at 37°C.
17. Set clock for 30 seconds before the zero.
18. Stir the incubation and recovery media again and place on ice in a small plastic beaker.
19. Start the clock and at ~15 seconds before zero, pipet into the first blank tube ~1µl of incubation medium. Stir with buzzer. As the clock hits zero, place the tube in the rack in the water bath. Then every 15 seconds add ~1µl of incubation medium to each tube consecutively (blanks, samples, homogenates), stir with buzzer, and place in water bath. The time can be longer or shorter than 15 seconds, depending on what you feel comfortable with, as long as you are consistent throughout the assay.
20. For the recovery tubes pipet ~1µl of recovery standard into each tube at the same time interval as above. The same pipet may be used as for the incubation medium, but it must be washed in between. Wash as quickly as possible, although the timing is not as critical for the recovery medium.
21. The tubes must now incubate in the water bath until the clock reaches 30 minutes.
22. Put all previously used chemicals back into the freezer. Discard the tube of Preincubation Medium and the homogenate dilution tube. Discard the Incubation Medium dilution tube in the waste radiation jug.
23. Take the same number of plastic scintillation vials as tubes used, and put 10 mls. Scintiverse/Scintilene Cocktail into each. To flush out the tubing on the automatic dispenser, always discard the first delivery. Kimble glass vials are also needed at the end of the assay, one for each blank and one for each recovery. These are filled with 10 mls. Scintiverse only.
24. At approximately 15 seconds before the clock reaches 30 minutes, remove the first blank tube from the water bath and add ~1.5µl 1N HCl. Stir tube with buzzer and place in rack on ice as the clock hits the 30 minute mark. Repeat this procedure with each tube consecutively and at the same time interval as was used to add the incubation medium.
25. Get Ethyl Acetate from refrigerator and place on ice in large plastic beaker.
26. Add ~75-77µl Ethyl Acetate to each tube, stir with buzzer and return to ice.
27. After the Ethyl Acetate has been added to all tubes, take rack to microcentrifuge. When placing tubes in centrifuge racks there must be an equal number of tubes on opposite sides of the roter. Be sure the centrifuge racks are pushed down all the way. Centrifuge the tubes for 5 minutes.
28. When centrifuge stops, immediately return tubes to rack on ice. Do not allow the tubes to sit in the centrifuge as the heat may cause chemical changes to occur.
29. From each tube withdraw ~65-67 µl top Acetate layer and pipet into the corresponding scintillation vials. To pipet into vials, place pipet on side of vial above fluid level. Do not immerse pipet, and blow out onto Micro Wipe between pipettings to reduce any carry over. Set cap on each vial after pipetting into it.

30. Place three of the glass vials in the plastic vial holder. Cut off the bottom part (safely above fluid level) of the incubation tubes and allow tip to fall into appropriate glass vial. Cap tightly and shake well until all fluid is out of tube tips. Discard the tops of the tubes into a waste radiation jug.
31. Repeat the above procedure for the blank tubes. Leave the remaining tubes in the rack (not on ice), and discard into waste radiation jug only after the assay has been counted once and appears normal.
32. Tightly screw caps on vials and label each cap by blank, sample number, homogenate or recovery. On the first vial put your initials, book and page number, and date.
33. Shake the box containing the vials from side to side to mix well.
34. Place vials in the scintillation counter with appropriate tower in front of first vial. Most assays will be counted at 20 min. for each vial for 3 runs. In the LS-8100, the tower for 20 minute counts is 3. For the LS-7500, it is tower 9. To start counting the assay, push the two RESET buttons simultaneously and then push AUTO COUNT.
35. Wash all pipets used in assay.
36. Do wipe test of work area.
37. After you have cleaned up your station and you are completely finished working with all radioactive materials, wash hands thoroughly.

# Protocol for Choline Acetyltransferase (ChAT) Assay

1. Turn on water bath and fill with deionized water until it reaches the top level of micro tube rack when sitting on top of metal rack. The water bath needs to warm up to 37.5°C.

2. Remove necessary chemicals from Forma freezer. These include:
   Incubation Substrate - [$^{14}$C] AcCoA (yellow label)
   Recovery Standard - [$^{14}$C] AChCl (green label)
   Preincubation Medium
   Rat Brain Homogenate
   1% Buffered BSA

3. Get styrofoam ice container full of ice.

4. When chemicals from step 2 have thawed, place immediately on ice.

5. If samples are in desiccator, place a piece of filter paper over opening and open to slowly release vacuum. This takes about 5 minutes, during which time the following dilutions can be made.

