NRC FORM 313 U.S. NUCLEAR REGULATORY COMMISSION
(6-2000)
10 CFR 30, 32, 33, 34, 35, 36, 39, and 40

APPLICATION FOR MATERIAL LICENSE

APPROVED BY OMB: NO. 3150-0120 EXPIRES: 08/31/2002

Estimated burden per response to comply with this mandatory collection request: 7.4 hours. Submittal of the application is necessary to determine that the applicant is qualified and that adequate procedures exist to protect the public health and safety. Send comments regarding burden estimate to the Records Management Branch (T-6 E6), U.S. Nuclear Regulatory Commission, Washington, DC 20555-0001, or by internet e-mail to bjs1@nrc.gov, and to the Desk Officer, Office of Information and Regulatory Affairs, NEOB-10202, (3150-0000), Office of Management and Budget, Washington, DC 20503. If a means used to impose an information collection does not display a currently valid OMB control number, the NRC may not conduct or sponsor, and a person is not required to respond to, the information collection.

INSTRUCTIONS: SEE THE APPROPRIATE LICENSE APPLICATION GUIDE FOR DETAILED INSTRUCTIONS FOR COMPLETING APPLICATION. SEND TWO COPIES OF THE ENTIRE COMPLETED APPLICATION TO THE NRC OFFICE SPECIFIED BELOW.

APPLICATION FOR DISTRIBUTION OF EXEMPT PRODUCTS FILE APPLICATIONS WITH:

DIVISION OF INDUSTRIAL AND MEDICAL NUCLEAR SAFETY
OFFICE OF NUCLEAR MATERIALS SAFETY AND SAFEGUARDS
U.S. NUCLEAR REGULATORY COMMISSION
WASHINGTON, DC 20555-0001

ALL OTHER PERSONS FILE APPLICATIONS AS FOLLOWS:

IF YOU ARE LOCATED IN:

CONNECTICUT, DELAWARE, DISTRICT OF COLUMBIA, MAINE, MARYLAND, MASSACHUSETTS, NEW HAMPSHIRE, NEW JERSEY, NEW YORK, PENNSYLVANIA, RHODE ISLAND, OR VERMONT, SEND APPLICATIONS TO:

LICENSING ASSISTANT SECTION
NUCLEAR MATERIALS SAFETY BRANCH
U.S. NUCLEAR REGULATORY COMMISSION, REGION I
475 ALLENDALE ROAD
KING OF PRUSSIA, PA 19406-1415

ALABAMA, FLORIDA, GEORGIA, KENTUCKY, MISSISSIPPI, NORTH CAROLINA, PUERTO RICO, SOUTH CAROLINA, TENNESSEE, VIRGINIA, VIRGIN ISLANDS, OR WEST VIRGINIA, SEND APPLICATIONS TO:

SAM NUNN ATLANTA FEDERAL CENTER
U. S. NUCLEAR REGULATORY COMMISSION, REGION II
61 FORSYTH STREET, S.W., SUITE 23T85
ATLANTA, GEORGIA 30303-8931

IF YOU ARE LOCATED IN:

ILLINOIS, INDIANA, IOWA, MICHIGAN, MINNESOTA, MISSOURI, OHIO, OR WISCONSIN, SEND APPLICATIONS TO:

MATERIALS LICENSING BRANCH
U.S. NUCLEAR REGULATORY COMMISSION, REGION III
801 WARRENVILLE RD.
LISLE, IL 60532-4351

ALASKA, ARIZONA, ARKANSAS, CALIFORNIA, COLORADO, HAWAII, IDAHO, KANSAS, LOUISIANA, MONTANA, NEBRASKA, NEVADA, NEW MEXICO, NORTH DAKOTA, OKLAHOMA, OREGON, PACIFIC TRUST TERRITORIES, SOUTH DAKOTA, TEXAS, UTAH, WASHINGTON, OR WYOMING, SEND APPLICATIONS TO:

NUCLEAR MATERIALS LICENSING SECTION
U.S. NUCLEAR REGULATORY COMMISSION, REGION IV
611 RYAN PLAZA DRIVE, SUITE 400
ARLINGTON, TX 76011-8064

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PERSONS LOCATED IN AGREEMENT STATES SEND APPLICATIONS TO THE U.S. NUCLEAR REGULATORY COMMISSION ONLY IF THEY WISH TO POSSESS AND USE LICENSED MATERIAL IN STATES SUBJECT TO U.S.NUCLEAR REGULATORY COMMISSION JURISDICTIONS.

1. THIS IS AN APPLICATION FOR *(Check appropriate item)*

- [ ] A. NEW LICENSE
- [ ] B. AMENDMENT TO LICENSE NUMBER _____
- [X] C. RENEWAL OF LICENSE NUMBER 45-25034-01

2. NAME AND MAILING ADDRESS OF APPLICANT *(Include ZIP code)*

John A. Hufnagel RSO
Washington and Lee University
116 North Main Street, Howe 301 B
Lexington, Virginia 24450

3. ADDRESS WHERE LICENSED MATERIAL WILL BE USED OR POSSESSED

Washington and Lee University
Science Center
116 North Main Street
Lexington, Virginia 24450

4. NAME OF PERSON TO BE CONTACTED ABOUT THIS APPLICATION

John A. Hufnagel

TELEPHONE NUMBER

(540) 458-8893

SUBMIT ITEMS 5 THROUGH 11 ON 8-1/2 X 11" PAPER. THE TYPE AND SCOPE OF INFORMATION TO BE PROVIDED IS DESCRIBED IN THE LICENSE APPLICATION GUIDE.

5. RADIOACTIVE MATERIAL
a. Element and mass number; b. chemical and/or physical form; and c. maximum amount which will be possessed at any one time.

6. PURPOSE(S) FOR WHICH LICENSED MATERIAL WILL BE USED.

7. INDIVIDUAL(S) RESPONSIBLE FOR RADIATION SAFETY PROGRAM AND THEIR TRAINING EXPERIENCE.

8. TRAINING FOR INDIVIDUALS WORKING IN OR FREQUENTING RESTRICTED AEAS.

9. FACILITIES AND EQUIPMENT.

10. RADIATION SAFETY PROGRAM.

11. WASTE MANAGEMENT.

12. LICENSE FEES *(See 10 CFR 170 and Section 170.31)*
FEE CATEGORY N.A. | AMOUNT ENCLOSED $

13. CERTIFICATION *(Must be completed by applicant)* THE APPLICANT UNDERSTANDS THAT ALL STATEMENTS AND REPRESENTATIONS MADE IN THIS APPLICATION ARE BINDING UPON

THE APPLICANT AND ANY OFFICIAL EXECUTING THIS CERTIFICATION ON BEHALF OF THE APPLICANT, NAMED IN ITEM 2, CERTIFY THAT THIS APPLICATION IS PREPARED IN CONFORMITY WITH TITLE 10, CODE OF FEDERAL REGULATIONS, PARTS 30, 32, 33, 34, 35, 36, 39, AND 40, AND THAT ALL INFORMATION CONTAINED HEREIN IS TRUE AND CORRECT TO THE BEST OF THEIR KNOWLEDGE AND BELIEF.

WARNING: 18 U.S.C. SECTION 1001 ACT OF JUNE 25, 1948 62 STAT. 749 MAKES IT A CRIMINAL OFFENSE TO MAKE A WILLFULLY FALSE STATEMENT OR REPRESENTATION TO ANY DEPARTMENT OR AGENCY OF THE UNITED STATES AS TO ANY MATTER WITHIN ITS JURISDICTION.

CERTIFYING OFFICER – TYPED/PRINTED NAME AND TITLE	SIGNATURE	DATE
Dr. Jeanine Stewart, Dean of College	Stewart	3-29-05

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TYPE OF FEE	FEE LOG	FEE CATEGORY	AMOUNT RECEIVED	CHECK NUMBER	COMMENTS
			$		
APPROVED BY				DATE	136921

WASHINGTON AND LEE
UNIVERSITY

Lexington, Virginia 24450-0303

www.wlu.edu

Department of Biology
(540) 458-8891
FAX (540) 458-8012

April 8, 2005

L A T
USNRC, Region I
475 Allendale Road
King of Prussia, Penn. 19406

Dear Licensing Team;

Please find enclosed in duplicate the renewal of our Material License # 45-25034-01 which expires May 31, 2005. The only significant change represented in the renewal is the retirement of James Donaghy Ph.D.. His retirement if official as of June 1, 2005, and we are requesting that his position as an "Authorized User" be filled by Kenneth E. Van Ness Ph.D.. Van Ness working with Donaghy has utilized the same protocols authorized for Donaghy for the past sixteen years, and we are requesting that Van Ness be authorized by our license to continue to employ the same protocols and isotope in his future research. Please find enclosed Dr. Van Ness's Curriculum Vitae, isotope protocols, and training experience. In addition to his past experience and training Van Ness has also received the " In-House program detailed in this renewal.

Sincerely,

John A. Hufnagel RSO

5. Radioactive Materials

Material	Form	Maximum Amount Possessed
Hydrogen 3 *	Any	20 millicuries
Carbon 14	Any	1 millicurie
Cobalt 60 **	Any	20 microcuries
Sulfur 35	Any	10 millicuries

* Tridiated Thymidine in aqueous solution
** Factory sealed source (disc), # 971-111-1, manufactured by Isotope Products Laboratories, Valencia California. Please see **"Attachment Six: Certification Documents for sources, Dosimetry Programs, and Instrument Calibration"**.

6. Purposes for which licensed material will be used:

Radioactive materials will be used both for basic scientific research and for instruction of undergraduates. Please refer to **"Attachment One: Protocols"** for detailed information.

7. Individuals responsible for the Radiation Safety Program and their training experience.

Please see **"Attachment Two: Authorized Users"** for information on authorized users. In addition to their previous professional experience, the "Authorized Users" in **"Attachment Two"** have received the "in-house" training program detailed in **"Attachment Five: Training and Safety Procedures"**. The same program is also used for students and other novices who will apply the protocols and isotopes detailed in this license.

Membership of the Radiation Safety Committee is as follows:

John A. Hufnagel, Science Laboratory Manager, Radiation Safety Officer and Chair of Radiation Safety Committee
Kenneth E. Van Ness, PhD., Professor of Physics
James C. Eason, PhD., Assistant Professor of Physics

8. Training for individuals working in or frequenting restricted areas

Please see **"Attachment Two: Authorized Users"** for specifics on training prior to employment at Washington and Lee University.

The "in-house" training program for students and other novices is a four phase program as follows:

Phase I: Lecture on the physics of radionuclides, routine laboratory procedures, emergency procedures, and experimental strategies by the Radiation Safety Officer.

Phase II: Viewing of three video cassettes produced by Indiana University Radiation Safety Office, Bloomington, IN.

Phase III: Discussion of the Radiation Safety Manual, with emphasis on Part III, Health Physics Procedures. Each person receives a copy of the manual.

Phase IV: Supervised "dry-run" of all relevant experimental procedures for researchers, or, for students, one of the radioisotopic dilution exercises detailed in **"Attachment One"**.

These four phases combined address at least once each item of the Regulatory Guide 10.7, item 15d (1-14). Please see **"Attachment Five: Training and Safety Procedures"** for greater detail of the training program.

9. Facilities and equipment.

Manipulations of radioisotopes are carried out in fume hoods lined with absorbent material ("labmat") using disposable gloves and aprons or lab coats. When radioisotopes equipment cannot fit into the fume hood (e.g. incubations of biological tissues in an incubator), only the minimal necessary procedures are to take place out of the fume hood. Trays and labmat are used to contain any potential spills. Decontamination materials (e.g. "Lift Off") and other spill kit items appropriate for the specific procedure are kept in each laboratory approved for use. Restricted rooms are marked in accordance with 10 CFR 20.

Material storage is in separate, isolated, locked cabinets or in freezers in locked restricted laboratories approved for radioisotope use marked with commercial radioactive warning signs. Most materials (i.e. except materials that are soft beta emitters) are kept in lead-lined locked boxes or in a lead brick pile in a locked storage room.

Authorized rooms are limited to the following:

Howe 102: Physics Research Lab
Howe 309: Biology Darkroom
Howe 312: Biology Teaching Lab
Howe 315: Biology Research Lab
Howe 304: Biology Hot Lab
Howe 306: Biology Instrumentation Lab

Each laboratory is equipped with fume hood(s), sink(s), and multiple chemically resistant research benches.

Please see **"Attachment Four: Room Plans"** for scale drawings and the "authorized users" utilizing that particular space.
10. Radiation Safety Program.

Radiation Monitoring Instruments:

2 BICRON "Surveyor M" Portable Count Rate Meters, with GM and scintillation probes, with count rate capability from 0 to 10^6. BICRON "APGM" probe – detects alpha particles > 3 MeV, beta particles > 45 MeV and gamma waves > 6 keV. BICRON "GILE" probe – NaI (T1) scintillator for low energy (< 60 keV) gamma detection. These instruments are used for monitoring of gamma producing materials and for all surveys.

3 SE International Monitor 4 survey meters with fixed internal probes are used for general monitoring during procedures. One each is assigned to the Departments of Biology, Chemistry, and Physics.

3 Victoreen 2000A Dosimeters and Chargers. These are personal pencil dosimeters for the use of those using gamma emitters or ^{35}S during procedures to determine absorbed doses.

1 Packard 2200 CA Liquid Scintillation Counter is used to count wipe survey membranes.

Calibration:

BICRON meters are calibrated yearly by Duratek Instrument Services (Kingston, TN). SE International meters are calibrated in house using a point source (these are used for qualitative warning purposes only, not for personal or physical surveying). The pencil dosimeters cannot be calibrated but must be replaced when they are no longer able to be properly "zeroed" by the charger. The scintillation counter is calibrated using commercial standards and a Packard calibration software program. Calibration is done by a factory trained Packard technician.

In addition, the Radiation Program contracts with Landauer, Inc, (Glenwood, Illinois) for a dosimetry program that includes the quarterly monitoring of room, ring, and whole body dosimeters. These are employed where isotopes if free form are used and stored on a regular basis. Please see **"Attachment Six: Certification Documents for Sources, Dosimetry Programs, and Instrument Calibration".**

"We will use instruments that meet the radiation monitoring instrument specifications published in Appendix M to NUREG – 1556, Vol. 7, 'Program-Specific Guidance About Academic, Research and Development, and Other Licenses of Limited Scope,' dated December 1999. Additionally, we will implement the model survey meter calibration program published in Appendix M to NUREG – 1556, Vol. 7, 'Program-Specific Guidance About Academic, Research and Development, and Other Licenses of Limited Scope,' dated December 1999. We reserve the right to upgrade our survey instruments as necessary."

Material Receipt and Accountability

In order to standardize procedures and avoid the mishandling or misplacement of deliveries of radioactive materials, The Radiation Safety Committee concurred that John Hufnagel RSO will in the future coordinate for all the science departments all purchase of said radioactive materials. The procedures will entail but not necessarily be limited to the following: the RSO when notified will make all orders personally by telephone; it will be specified to the vendor that the order is to be sent by Federal Express; the vendor will be required to specify the date of shipment and that date must allow for the arrival of the order sometimes during normal working hours Monday – Friday; further, it will be specified to the vendor that the order delivery address must include Room 301B Howe Hall, attention John Hufnagel RSO; and finally the RSO will notify the local area Federal Express Office that all deliveries of radioactive materials are to be delivered on to Room 301 B Howe Hall and signed for by the RSO or another member of the Biology Department faculty or staff. If a shipment is inadvertently delivered to another location on campus, the University Mail Room for example, it will be the responsibility of the RSO to respond, go to the location, and receive shipment.

In addition, the existing stock of radioactive isotopes that are authorized by the license will be physically inventoried not less than twice a year on intervals that will not exceed six months. This inventory will be documented and performed by the RSO, John Hufnagel.

Occupational Dose

"We have done a prospective evaluation and determined that unmonitored individuals are not likely to receive, in one year, a radiation dose in excess of 10% of the allowable limits in 10 CFR Part 20."

In our programs utilizing isotopes, we have only one occasion when unmonitored individuals are exposed to radiation. That is in the process of teaching Laboratories # 11 & 12: Radiation Safety Workshop for Biology 215, Biochemistry of the Cell. In the process of the dilution exercise, each student is exposed externally to 7.5 μ curies of radiation. Please see **"Attachment Seven: Exposure of Unmonitored Individuals."**

Isotopes are stored in areas that are locked at all times, are not accessible to the general public or student population, and are used primarily for professional research. When students are present in these areas they are under the direct supervision of one of the "Authorized Users" permitted in this license. Please see **"Attachment Four: Room Plans."**

Safe Use of Radionuclides and Emergency Procedures

Please see **"Attachment Three: Radiation Safety Manual"** and **"Attachment Five: Training and Safety Procedures".**

Survey

"We will implement the model leak test program published in Appendix R to NUREG – 1556, Vol7, "Consolidated Guidance about Materials Licenses: 'Program-Specific Guidance About Academic, Research and Development, and Other Licenses of Limited Scope,' dated December 1999."

Frequency and content of routine surveys

Surveys consist of (1) "general" surveys using a survey meter and (2) "wipe" surveys of a 100 cm square area with a membrane and subsequent scintillation counting.

General surveys are conducted of all areas of a room where radionuclides have been used (benches, sinks, floors, hoods) after each use of the radionuclide. Survey meters are kept available for each user to perform these surveys. In some cases, where a radioactive protocol is done only once a year (in teaching situations) the survey is performed annually, immediately following the procedure. In other cases, the survey is performed daily at the end of the day. The Radiation Safety Officer also performs general surveys at random times, approximately every ninety days, except when no radioactive procedures have taken place since the previous survey.

Wipe surveys of benches, floors, sinks, and hoods are conducted twice annually, or immediately following the discovery of a "hot spot" during a general survey. A hot spot is defined as any portion of a lab which shows radioactivity above background. Background varies on the survey meters for 200 to 400 counts/min. Counts, therefore, above 400/minute trigger an immediate wipe survey. Wipe surveys units are dpm/100cm^2. Background is approximately 30 dpm (see results of a recent wipe survey below). Decontamination efforts (or licensed disposal of point sources which fail a leak test) are initiated when there is a two-fold increase (60 dpm). Decontamination is performed using a commercial product for the purpose ("Lift Off") with repeated wipes until background is achieved.

Please see **"Attachment Five: Training and Safety Procedures"** for example of annual wipe test survey in Room Howe 312. This test follows Lab 12 of Biology 215, Radiation Safety Workshop.

Please see **"Attachment Three: Radiation Safety Manual"** for details of the radiation safety program.

11. Waste Management.

For Sulfur 35, we will use the *Decay-In-Storage* model waste procedures that are published in Appendix T to NUREG – 1556, Vol. 7, 'Program-Specific Guidance About Academic, Research and Development, and Other Licenses of Limited Scope,' dated December 1999.

For Carbon 14 and Hydrogen 3 (Tridiated Thymidine), we will use the *Disposal of Liquids Into Sanitary Sewerage* model waste procedures that are published in Appendix T to NUREG – 1556, Vol. 7.

The University purchases approximately 40,000,000 gallons of water annually. After accounting for water lost to irrigation and condensation in the heating and cooling plant, the

Maintenance Department estimates that the University releases, in a very conservative estimate, at least 55,000 gallons of effluent daily into the public sewer system. In addition the chemical waste from the Science Center is flushed through a chemical dilution tank before being released into the general sewer system.