6. Wash mouthpiece of pipet tubing well with warm water and soap. Once you have started the assay if you remove the mouthpiece from your mouth, do not let it hang or touch any surfaces. If it accidentally does, be sure to wash again before using.

7. Take three small glass test tubes and label I (incubation medium), R (recovery medium), and H (homogenate dilution).

8. Mix up the incubation medium in the above labeled tube I by diluting ~15 µl [$^{14}$C]AcCoA with ~65 µl Preincubation Medium. Mix up as much as needed for the number of tubes in the assay, keeping the same proportions. Always mix up at least 15-20 µl over what will actually be used in assay. Be sure to mix well with vortex. Place on ice.

9. Mix up the recovery medium in the above labeled tube R by diluting ~1.5 µl [$^{14}$C]AChCl with ~32 µl Preincubation Medium. Mix well with vortex. Place on ice.

10. Mix up the Homogenate Dilution in the above labeled tube H. Vortex the homogenate tube very well before pipetting. Use a ~3.3 µl pipet with a fairly large constriction and tip, or the homogenate will clog up the pipet. Dilute with ~32 µl 1% Buffered Bovine Serum Albumin. Vortex until well mixed. Place on ice. Each homogenate tube can be thawed three times, so be sure to label the tube with the thaw number. After the third thaw dispose of the tube.

11. Pipet ~1 µl of the homogenate dilution into each of three plastic micro tubes labeled H1, H2, H3. The homogenate dilution should be pipetted into the bottom of the tubes and be in one droplet. Place the tubes immediately on ice. The homogenate dilution should be vortexed between each pipet delivery.



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12. Label three plastic micro tubes for blanks (B1, B2, B3) and three for the recoveries (R1, R2, R3).

13. If samples are in desiccator, carefully remove them once the vacuum has been released. To remove desiccator lid, carefully slide lid off bowl. If lid will not slide, place a razor blade between the lid and bowl and tap with the gray plastic cap until the seal breaks.

14. Check all tubes under the microscope at 6X to make sure that samples are still at bottom of tubes.

15. Place the tubes in a rack in the following order: 3 Blanks, Tissue Samples, 3 Homogenates (leave these 3 on ice for now, skip 3 spaces in rack), 3 Recoveries.

16. Check water bath to make sure temperature is at 37.5°C.

17. Set clock for 30 seconds before the zero.

18. Stir the incubation and recovery media again and place on ice in a small plastic beaker.

19. Start the clock and at ~15 seconds before zero, pipet into the first blank tube ~5 µl of incubation medium. As the clock hits zero, place the tube in the rack in the water bath. Then every 15 seconds add ~5 µl of incubation medium to each tube consecutively (blanks, samples, homogenates), and place in water bath. The time can be longer or shorter than 15 seconds, depending on what you feel comfortable with, as long as you are consistent throughout the assay.

    When pipetting into the tubes with the tissue samples be sure that there are no air bubbles at the bottom of tube, as this can prevent the incubation medium from reaching the sample. If there is a bubble, tap the bottom of the tube with your finger until the bubble rises.

    When the incubation medium is added to the homogenate tubes, they need to be stirred with the buzzer.

20. For the recovery tubes pipet ~5 µl of recovery medium into each tube at the same time interval as above. The same pipet may be used as for the incubation medium, but it must be washed in between. Wash as quickly as possible, although the timing is not as critical for the recovery medium.

21. The tubes must now incubate in the water bath until the clock reaches 30 minutes.

22. During this time, fill another rack with as many tubes as used before and label in the same order.

23. Using the Eppendorf Repeater with the radioactive label, pipet 100 µl Tetraphenyl Boron (TPB) in 10mM $NaPO_4$ Buffer (in refrigerator) into each tube. If there is a gap in the fluid, vortex tube to get rid of it. Cover and place the rack of tubes either on ice or in the refrigerator.

24. If you have time you can also put all previously used chemicals back into the freezer. Discard the tube of Preincubation Medium and the homogenate dilution tube. Discard the Incubation Medium and Recovery Medium dilution tubes in waste radiation jug.

25. Get $15^{mg}$/ml Tetraphenyl Boron (TPB) in 3 Heptanone from freezer part of refrigerator and place on ice in large plastic beaker.

26. At approximately 15 seconds before the clock reaches 30 minutes, remove the first blank tube from the water bath and add ~40-42 µl $15^{mg}$/ml TPB in 3 Heptanone. Stir tube with buzzer and place in rack on ice as the clock hits the 30 minute mark. Repeat this procedure with each tube consecutively and at the same time interval as was used to add the incubation medium.

27. After the TPB Heptanone has been added to all tubes, take rack to micro-centrifuge. When placing tubes in centrifuge racks there must be an equal number of tubes on opposite sides of the roter. Be sure the centrifuge racks are pushed down all the way. Centrifuge the tubes for 5 minutes.

28. While tubes are centrifuging, take the same number of plastic scintillation vials as tubes used, and put 10 mls. Scintiverse/Scintilene Cocktail into each. To flush out the tubing on the automatic dispenser, always discard the first delivery. Kimble glass vials are also needed at the end of the assay, one for each blank and one for each recovery. These are filled with 10 mls. Scientiverse only.

29. When centrifuge stops, immediately return tubes to rack on ice. Do not allow the tubes to sit in the centrifuge as the heat may cause chemical changes to occur.

30. Place wash tubes containing TPB in 10mM $NaPO_4$ Buffer on ice (if taken from refrigerator). From each incubation tube withdraw ~35-37 µl of top Heptanone layer and pipet into corresponding wash tube. Mix each tube well with buzzer and place on ice. Between each withdrawal blow pipet out onto a Micro Wipe to reduce any carry over.

31. Centrifuge wash tubes for 5 min. following the previous centrifuge directions.

32. From each wash tube withdraw ~29-31 µl top Heptanone layer and pipet into the corresponding scintillation vials. To pipet into vials, place pipet on side of vial above fluid level. Do not immerse pipet, and blow out onto Micro Wipe between pipettings to reduce any carry over. Set cap on each vial after pipetting into it.

33. Place three of the glass vials in the plastic vial holder. Cut off the bottom part (safely above fluid level) of both the incubation and wash recovery tubes and allow tip to fall into appropriate glass vial. Cap tightly and shake well until all fluid is out of tube tips. Discard the tops of the tubes into a waste radiation jug.

34. Repeat the above procedure for the blank tubes, except cut off the tip of the incubation tube only. Leave the remaining tubes in the rack (not on ice), and discard into waste radiation jug only after the assay has been counted once and appears normal.

35. Tightly screw caps on vials and label each cap by blank, sample number, homogenate or recovery. On the first vial put your initials, book and page number, and date.

36. Shake the box containing the vials from side to side to mix well.

37. Place vials in the scintillation counter with appropriate tower in front of first vial. Most assays will be counted at 20 min. for each vial for 3 runs. In the LS-8100, the tower for 20 minute counts is 3. For the LS-7500, it is tower 9. To start counting the assay, push the two RESET buttons simultaneously and then push AUTO COUNT.

38. Wash all pipets used in assay.

39. Do wipe test of work area.

40. After you have cleaned up your station and you are completely finisned working with all radioactive materials, wash hands thoroughly.

NRC Form 782
(1-82)

U.S. NUCLEAR REGULATORY COMMISSION

# MATERIALS DATA INPUT – INDUSTRIAL, MEDICAL, SOURCE/SPECIAL NUCLEAR

## A. TYPE OF ACTION AND IDENTIFICATION CODES

| | NEW LICENSE | | AMENDMENT TO RENEW LICENSE | | AMENDMENT TO TERMINATE | | VOID | DOCKET NUMBER | | MAIL CONTROL NUMBER | CHANGE NAME/ADDRESS ("X" box) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | NEW LICENSE AND NEW LICENSEE | XX | OTHER AMENDMENT | | CLERICAL CHANGE NO AMENDMENT | 4 | | 030-14781 | | 460534 | |

## B. INDICATIVE INFORMATION

| | | |
|---|---|---|
| INDIVIDUAL LICENSEES | NAME (Last, First, Middle) | NAME (Last, First, Middle) |
| | NAME (Last, First, Middle) | NAME (Last, First, Middle) |
| | NAME (Last, First, Middle) | NAME (Last, First, Middle) |

Ok LFMB
no fee pd

| | | | | |
|---|---|---|---|---|
| ORGANIZATION LICENSEES 5 | ORGANIZATION NAME (Alphabetic Sequence) Oral Roberts University | | | |
| | DEPARTMENT OR BUREAU | | | |
| ADDRESS 5 | BUILDING, STREET 7777 S. Lewis Ave. | CITY Tulsa | STATE OK | ZIP CODE 74171 |

| 6 | TYPE OF APPLICANT | | U.S. GOVERNMENT AGENCY | DATE REQUEST RECEIVED | INSTITUTION CODE | PENDING PROG. CODE | ACTUAL PROG. CODE |
|---|---|---|---|---|---|---|---|
| | | | INDIVIDUAL LICENSEE | | | | |
| | | 333 | ORGANIZATIONAL LICENSEE | 02/13/85 | 18282 | | |