Please see **"Attachment Three: Radiation Safety Manual, III F"** (pg. 9) for details of waste management.

12. Fee. N. A.

Attachment One: Protocols

DNA Sequencing Protocols

Employed By
Maryanne Simurda

PROTOCOL 1: BUFFER GRADIENT POLYACRYLAMIDE GELS

REFERENCES: Biggin, M.D., Gibson, T.J., Hong, G.F. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 3963-3965; Sanger, F., Coulson, A.R. (1978) FEBS Letters 87, 107-110.

The logarithmic relationship between the length of a DNA fragment and its mobility on polyacrylamide gels produces a very large spacing between the consecutive bands of a sequence ladder at the bottom of the gel but an ever decreasing spacing at the top of the gel. By increasing the salt concentration toward the bottom of the gel a potential difference gradient can be set up which has a lower voltage drop over the bottom part of the gel. This retards the smaller faster moving fragments, not only evening out the spacing between consecutive bands up the gel but resulting in the fractionation of the larger fragments through a greater length of gel, increasing the resolving power of the gel. In practice the best compromise between maximum resolution and decreasing band spacing, which makes reading difficult, is found to be a gradient over the lower third of the gel. The use of these buffer gradient polyacrylamide gels has increased the sequence information obtainable from each gel run by over 30%.

We will prepare a 0.5-5.0X TBE gradient across a 6% acrylamide gel which will be used for the sequence reactions performed tomorrow. The gradient covers the lower third of the gel and is generated in a pipet by limited mixing of two solutions.

SOLUTIONS:

10X TBE (1 liter)

108g Tris
55g Boric acid
9.3g EDTA (Na_2)
Make up to 1 liter with deionized water

25% AMPS

25% ammonium persulfate in water
Keeps several months at 4°C

40% Acrylamide (1 liter)

380g Acrylamide
20g N'N'-methylenebisacrylamide
Make up to 1 liter with deionized water
Stir gently with 20g mixed bed resin ~1 hr
(eg. Amberlite MB 1)
Filter through sintered glass funnel to remove resin
Store at 4°C

0.5X TBE 6% Gel Mix (500 ml)

75ml 40% Acrylamide
25ml 10X TBE
230g Urea
Make up to 500ml with deionized water
Keeps 3-4 weeks at 4°C filter

5.0X TBE 6% Gel Mix (200 ml)

30ml 40% Acrylamide
100ml 10X TBE
92g Urea
10mg Bromophenol blue
Make up to 200ml with deionized water
Keeps 3-4 weeks at 4°C filter

PROCEDURE:

1. Carefully clean one pair of glass gel plates.

2. Wearing gloves and working in a fume hood, silanize the smaller plate by spreading approximately 2ml of silanizing solution (2% dimethyldicholorosilane in carbon tetrachloride) over the inner surface of the glass plate with a tissue and allow to dry.

   NOTE: Treat only one side of one plate!

3. After wiping clean with 95% ethanol assemble the glass plates using Plastikard side spacers and vinyl tape. Clamp around gel with fold back binder clip.

4. For a 40cm x 20cm gel, into two separate beakers put 6ml 5x TBE gel mix and 35ml 0.5x TBE gel mix.

5. Initiate polymerization by adding first 12µl (to the 5x) and 70µl (to the 0.5x) of 25% ammonium persulfate and then adding 7.2µl (5x) and 42µl (0.5x) of TEMED.

6. Take up into a 50ml plastic syringe 22ml of the 0.5x TBE gel mix. Set aside.

7. Using a pipette controller, take up into a 10ml pipette first 6ml of 0.5 TBE gel mix and then 6ml of 5x TBE gel mix. Form a rough gradient by allowing 2-3 air bubbles to pass through the interface.

8. Pour the gradient down the inner edge of the gel plates held at an angle of around 45°, adding the gel mix slowly and trying to maintain an even flow. When the pipette is empty, lower the plates to horizontal to stop the flow while the syringe is being picked up.

9. Add the remaining 0.5x TBE gel mix from the syringe to fill the glass plates.

10. Put the slot former in place, clamp the edges of the gel plates with foldback clips (only over side spacers), and leave the gel at least 30 minutes to polymerize.

11. Wash out the syringe and pipette before the gel sets (they can be reused) but keep the rest of the 0.5x TBE gel mix for topping up if necessary.

12. If storing the polymerized gel overnight, cover the top of gel with Saran wrap to prevent drying out.

MATERIALS:

Chemicals:

TBE
Acrylamide
N'N'-methylenebisacrylamide
Amberlite mixed bed resin
Ultra pure urea
Bromophenol blue
Ammonium persulfate
TEMED
Silanizing solution

Equipment:

1 set of glass plates
Disposable gloves
Tissues
Fumehood
Plastikard (side spacers and slot former)
Vinyl tape
20ml beaker
50ml beaker
Magnetic stirrer
Magnetic stir bars
Gilson p20
Gilson p200
50ml plastic syringe
10ml disposable pipette
Pipette controller
Foldback clips
Sintered glass filter funnel
Saran wrap

TIPS:

Ensure the glass plates are scrupulously clean to avoid air bubbles when pouring the gel, the silane coating also aids bubble-free pouring.

Keep the flow as smooth as possible, interruptions will introduce air bubbles.

Often if the bubbles are few, they can be removes or pushed to the side with a length of emulsion stripped X-ray film. If the bubbles are in the gradient part of the gel or there are many of them, it is often easier to pour another gel.

Try to pour the gradient gel mix as a 5cm wide flow; this ensures that any severe irregularities of the gradient down the pouring edge are outside the area used for running samples.

When topping up with the syringe, move the syringe along the top as the plates fill, this helps stop "kickback" of the gradient mix up the other edge of the plate.

Don't worry about irregularities in the gradient. They seldom pose any serious problem to reading the autoradiograph.

Polymerization time can be increased by decreasing the volume of the catalyst, TEMED, which is added. This may be a good idea for the first few attempts until confidence is gained.

We will be using ^{35}S-alpha-thio-dATP in preference to the more commonly used ^{32}P analog. Using S label has several distinct advantages:

1) The low energy particles produced by ^{35}S decay result in less "spread" than when using ^{32}P, consequently the bands are much sharper.

2) The lower energy of ^{35}S also produces less damage from radiolysis.

3) Radioactive decay of alpha-^{32}P-labelled nucleotides, incorporated into DNA molecules, breaks the phosphate backbone. This produces fragments with non-specific 5' ends which give a dark background smear on sequence autoradiographs.

4) The energy of ^{35}S emission is around 10% that of ^{32}P and therefore there is a considerable safety advantage to using ^{35}S.

5) The half life of ^{35}S is around six times longer than that of ^{32}P, so very little is wasted from it getting too "cold".

SOLUTIONS:

T.E.

10mM Tris-HCl, pH 8.0-8.5
0.1mM EDTA (Na_2)

T.M.

100mM Tris-HCl, pH 8
50mM $MgCl_2$

NTPs and Mixes using Amersham kit reagents

10mM ddNTPs (provided)

Dilutions of 10mM ddNTPs

ddTTP 10ul -> 200ul
ddCTP 2ul -> 1000ul
ddGTP 5ul -> 1000ul
ddATP 1ul -> 1000ul

10mM dNTPs (provided)

0.5mM dNTPs

10ul -> 200ul dilution of 10mM stocks in water

NTP Mixes

	T Mix	C Mix	G Mix	A Mix
0.5mM dTTP	3	60	60	60
0.5mM dCTP	60	3	60	60
0.5mM dGTP	60	60	3	60
diluted ddTTP	200	-	-	-
diluted ddCTP	-	200	-	-
diluted ddGTP	-	-	200	-
diluted ddATP	-	-	-	200
T.E.	60	60	60	60

Formamide Dye Mix

100ml Formamide (deionized with mixed bed resin)
0.1g Xylene cyanol F.F.
0.1g Bromophenol blue
2ml 0.5M EDTA (Na_2), pH 8.0

10X TBE (1 liter)

108g Tris
55g Boric acid
9.3g Na_2EDTA
Make up to 1 liter with deionized water

PROCEDURE:

1. For each template to be sequenced, to a 1.5 ml microfuge tube add:
   2 µl Primer (0.2 pmol)
   1 µl T.M. (Buffer)
   5 µl Water
   Scale this up by the number of templates to be sequenced.

2. Using a disposable microtiter tray such as Falcon 9311, assign vertical columns (12 wells) as T, C, G, A.

3. Assign a horizontal row of 'T', 'C', 'G', 'A' for each template to be sequenced.

4. To each well add 2 µl of primer/TM mix.

5. To each of the appropriate horizontal row of wells 'T', 'C', 'G', 'A' add 2 µl of the appropriate template DNA.

6. Cover the wells with a layer of Saran wrap.

7. Centrifuge the tray briefly to mix and place in an oven at 55°C for at least 45 minutes.

8. Centrifuge the tray briefly to concentrate any condensation and remove the Saran wrap.

9. To the appropriate vertical column dispense, close to the rim, 2 µl of the appropriate NTP mix (i.e. T mix → 'T').

10. Wearing gloves dilute 4 µCi of ^{35} Sα thiodaTP (for each template to be sequenced) in a small silanized glass tube to 0.5 µCi/µl with 12.5 mM DTT and place on ice.

11. To the diluted 35S daTP add Klenow fragment DNA Pol I to 0.25 u/µl.

12. WITHOUT DELAY, dispense 2 µl of ^{35}S/Klenow to each well, avoiding the nucleotide drop, and centrifuge as briefly as possible to mix and leave at 37°C for 20 minutes.

13. Add to each well 2 ul of 0.25 mM dNTP chase solution and centrifuge very briefly to mix and leave at 37°C for 20 minutes.

14. Add to each well 2 µl of formamide dye and centrifuge to mix

15. Just before loading, heat denature the reactions by placing the tray in an oven at 80°C for 15 minutes.

16. Remove the slot former from the gel and wash the slots to remove any unpolymerized acrylamide.

17. Using foldback clips clamp the gel to the electrophoresis apparatus and fill the top and bottom tanks with 1.0x TBE.

18. Using a Pasteur pipette flush the slots immediately and again just before loading.

19. With a drawn-out length of polypropylene tubing or glass capillary and a mouthpiece load 1.5-2.0 µl of each sample onto the gel (preferably in the order T, C, G, A) noting the order of the clones.

20. Connect the apparatus to a power supply (positive to the bottom) and run at constant power of around 35-37 Watts until the bromophenol blue marker is just off the bottom of the gel, around 2.5 hours

21. Disconnect the power, drain off the buffer, take the gel/plates from the apparatus and completely remove the vinyl tape.

22. Pry apart the glass plates gently, using a spatula, trying to keep the gel stuck to the larger plate.

23. Slowly immerse the gel/plate in 10% acetic acid and 10% methanol in water and leave for at least 15 minutes.

24. Trying to keep the gel stuck to the glass plate, remove them the acetic acid and drain well.

25. Transfer the gel to 3 MM paper by placing paper on the gel and, after applying gentle pressure over the whole area of the gel, gently peeling the paper/gel off the glass plate.

26. Cover the gel with Saran wrap, trim the edges to fit the gel drier and dry at 80°C under vacuum for at least 15 minutes.

27. Peel off the Saran wrap and label the gel with radioactive ink. Place the dried gel in a film cassette in direct contact with X-ray film and leave overnight.

MATERIALS:

Chemicals:

TE
dNTPs
ddNTPs
Synthetic oligonucleotide primer
TM
^{35}S-alpha-thio dATP (400 Ci/mMol)
Klenow fragment DNA Pol I
Amberlite mixed bed resin
TBE
Formamide dyes
Acetic acid
Dithiotheitol

Equipment:

1.5 ml microfuge tubes
Microtiter trays (non-sterile, U well)
Benchtop centrifuge
Rotor for above (microtiter tray)
Oven at 55°C
Oven at 80°C
Gilson p20
Gilson p200
Disposable gloves
Radioactive waste bin
Glass capillaries (eg. Gallenkamp MFB-210-538L) or polypropylene tubing
Mouthpiece
Electrophoresis apparatus
Power supply
Gel fixing tray
3 MM paper
Gel drier
Vapor traps and vacuum pump
Saran wrap
Film cassettes
X-ray film
Radioactive ink pen

NOTE:

NTPs and Mixes (non-kit variety)

1CmM ddNTPs

ddTTP 6.1 mg/ml in T.E.
ddCTP 5.8 mg/ml
ddGTP 6.2 mg/ml
ddATP 6.2 mg/ml

50mM dNTOPs

dTTP mg/ml in T.E.
dCTP mg/ml
dGTP mg/ml
dATP mg/ml

0.5mM dNTPs

1-100 dilution of 50mM stocks in T.E.

dNTP Chase

0.25 mM dttp
0.25 mM dCTP
0.25 mM dGTP
0.25 mM dATP
From 50mM stocks in T.E.

NTP Mixes

	T	C	G	A
	Mix	Mix	Mix	Mix
0.5mM dTTP	2.5	50	50	50
0.5mM dCTP	50	2.5	50	50
0.5mM dGTTP	50	50	2.5	50
10mM ddTTP	5	-	-	-
10mM ddCTP	-	8	-	-
10mM ddGTP	-	-	16	-
10mM ddATP	-	-	-	1*
T.E.	100	100	100	50

*3 when using ^{32}P-dATP

Recipe for 4 ul pe Klenow

TE	54.0	81.0	72.0
.1M DTT	8.0	12.0	10.7
35	3.3	4.95	4.4
Klenow	2.1	3.15	2.8
	67.4	101.1	89.9
	(8)	(12)	(11)

TIPS:

The majority of problems associated with sequence reactions can be attributed to the Klenow DNA Pol I. It needs to be treated very gently and kept cold as much as possible, especially when diluted.

Combined nucleotide solutions keep a long time at -20°C. Once the ratio of deoxy to dideoxy has been properly titrated the combined solution can be stored as aliquots at -20°C.

When using combined solutions the volume added is not critical since the nucleotide ratios will remain constant. Where possible use repetitive dispensers, these greatly increase the speed of additions. This means more reactions can be performed simultaneously, the diluted polymerase is added to the reactions very quickly and there is less evaporation.

The reactions can be fine-tuned. For example, take a template of average DNA concentration (as assayed on a mini-gel) and optimize the amount of primer for this average DNA concentration. This primer concentration can be used routinely for all sequence reactions. The darkness of bands after an overnight exposure can be altered by scaling the primer/template volumes.

When heat denaturing at 100°C never exceed 3 minutes. The dark background on sequence autoradiographs increases with time, presumably due to degradation.

If using ^{32}P-labeled dATP don't store reactions to run later. Radioactive decay of ^{32}P-labeled DNA breaks the phosphate backbone, contributing to background on autoradiographs.

Ensure adequate fixing. This dialyses out the urea which otherwise prevents proper drying and results in sticking of the gel to X-ray films.

When using ^{35}S, the Saran wrap must be removed before autoradiography as the low energy particles of ^{35}S decay cannot penetrate this effectively.

If on opening the gel plates the gel tends to stick to both plates, pry them apart while submerged in the acetic acid. After fixing, position the gel centrally on the glass plate and remove the gel/plate with the aid of a piece of plastic netting to prevent the gel escaping. The gel can then be transferred to paper and dried as normal.

PROTOCOL 3: READING DIDEOXY SEQUENCES

After developing the X-ray films of yesterday's dideoxy sequences, we will make a first analysis of any problems which arise and then read what sequences we can, comparing our readings with previously determined sequences.

Reading sequence autoradiographs accurately is a skill which only comes with practice and experience. When reading dideoxy sequences there are a few guidelines which may help where problems or ambiguities arise.

A. GENERAL

1. Mark out the four tracks of the sequence to avoid confusion with tracks from other sequences.

2. Identify the ends of the insert, this avoids hours spent reading vector sequences.

3. It is equally important to read the band spacings as well as the bands themselves. The spacing can tell you how many bands to expect between two points and can highlight fragments running anomalously.

B. BAND INTENSITY

1. Single C bands are generally weak.

2. In a run of C bands the first is normally very weak and the second is strongest. Occasionally when preceded by a G the first band can be of normal intensity.

3. Weak G bands are sometimes seen when preceded by a T.

4. In a run of A bands the first is often stronger.

C. ARTIFACTS

1. Artifact bands are almost always indicative of a system not working properly and under ideal conditions they should pose no problem.

2. Extra C band between T and G in sequence TGCC.

3. Extra C and T bands opposite A in the sequence GCA.

4. Artifact A band preceding real A. This most probably results from an inadequate chase step due to the enzyme failing. The chains terminating not because of incorporation of a dideoxy but because the rate of ^{35}S-dATP incorporation is limited both by its low concentration and by the reduced efficiency with which it is used as a substrate by DNA Pol I.

5. A series of very dark "pile-up" band across all four tracks. Fragments most probably resulting from failure of the polymerase to traverse a region of conformation change in the template e.g. base stacking of G nucleotides. These pile-up bands can often be remedied by lowering the salt concentration of the reaction buffer, this destabilizes the base interactions.

D. ANOMALOUS BAND SPACING

1. Irregularities in the gradient can produce minor band spacing anomalies on the gel.

2. "Smiling" of the bands across the gel results from the difference in heat losses between the center and the edge of the gel. This smiling can be greatly reduced by avoiding the very edge of the gel. Gradient gels, because of their retarding effect also tend to reduce the smiling effect.

3. Even quite short stretches of complementarity at the ends of the fragments can cause them to run as fast on gels as shorter single stranded fragments. This is seen on autoradiographs as a region of narrow band spacing, often with bands in adjacent tracks running at the same position, followed by a region of unusually wide spacing. This can be the most serious problem encountered when DNA sequencing, as bands can be missed completely. For this reason it is very important that the sequence be determined entirely from both strands (the compression is displaced on the other strand).

Carbon 14 and Hydrogen III Protocols

Employed By
John Jay Wielgus

Sample protocol for use of ^{3}H-leucine; ^{35}S will not be used for our purposes.

Labeling and extraction of epidermal proteins. The larvae or pharate pupae were anesthetised in water, then surface sterilized in two changes of 70% alcohol followed by two rinses in sterile distilled water. The dorsal abdominal integument was removed to Grace's medium (GIBCo) lacking leucine and methionine at 25°C. The adhering fat body, Verson's glands and most of the muscles were dissected away. The time required for the dissection was 15–20 min depending of the stage of the animal. The cleaned attached segments (A2, 3 and 4) were divided in half along the anterior to posterior axis. One piece was immediately transferred to leucine-deficient Grace's medium containing 500 µCi/ml ^{3}H-leucine (60 Ci/mmol; New England Nuclear) and the other piece to methionine-deficient Grace's medium containing 300 µCi/ml of high specific activity ^{35}S-methionine (1000 Ci/mmol; New England Nuclear). The tissue was labeled for 1 h at 25° C with aeration and gentle shaking as described previously (Riddiford 1978). At 1 h the integument was removed from radioactive medium, rinsed, then chased in complete Grace's medium for 15 min at 25° C as above. Following the chase the tissue was rinsed in ice-cold Grace's medium, then pinned to an ice-chilled metal dissecting dish, containing Grace's medium, to remove the remaining muscle fragments.