SECONDARY PROGRAM CODES (As required)

| #1 | #2 | #3 | #4 | #5 |
|---|---|---|---|---|
| | | | | |

No work done on this.

| 7 | LICENSE NUMBER | DATE LICENSE ISSUED OR ACTION COMPLETED | EXPIRATION DATE | |
|---|---|---|---|---|
| | 35-18282-01 | 4/30/85 | Amendment not nec. | |

## C. STATISTICAL INFORMATION

| MEDICAL CATEGORY | | FOR HUMAN USE ONLY | | FOR HUMAN AND NONHUMAN USE | | FOR NONHUMAN USE ONLY |
|---|---|---|---|---|---|---|

POSSESSION OF THE MATERIAL IS AUTHORIZED IN ONE OF THE FOLLOWING AREAS:

| AND/OR IN THE STATE(S), TERRITORY(IES), COUNTRY CHECKED (At right) | SAME AS "STATE" IN ADDRESS | | ALL STATES | | ALL NON-AGREEMENT STATES | |
|---|---|---|---|---|---|---|
| | AL - ALABAMA | GA - GEORGIA | MD - MARYLAND | NJ - NEW JERSEY | SC - SOUTH CAROLINA | WY - WYOMING |
| | AK - ALASKA | HI - HAWAII | MA - MASSACHUSETTS | NM - NEW MEXICO | SD - SOUTH DAKOTA | |
| | AZ - ARIZONA | ID - IDAHO | MI - MICHIGAN | NY - NEW YORK | TN - TENNESSEE | AS - AMERICAN SOMOA |
| | AR - ARKANSAS | IL - ILLINOIS | MN - MINNESOTA | NC - NORTH CAROLINA | TX - TEXAS | CZ - CANAL ZONE |
| | CA - CALIFORNIA | IN - INDIANA | MS - MISSISSIPPI | ND - NORTH DAKOTA | UT - UTAH | GU - GUAM |
| | CO - COLORADO | IA - IOWA | MO - MISSOURI | OH - OHIO | VT - VERMONT | PR - PUERTO RICO |
| | CT - CONNECTICUT | KS - KANSAS | MT - MONTANA | OK - OKLAHOMA | VA - VIRGINIA | VI - VIRGIN ISLANDS |
| | DE - DELAWARE | KY - KENTUCKY | NB - NEBRASKA | OR - OREGON | WA - WASHINGTON | |
| | DC - WASHINGTON DC | LA - LOUISIANA | NV - NEVADA | PA - PENNSYLVANIA | WV - WEST VIRGINIA | CN - CANADA |
| | FL - FLORIDA | ME - MAINE | NH - NEW HAMPSHIRE | RI - RHODE ISLAND | WI - WISCONSIN | |

## D. POSSESSION LIMITS OF SOURCE AND SPECIAL NUCLEAR MATERIALS AND TRITIUM

| SOURCE MATERIAL CEILING | G - GRAMS / Kg - KILOGRAMS | SNM CEILING | G - GRAMS / Kg - KILOGRAMS | IF FOR POWER REACTOR ("X" here) |
|---|---|---|---|---|
| | | | | |

| *MATERIAL | AMOUNT | UNIT | CONFIG. | ENRICH | *MATERIAL | AMOUNT | UNIT | CONFIG. | ENRICH |
|---|---|---|---|---|---|---|---|---|---|
| U5 - U235 | | G / Kg | S / UNS | | | | G / Kg | S / UNS | |
| U3 - U233 | | G / Kg | S / UNS | | | | G / Kg | S / UNS | |
| PU-Plutonium | | G / Kg | S / UNS | | | | G / Kg | S / UNS | |
| UR-Uranium | | G / Kg | S / UNS | | | | G / Kg | S / UNS | |
| TH-Thorium | | G / Kg | S / UNS | | | | G / Kg | S / UNS | |
| | | G / Kg | S / UNS | | | | G / Kg | S / UNS | |
| | | G / Kg | S / UNS | | | | G / Kg | S / UNS | |

| H3-Tritium | | CURIES / MILLICURIES / MICROCURIES | RIS CODES | |
|---|---|---|---|---|
| | | | | ML40 |

*Use two-digit codes S-SEALED, UNS-UNSEALED

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(1-82)

1 – FILE COPY