The epidermis was then scraped from the cuticle and either frozen at –80° C or immediately homogenized. For one-dimensional gel samples the tissue was homogenized for 2 min in 100–150 µl/segment of boiling 0.0625 M Tris pH 6.8, 2% sodium lauryl sulfate (SDS), 0.001 M phenylmethylsulfonylfluoride (PMSF) (Calbiochem). For samples to run on two-dimensional gels, the tissue was homogenized at 4° C in 100–150 µl/segment of 0.0625 M Tris pH 6.8, 1% Nonidet P-40 (NP-40; Particle Data Laboratories) and 0.001 M PMSF. The homogenates were centrifuged at 10,000 rpm for 15 min at 2° C in an HB-4 rotor (Sorvall). The supernatants were collected, 5 µl aliquots were taken from each for determination of trichloroacetic acid (TCA)-precipitable radioactivity, and the remaining portions frozen at –80° C until immunoprecipitation or electrophoresis..

Determination of DNA synthesis

[Methyl-^{3}H]thymidine (49 Ci/mmole was diluted 1:1 with equimolar unlabelled thymidine (dT) in Grace's medium to obtain a final specific activity of 24.5 Ci/mmole. Larvae were anesthesized with CO_2 and pieces of integument (~ 2 × 3 mm) were dissected from the dorso-lateral part of the 3rd abdominal segment and cleaned of adhering tissues (WIELGUS and GILBERT, 1978). Three pieces of integument were taken from each larva so that duplicate incubations could be performed and 'time zero' [^{3}H] dT incorporation (background) determined. After a 15 min wash in Grace's medium each tissue piece was incubated in 0.5 ml of Grace's medium containing [^{3}H] dT at 25°C in a shaking water bath. Incubations were terminated by the addition of 2.0 ml absolute ethanol; in the case of background samples, the ethanol was added prior to the introduction of the tissue. Following incubation the samples were stored at 4°C until extracted for DNA.

All procedures for the extraction of DNA (i.e., localization of the DNA into the acid insoluble fraction of whole tissue homogenates) were carried out at 0–4°C unless stated otherwise. Each tissue piece was homogenized in 0.5 ml 0.5 N percholoric acid (PCA). The homogenate and 3 subsequent 0.5 ml PCA washes of the homogenizer were centrifuged at 5000 *g* for 20 min and the pellet resuspended in 2.0 ml 0.5 N PCA and recentrifuged. The wash was repeated and the final pellet was resuspended in 2.0 ml 0.35 N KOH for hydrolysis of RNA at 37°C for 3 hr. Subsequently, DNA was precipitated by the addition of 0.07 ml of 11.2 N PCA and the tube was placed on ice for 15 min. After centrifugation as before, the pellet was resuspended in 1.0 ml of 1.6 N PCA. Continuous vortexing was utilized to keep the precipitate uniformly suspended while 0.5 ml and 0.25 ml aliquots were removed for DNA estimation and radioassay, respectively. It was not necessary to solubilize the DNA by hydrolysis since it dissolved in the PCA within 4 hr at 30°C.

DNA was estimated using the diphenylamine method of BURTON (1956) as modified by GILES and MYERS (1965); 14 hr were allowed for colour development. Standards were prepared from calf thymus DNA which were then calibrated by u.v. absorption (MCCLURE *et al.*, 1976). Accuracy of DNA estimation by this method was tested on both fresh beef and mouse liver and was found to be within 10% of previously reported values.

The 0.25 ml aliquot was suspended in 3.0 ml Aquasol-2 (New England Nuclear) and radioassayed (Packed liquid scintillation spectrometer; Model 3380). Specific activity was calculated for each sample prior to the subtraction of background (activity of the 'time zero' corresponding tissue).

Since muscle is a minor contaminant of the integument used in these experiments, intersegmental muscle from each day of the instar was incubated as above and the specific activity of extracted muscle DNA was then determined. In no case was the specific activity of muscle DNA significantly above background levels. Although muscle was found to contribute to the total DNA pool of the integument in separate tests, this contribution did not vary with developmental stage and corrections were therefore unnecessary.

To ensure that the radioactivity associated with the PCA insoluble fraction was [^{3}H]-dT, aliquots of the PCA fractions were pooled, and the DNA solubilized at 30°C for 4 hr. After centrifugation, the supernatant was dialyzed against distilled water at 4°C to remove salts and PCA. Subsequent to evaporation the residue was dissolved in 3.0 ml 6 N HCl and hydrolyzed to the free bases at 120°C for 2 hr (DAVIDSON, 1965). The products of hydrolysis were dissolved in water saturated *n*-butanol after flash evaporation of the HCl and co-chromatographed with standards using thin layer chromatography in a solvent system of *n*-butanol: acetic acid:water (50:25:25, v/v).

After identification under u.v. of the 4 bases obtained by hydrolysis, the TLC plate was fractionated (0.5 cm sections) and each section was radioassayed. Radioactivity was associated only with the thymine standard, thus indicating that the label incorporated was indeed [^{3}H] dT.

Sample protocol for use of ^{3}H-thymidine

Protocol for Phase IV, Appendix D

Source: Clark, John M., Jr. and Robert L. Switzer. Experimental Biochemistry. Second edition. W. H. Freeman and Company. New York. 1977.

EXPERIMENT 21

Determination of Cholesterol by Radioisotopic Dilution Analysis

THEORY

Isotope dilution analysis is a unique approach to measuring the concentrations of individual components in complex mixtures. In conventional analytical procedures you isolate each constituent in a pure state with 100% recovery. However, as the complexity of the mixture to be analyzed increases, it become technically difficult or impossible to realize 100% recovery and 100% purity simultaneously. Isotope dilution methods allow quantitative analyses, provided any measurable quantity of the component in question can be recovered in the pure state.

Depending upon the problem at hand, you can employ one of two methods for quantitative analyses. When the mixture contains amounts of the desired component that are sufficient to permit isolation of a measurable amount, you can add to the mixture a small, measured weight of the labeled form of the compound to be analyzed. After subsequently isolating the compound from the mixture and counting it, you can calculate the original amount present if you know the specific activities of the added and isolated materials.

Alternatively, there are many instances in biological experiments in which you want to determine the amount of a trace quantity of a radioactive constituent in a mixture of radioactive compounds. Here you initially add to the labeled mixture an amount of the nonradioactive form of the desired compound sufficient to permit isolation. You then purify the compound to constant specific activity. The amount of radioactive compound originally present in the mixture may be calculated from the amount of carrier added and the specific activity of the material isolated.

Both approaches must fulfill the following requirements for validity: there must be complete admixture of labeled and unlabeled forms of the compound being assayed, the isolated material must be both chemically and radiochemically pure, and the specific activity of the isolated materials must be known.

The theory of radioactive isotope dilution analysis is very simple. If a radioactive tracer is mixed with the corresponding unlabeled compound, the amount of

activity per gram of the substance will be reduced. In other words, the radioactive material will be diluted with nonradioactive material. If the reduction in activity per gram can be measured, the amount of diluting material added can be calculated.

If we consider the tracer compound C* with an activity A_0 and a mass W_0, we can calculate the specific activity (S_0) of the compound from these two values.

$$S_0 = \frac{A_0}{W_0}$$

The inactive form of the same compound (C) has no activity but it has a mass W_u. If we mix C* and C, the total activity is still A_0 but the mass is $W_0 + W_u$. The new specific activity is therefore

$$S_1 = \frac{A_0}{W_0 + W_u}$$

Provided that we know A_0 and W_0 and can measure S_1, the value of W_u can be calculated.

$$W_u = W_0\left[\frac{S_0}{S_1} - 1\right]$$

The great advantage of this method is that it is not necessary to separate the whole of $W_0 + W_u$ to measure S_1. Provided that the labeled compound and the inactive component are in the same chemical form and have been thoroughly mixed, the specific activity (S_1) is independent of the amount of material used to measure it. Therefore, as long as some of the component can be separated in a pure state the amount of inactive material in the sample can be determined.

This experiment uses radioisotope dilution analysis to determine the cholesterol content of a natural or synthetic unknown lipid mixture. Cholesterol can be isolated from this mixture in one of several ways. In this case the cholesterol is conveniently isolated by precipitation with digitonin, a naturally occurring steroid glycoside (Figure 21-1). Digitonin forms a specific one-to-one molecular complex with 3-β sterols (such as cholesterol). This complex then precipitates from the mixture in nonpolar solvents. The washed precipitate is sufficiently pure to be used for determination of the specific activity of cholesterol (after correction for the mass contributed by digitonin).

Digitonin may also be used for quantitative determination of cholesterol and cholesterol esters present in lipid mixtures. An initial separation of polar lipids is advisable in these cases in order to facilitate the desired quantitative precipitation of cholesterol. The precipitate can then be analyzed by the colorimetric Liebermann-Burchard test for 3-OH, Δ^5 sterols (see Experiment 19). You can distinguish cholesterol esters from free cholesterol by determining the yield of cholesterol before and after alkaline hydrolysis of the mixture. Because digitonin specifically precipitates free cholesterol, the increase in cholesterol after hydrolysis is a measure of the content of cholesterol esters.

EXPERIMENTAL PROCEDURE

Materials

Acetone:absolute ethanol (1:1)
^{14}C- or ^{3}H-cholesterol, commercial material diluted in 1:1 acetone:ethanol to approximately 200,000 cpm/ml
Scintillant fluid
Complex lipid mixture containing cholesterol
Commercial solubilizer (e.g., Soluene-100)
Absolute ethanol
0.5% Digitonin in 50% aqueous ethanol
Steam bath
Scintillation counter and vials

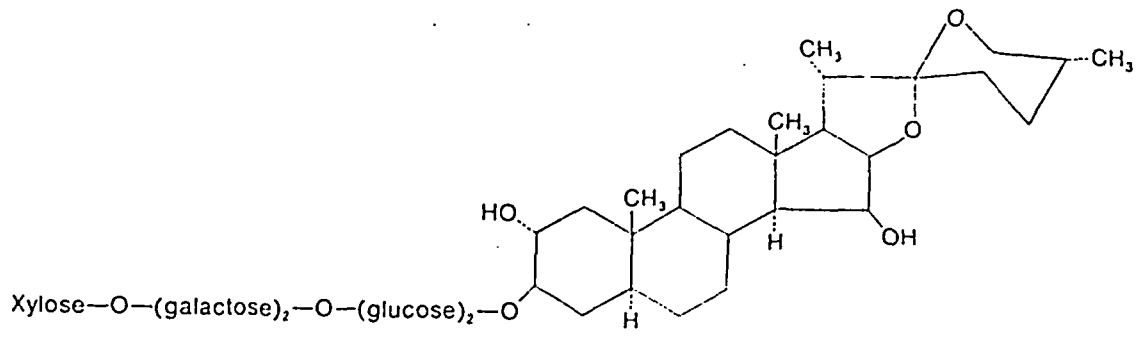

FIGURE 21-1
Digitonin ($C_{56}H_{92}O_{29}$), MW 1,221.

WARNING
Fire hazard. There must be no open flames in the laboratory during this experiment.

Isolation of Radioactive Cholesterol

Obtain a solution of radioactive cholesterol of precisely known radioactivity (A_0) and concentration (W_0) per ml. Add exactly 0.50 ml of the radioactive cholesterol solution to a vial of complex lipid mixture containing an unknown amount of cholesterol, i.e., a mixture prepared by dissolving 20 to 50 mg (weigh accurately!) of lipid in 2 ml acetone : absolute ethanol and mixing thoroughly.* To this mixture add 40 ml of 0.5% digitonin in 50% aqueous ethanol. Warm the solution for a few seconds on a steam or a 75° C water bath, and then allow the suspension to stand at 0–5° C for 1 hr.† Collect the precipitate by centrifugation (1,500 × *g*, 5 min). Wash the precipitate once with 25 ml of cold absolute ethanol. Dry the precipitate *thoroughly* in air, dispersing the solid with a glass rod or spatula. Then dry further overnight in a vacuum dessicator or dry at 100° C for 30 min.

Determination of the Specific Radioactivity of the Isolated Cholesterol

Weigh approximately 10 mg (the precise total is not critical, but should be determined to within 0.1 mg) of the dried digitonide into a tared scintillation vial. Dissolve the solid by addition of 0.5 ml solubilizer to

WARNING
Solubilizer is a painful irritant: do not pipette by mouth.

the vial, capping the vial tightly and agitating the contents until solution is complete. Warm the vial if necessary to dissolve the solid material. The suspension may be slightly cloudy, but should not be milky or yellow. Add 10 ml of scintillant solution and allow the vial to stand in your desk for an hour to minimize chemiluminescence. (Note: Solubilizers such as Soluene-100 contain quaternary alkyl or aryl ammonium salts, which disrupt and hold in solution insoluble materials and biological tissues. Such alkaline solutions tend to produce chemiluminescent reactions in oxygen-containing scintillant solutions. You prevent this here by including benzoic acid in the scintillant to neutralize the excess alkali and by allowing the sample to stand until chemiluminescent reactions are complete.) Then count the radioactivity in your sample. Do not delay counting, as discoloration or precipitation may occur and interfere with the measurement.

From the weight of the cholesterol digitonide sample counted and the observed radioactivity, calculate the observed specific activity (in cpm/mg) of the digitonide. Calculate the specific activity of the cholesterol in the digitonide by correcting for the fraction of the total mass that is cholesterol; that is, multiply the observed specific activity of the digitonide by (1,229 + 386)/386.

It is necessary to calculate the true specific activity of the cholesterol by determining the efficiency of counting under your conditions. Do this as follows.*

1. Count the sample containing cholesterol digitonide, solubilizer, and scintillant fluid.

2. To the same sample, add a known volume of cholesterol of known specific activity (dpm). For example, use 0.50 ml of the same radioactive cholesterol solution used at the start of the experiment. Mix the solution. Allow to stand for 15 min to 1 hr.

3. Count the sample again.

4. Subtract the counts obtained in step 1 from those observed in step 3.

5. The difference in counts observed after adding the standard radioactive cholesterol (4) divided by the true radioactivity added (dpm) is the efficiency of counting under your conditions.

The observed specific radioactivity of the cholesterol (cpm/mg) divided by the efficiency of counting yields the true specific activity of your isolated cholesterol (S_1, in dpm/mg).

Concentration of Cholesterol in the Unknown Lipid Mixture

The radioactivity (A_0, in dpm) and mass (W_0, in mg) of the cholesterol added to the lipid sample will be provided by the instructor. Calculate the specific radioactivity of the cholesterol (S_0). From S_0 and your value of S_1, calculate the mg of cholesterol in your lipid sample. Express the cholesterol content of the sample as mg cholesterol per mg total sample. De-

*If the unknown vials contain known quantities of lipid in acetone/ethanol solution, the radioactive cholesterol can be added directly to the vial.

†The solution can stand for as little as 20 min if you are pressed for time.

*If standard radioactive cholesterol is not available in sufficient quantity, the efficiency can be determined by one experiment for the entire class: all students will [illegible] identical conditions.

scribe possible sources of error in your determination and estimate their magnitude.

EXERCISES

1. In the introduction the relationship,

$$W_u = W_0\left|\frac{S_0}{S_1} - 1\right|$$

is discussed. From the definitions given, prove the validity of this equation.

REFERENCES

Dittmer, J. C., and M. A. Wells. 1969. Quantitative and Qualitative Analysis of Lipids and Lipid Components. Lipids Methods in Enzymology, Vol. 14). New York: Academic Press.

Sperry, W. M., and M. Webb. 1950. A Revision of the Schoenheimer-Sperry Method for Cholesterol Determination. *J. Biol. Chem. 187*:97.

Wang, C. H., and D. L. Willis. 1965. *Radiotracer Methodology in Biological Science.* Englewood Cliffs, N.J.: Prentice-Hall.

Cell and Molecular Biology 316: Radioisotope Dilution Lab Exercise

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Rather than the cholesterol determination exercise given in Clark and Switzer, perform the following to determine the specific activity of N-acetylglucosamine and the accuracy of your pipetting:

1) Using your 100 µl Hamilton syringe, measure out 100 µl of stock ^{14}C into a scintillation vial; set up 4 other vials. Do not write on the vials.

2) Add 900 µl 5% ethanol (5:95/v:v) to each of the vials; cap and vortex carefully!

3) Initiate a "serial dilution" by transferring 100 µl from vial 1 to vial 2; vortex.

4) Transfer 100 µl from vial 2 to vial 3, cap and vortex, then repeat the procedure until all the vials are done; what is the dilution ratio?

5) Add 5.0 ml of scintillation fluid to each vial and vortex. Count the samples.

6) Your report should be abbreviated; answer: (a) what was the original (stock) dpm/ml (specific activity)? (b) what are the number of curies in 1.0 ml of stock solution? (c) what is the "r" value of your pipetting? Your report should have an introduction, the present sheet (as Materials and Methods), the scintillation courter printout (as data), short calculations for specific activity and a plot of dpm/ml vs. vial # as Analysis, and a one (1) sentence discussion followed by the Reference section.

Technique is critical: use gloves and aprons; follow contamination avoidance procedures as specified by the NRC regulations!!!

Cobalt 60 Protocols

Employed By
Kenneth E. Van Ness

Dr. Kenneth E. Van Ness first worked with radioactive materials in 1966 at the University of Pittsburgh School of Medicine, doing research in the field of health physics. As an undergraduate physics major at Bucknell University, Dr. Van Ness took a course in nuclear and atomic physics. Since 1989 he has been working with Dr. James Donaghy, currently a retiring member of the Radiation Safety Committee, in the field of positron annihilation spectroscopy. Their research involves the use of a Na-22 source to study the molecular structure of polymeric materials. A Co-60 source is used occasionally to check the resolution of the spectrometer's timing circuitry. Dr. Van Ness is fully trained in the handling of these sources.

RADIOACTIVE SOURCE PREPARATION AND USE

James J. Donaghy
Department of Physics and Engineering

The radioisotopes to be used are Sodium-22 and Cobalt-60. One Co-60 source will be required. It will have a strength of about 10 microcuries and will be encased in plastic. The sodium sources will be of two types:

a) A 10 microcurie liquid source deposited directly on the sample. After completing the measurements, the samples will be stored in a lead container. A special room will be set aside for preparing the sources and storing the old samples.

b) A commercially prepared source containing 50-100 microcuries of Sodium-22 sandwiched between thin (about 1 mg/cm^2) metal or plastic foils. When in use this source will be housed in a shielded dewar. The laboratory in which the source is used will be secure against accidental entry, and appropriate warning signs will be posted at the entrance.

Attachment Two: Authorized Users

Authorized users are limited to the following four individuals.

1) John A. Hufnagel, Science Laboratory Manager and Radiation Safety Officer.

2) John Jay Wielgus, PhD. Professor of Biology.

   Isotopes used: Hydrogen 3 (Aqueous Thymidine), 20 millicuries and Carbon 14, 1 millicurie

3) Kenneth E. Van Ness, PhD. Professor of Physics. *

   Isotope used: Cobalt 60 (sealed source), 20 microcuries

4) Maryanne Simurda, PhD. Assistant Professor of Biology.

   Isotope used: Sulfur 35, 10 millicuries

These individuals in addition to their previous professional experience and training have received the "in-house" training program detailed in **"Attachment Five: Training and Safety Procedures"**.

* This list reflects the fact that James Donaghy, originally designated an authorized user, will retire as of 31 May 2005 and has, therefore, been omitted from this renewal request. We are requesting that Kenneth E. Van Ness be designated an authorized user in place of Dr. Donaghy. Dr. Van Ness will be employing in his own research the same protocols and radioactive sources employed by Dr. Donaghy. Dr. Van Ness, under the supervision of Dr. Donaghy, has employed these protocols for sixteen years.

Curricula vitae follows this page.

John A Hufnagel

Position: Radiation Safety Officer and Laboratory Manager for the Sciences

Education: Bachelor of Science, Fairfield University. Major in biology and minor in chemistry

Experience: Twenty-two years at Washington and Lee as technician and laboratory manager. Two years as assistant Radiation Safety Officer under Dr Barlow Newbolt from 1996 – 98, and full RSO duties from 1998 till present.

Training; Under direction from Mr. Wade Loo of the NRC, I attended a five day radiation safety training program by Radiation Safety & Control Services Inc., Portsmouth, New Hampshire. This training in conjunction with two years experience as assistant RSO was deemed adequate for assignment to full duties as RSO. Please see following copies of course outline and certificate.

Radiation Safety & Control Services, Inc.

Radiation Safety Officer Training Course

June 10 - 14, 1996
Portsmouth, New Hampshire

Course Schedule

Monday, June 10, 1996

8:30	Introduction and Course Objectives
9:15	Math Review
10:00	*Break*
10:15	Nuclear Physics Review
12:00	*Complimentary Lunch*
1:00	Radiation and Radioactive Material
2:30	*Break*
2:45	Interaction of Radiation With Matter
5:00	*Class Ends*
5:30	*Social Hour*

Tuesday, June 11, 1996

8:00	Interaction of Radiation with Matter (con't)
10:00	*Break*

Radiation Safety & Control Services, Inc.

Radiation Safety Officer Training Course

June 10-14, 1996
Portsmouth, New Hampshire

Course Schedule

Tuesday, June 11, 1996 (Con't)

10:15	Radiation Exposure and Dose
12:00	*Lunch*
1:00	Biological Effects of Radiation
2:30	*Break*
2:45	Radiological Hazards
5:00	*Class Ends*

Wednesday, June 12, 1996

8:00	Radiological Hazards (con't)
10:00	*Break*
10:15	Radiological Hazards (con't)
12:00	*Lunch*
1:00	Principals of Radiation Detection

Radiation Safety & Control Services, Inc.

Radiation Safety Officer Training Course

June 10 - 14, 1996
Portsmouth, New Hampshire

Course Schedule

Wednesday, June 12, 1996 (con't)

2:30	*Break*
2:45	Principals of Radiation Detection (con't)
5:00	*Class Ends*

Thursday, June 13, 1996

8:00	Operational Radiation Safety Program
10:00	*Break*
10:15	Operational Radiation Safety Program (Con't)
12:00	*Lunch*
1:00	Operational Radiation Safety Program (Con't)
2:30	*Break*
2:45	Planning For Emergencies
5:00	*Class Ends*

Radiation Safety & Control Services, Inc.

Radiation Safety Officer Training Course

June 10 - 14, 1996
Portsmouth, New Hampshire

Course Schedule

Friday, June 14, 1996

8:00	Nuclear Regulatory Commission Regulations
10:00	*Break*
10:15	Transportation of Radioactive Material
12:00	*Class Commencement*

Radiation Safety & Control Services, Inc.

Awards this certificate to

John Hufnagel

in recognition of satisfactory completion of

RADIATION SAFETY OFFICER TRAINING COURSE

Portsmouth, New Hampshire

June 10 - 14, 1996

Frederick P. Straccia

James P. Tarzia

Eric L. Darois

Curriculum Vitae

John Jay Wielgus
Professor of Biology
[redacted]

Education

PhD Northwestern University, August 1977
MS Northwestern University, June 1974
BA The University of Illinois at Chicago, June 1969

Fields of Specialization

Developmental Biology
Insect Biochemistry
Hormonal Control of Cell Function

Professional Experience

Morton Upper Grade Center, Chicago IL; 1969 to 1971; Special Education Teacher

Washington and Lee University, Lexington VA; 1977 to present; Professor of Biology

Teaching Areas

Developmental Biology
Endocrinology
Cell and Molecular Biology
The Biology of Cancer
Human Anatomy and Physiology

Awards and Honors

Graduate Teaching Assistantship; 1974 to 1977; Northwestern University

Chairman's Citation for Teaching Excellence, 1975; Northwestern University

Research Corporation Cottrell College Science Research Reward, 1985 to 1987

Who's Who in the South and Southeast

Who's Who in Science and Engineering

Professional Memberships

American Association for the Advancement of Science

Professional Activities: Papers to Professional Groups

Wielgus, J.J. and L.I. Gilbert. Regulation of intermolt endocuticular deposition in *Manduca sexta.* American Society of Zoologists Meeting, Tulane University, May 1976.

Wielgus, J.J., L.B. Aden and R.M. Franks. Characterization and Site of Synthesis of a Hemolymph Trophic Factor in the Tobacco Hornworm, *Manduca sexta.* Invited presentation at the Congress on Cell and Tissue Culture (Meeting of the Tissue Culture Association), San Diego CA, June 1993.

Professional Activities: Papers to Lay Groups

"The Biology of Cancer"; American Cancer Society Seminar, Lexington VA; January 1984.

"The Ethics of In Vitro Fertilization"; St. Patrick's Church, Lexington VA; October 1987.

"In Vitro Fertilzation: How and Why"; the Lexington Kewanis Club, Lexington VA, April 1988.

"Third Generation Pesticides"; Harrisonburg High School, Harrisonburg VA, December 1989.

Professional Publications

J.J. Wielgus & L.I. Gilbert. Regulation of intermolt endocuticular deposition in *Manduca sexta. Am. Zool. 16:*187 (1976) (Abstract).

S.L. Smith, W.E. Bollenbacher, D.Y. Cooper, H. Schleyer, J.J. Wielgus & L.I. Gilbert. Characterization of ecdysone 20-monoxgenase activity in *Manduca sexta. Am. Zool. 17:*928 (1977) (Abstract).

W.E. Bollenbacher, S.L. Smith, J.J. Wielgus and L.I. Gilbert. Evidence for an α-ecdysone cytochrome p-450 mixed function oxidase in insect fat body mitochondria. *Nature 268:* 660-663 (1977).

J.J. Wielgus and L.I. Gilbert. Epidermal cell development and control of cuticle deposition during the last larval instar of *Manduca sexta. J. Insect Physiol. 24:*629-637 (1978).

Professional Publications, continued

J.J. Wielgus, W.E. Bollenbacher & L.I. Gilbert. Correlations between epidermal DNA synthesis and haemolymph ecdysteroid titre during the last larval instar of the tobacco hornworm, *Manduca sexta. J. Insect Physiol. 25:* 9-16 (1979).

S.L. Smith, W.E. Bollenbacher, D.Y. Cooper, H. Schleyer, J.J. Wielgus & L.I. Gilbert. Ecdysone 20-monooxygenase: Characterization of an insect cytochrome p-450 dependent steroid hydroxylase. *Molec.&Cell. Endocrinol. 15:* 111-113 (1979).

J.J. Wielgus. Stimulation of intermoult cuticle deposition by a haemolymph trophic factor in the tobacco hornworm, *Manduca sexta. Insect Biochemistry 13:*313-322 (1983).

J.J. Wielgus, G.A. Caldwell, R.L. Nichols & C.F White. Purification, properties, and titer of a hemolymph trophic factor in larvae and pupae of *Manduca sexta. Insect Biochemistry 20:* 65-72 (1990).

J.J. Wielgus, L.B. Aden & R.M. Franks. Characterization and site of synthesis of a hemolymph trophic factor in the tobacco hornworm, *Manduca sexta. In Vitro Cellular & Developmental Biology 29A:* 31A (1993) (Abstract).

J.J. Wielgus, L.B. Aden & R.M. Franks. Site of synthesis and phylogenetic distribution of a hemolymph trophic factor of the tobacco hornworm, *Manduca sexta.* Submitted to: *In Vitro Cellular and Developmental Biology* (1993).

Kenneth E. Van Ness

Department of Physics and Engineering
Washington and Lee University
Lexington, Virginia 24450
February 18, 2005

(540)458-8887 (phone)
(540)458-8884 (fax)
vannessk@wlu.edu

Education

Ph.D., Mechanics and Materials Science, Rutgers, The State University of New Jersey.

B.S., Physics, Bucknell University.

Professional Experience

Professor of Physics and Engineering, Washington and Lee University, 1997-present

Associate Professor of Physics and Engineering, Washington and Lee University, 1991 - 1997.

Assistant Professor of Physics and Engineering, Washington and Lee University, 1986-1991.

Research Interests

Current interests include experimental and theoretical studies of polymer blends, both miscible and immiscible. Studies may be further divided into two general areas: (1) the study of the connection between thermodynamics and viscous behavior for miscible polymer blends, and (2) the measurement and prediction of thermal and mechanical properties for solid polymers produced from the blending of two or more immiscible polymers. Polymer melts are studied by squeeze-plate and capillary rheometry. Experimental methods used to characterize these materials include **scanning electron microscopy**, dynamic mechanical analysis, **positron lifetime spectroscopy**, infrared spectroscopy, differential scanning calorimetry and **x-ray diffraction**. Theoretical approaches to the study of the viscous behavior of miscible blends include the development of statistical mechanical models based upon the cell theory of the liquid state, and the derivation of blend viscosity in terms of phase-transition properties. A theoretical approach to the prediction of long-term mechanical behavior of polymeric solids involves the derivation and extension of a mathematical relation for short-term viscoelastic behavior to include long-term effects.

Publications

Chapter

Van Ness, K.E. and Nosker, T.J. "Commingled Plastics", chapter nine in Plastics Recycling, ed. R.J. Ehrig, Hanser Publications, Munich, Germany, pp. 187-229(1992).

Patents

Nosker, T.J., Renfree, R.W., Fernandes, J.R., Van Ness, K.E., and Saba, R.G., Recycled Generic Polymers and Polymer Alloys and Blends Derived from Mixed Waste Plastics Suitable for Blow Molding, Patent No. 8,236,447, 11/00.

Morrow, D.R., Nosker, T.J., Van Ness, K.E., and Renfree, R.W., Polystyrene and Polyolefin Plastics Composite Derivable From Recycled Plastics, Patent Number: 5,298,214, March 29, 1994.

Software

Software: "Non-Linear Strain Energy Equivalence Theory: Creep Prediction Software for Engineered Materials", K. Van Ness, J.K. Lynch, and A. Branzoi, Published by the Center for Advanced Materials via Immiscible polymer Processing, New Brunswick, NJ, 2004.

Articles

"Creep Prediction Using The Non-Linear Strain Energy Equivalence Theory", J. K. Lynch et. al, Rutgers University, and K.E. Van Ness, Washington and Lee University, Proceedings of the 62nd Annual Technical Conference of the Society of Plastics Engineers, Vol. II, pp. 1927-1932, May, 2004

"Applications of Functional Nanocomposite"s, T. Tsakalakos, R. L. Lehman, T. N. Nosker, J. D. Idol, R. Renfree, J. Lynch, K. E. Van Ness, M. DaSilva, S. Wolbach, E. Lee, Synthesis, Functional Properties and Applications, pgs 676-691, Kluwer Academic Publishers. Printed in the Netherlands,128, NATO Science Series II, 2003.

"On Synthesis and Properties Of Nanocomposites", T. Tsakalakos, R. L. Lehman, T. N. Nosker, J. D. Idol, R. Renfree, J. Lynch, K. E. Van Ness, M. DaSilva, S. Wolbach, E. LeeD-Y. KimM. Muhammed, NANO2002, Processing, ISBN 0-9746216-0-9, pp 1-10, 2003.

Jayant Joshi, R. L. Lehman, K. VanNess, T. J. Nosker, Kim Le, "Mechanical Grafting: The Generation of Mechanically Intimate Blends with Enhanced Engineering Properties", Proceedings of the Fifth National Graduate Research Polymer Conference, American Chemical Society, June (2003)

J.K. Lynch, K.E.Van Ness, T.J. Nosker, and R.W. Renfree, "Stress relaxation behavior & prediction of an immiscible polymer blend", Proceedings of the International Conference on Composites/Nano Engineering (ICCE, 10) 2003.

Hmel, P.J., Kennedy, J.Q., Goroglas, M., Seelbaugh, J.P., Morrissette, C.R., Van Ness, K.E., and Reid, T.J., "Physical and thermal properties of blood storage bags: implications for shipping frozen components on dry ice," Transfusion 2002, 42: 836-846.

J.Creek and K.E. Van Ness, Modulus as a Function of Composition for Miscible Polymer Blends, Proceedings of the Annual Meeting of the Materials Research Society, April 17, 2001.

J.K. Lynch, K.E. Van Ness, T.J. Nosker, R.W. Renfree, and M.J. Miraglia. Creep Behavior and Prediction of an Immiscible Polymer Blend. Proceedings of the Eighth International Conference on Composites Engineering, ICCE/8, 08/2001.

Van Ness, K.E., Nosker, T.J., Renfree, R.W., Lynch, J., and Kalista, S.J., Stress-Relaxation of Polyolfefin-Based, Oriented, Glass-Fiber Materials, Proceedings of the Society of Plastics Engineers Annual Technical Conference, 57(3), 2833-2637, 1999.

Nosker, T.J., Renfree, R.W., and Van Ness, K.E., The Development of Polyolefin-Based, Oriented, Glass-Fiber Building Materials, Proceedings of the Society of Plastics Engineers Annual Technical Conference, 57(3), 2838-2841, 1999.

Van Ness, K.E., Nosker, T.J., Renfree, Killion, J.R., Long-term Creep of Commercially Produced Plastic Lumber, Proceedings of the Society of Plastics Engineers Annual Technical Conference, 56(3), 2916-2920, 1998.

Nosker, T.J., Renfree, R.W., Lutz, M., Gillespie, B., Lampo, R., Van Ness, K.E., and Lynch, J., A Performance-based Approach to the Development of a Recycled Plastic/Composite Crosstie, Proceedings of the Society of Plastics Engineers Annual Technical Conference, Atlanta, 56(3), 2912-2916, 1998.

Van Ness, K.E., Nosker, T.J., Renfree, R.W., Sachan, R.D., Lynch, J.K., and Garvey, J.J., Creep Behavior of Commercially Produced Plastic Lumber, Proceedings of the Society of Plastics Engineers Annual Technical Conference, 55(3), 3128-3125, 1997.

Van Ness, K.E. and Couchman, P.R., Cell Theory calculation of Surface Tension of Simple Liquids Solely from Bulk Properties, Journal of Colloid and Interface Science, 182, 110-116(1996).

Nosker, T.J., Renfree, R.W., Van Ness, K.E. and Sachan, R.D., Predictive Techniques for Commingled Plastic Properties, Proceedings of the Society of Plastics Engineers Annual

Van Ness, K.E., Hocking, S.K., Nosker, T.J., Renfree, R.W., and Sachan, R.D., Morphological Characteristics of Commercially Produced Recycled Plastic Lumber, Society of Plastics Engineers Annual Technical Conference Proceedings, 53(3), 3704-3708, 1995.

Nosker, T.J., Renfree, R.W. and Van Ness, K.E., Refined Commingled Processing of Post-Consumer Plastic Blends from an All Rigid Container Collection System, Society of Plastics Engineers Annual Technical Conference Proceedings, 52(3), 2881-2884, 1994.

Van Ness, K.E., Edwards, C.C., Nosker, T.J. and Renfree R.W., Morphology of Blends of Post-Consumer PET with Curbside Tailings, Society of Plastics Engineers Annual Technical Conference Proceedings, 52(3), 3064-3068, 1994.

Van Ness, K.E., Fielder, W.T., Strickler, L.W., Xanthos, M., Nosker, T.J., and Renfree, R.W., Properties and Microstructure of Impact Modified Post-Consumer Plastics, Society of Plastics Engineers Annual Technical Conference Proceedings, 51(3), 1764-1768, 1993.

Renfree, R.W., Nosker, T.J., Morrow, D.R., and Van Ness, K.E., Physical Characteristics of the Dual Phase Region in Mixtures for Recycled Polystyrene/Curbside Tailings Materials, Society of Plastics Engineers Annual Technical Conference Proceedings, 51(3), 920-924, 1993.

Renfree. R.W., Nosker, T.J., Morrow, D.R., and Van Ness, K.E., Thermal Characteristics of the Dual Phase Region in Mixtures for Recycled Polystyrene/Curbside Tailings Materials, Society of Plastics Engineers Annual Technical Conference Proceedings, 51(3), 925-929, 1993.

Nosker, T.J., Renfree, R.W., Lampo, R., and Van Ness, K.E., The Use of Commingled Plastic Lumber as Construction Materials, Society of Plastics Engineers Annual Technical Conference Proceedings, 51(3), 929-933, 1993.

Xanthos, M., Nosker, T.J., and Van Ness, K.E., Compatibilization for Reuse of commingled Post-Consumer Plastics, Compalloy Proceedings, 1992, Short Hills, NJ, April, 1992.

Renfree, R.W., Nosker, T.J., Morrow, D.R., Van Ness, K.E., Wyatt, E.D.*, and Suttner, L.W., Dual Phase, Co-continuous Morphology from Mixtures of Recycled Polystyrene/Curbside Tailings Materials, Society of Plastics Engineers Annual Technical Conference Proceedings, 50(3), 1854-1858, 1992.

Van Ness, K.E., Surface Tension and Surface Entropy for Polymer Liquids, Polymer Engineering and Science, 32(2), pp. 122-129, 1992.

Applebaum, M.D., Nosker, T.J., Renfree, R.W., Morrow, D.R., and Van Ness, K.E., Properties of Refined Reinforced Compounded Post-consumer Plastics, Proceedings Society of Plastics

Engineers Regional Technical Conference, 1991.

Applebaum, M.D., Van Ness, K.E., Nosker, T.J., Renfree, R.W., and Morrow, D.R., The Properties of Fiber Reinforced Refined Post-Consumer Plastics Compounded on a Non-intermeshing Twin Screw Extruder, Proceedings Society of Plastics Engineers Annual Technical Conference, 1991.

Nosker, T.J., Van Ness, K.E., Renfree, R.W., Morrow, D.R. and Donaghy, J.J., Properties and Morphologies of Recycled Polystyrene/Curbside Tailings Materials, Proceedings Society of Plastics Engineers Annual Technical Conference, 1991.

Morrow, D.R., Nosker, T. J., Donaghy, J.J., Renfree, R.W., and Van Ness, K.E. (principal author), Composite Structures Made from Recycled Polymer Blends, Nature, 350, p.563, 1991.

Donaghy, J.J., Van Ness, K.E., Thompson, M.E.*, Cassada, D.C.*, Nosker, T.J., Morrow, D.R., and Renfree, R.W., Positron Lifetimes in Blends of Recycled Polystyrene and Polyethylene, phys. stat. sol. (a), 124, pp. 67-74, 1991.

Donaghy, J.J. and Van Ness, K.E., A Trapping Model Analysis of the Positron Lifetime Spectra of Polymers, physica status solidi (a), 116(2), K213(1989).

Van Ness, K.E. and Couchman, P.R., Surface Tension of Polymer Liquids, Polymer Science and Engineering, 27, 324(1987).

Funded Research

"Advanced Functional and Structural Materials from Immiscible Polymer Blends (AMIPP)", with Rutgers University and Princeton University, funded by New Jersey Commission on Science and Technology, 2002-current.

"Viscous Behavior of Miscible Polymeric Blends", The Jeffress Memorial Trust, 1999-2001.

VITA

NAME: Maryanne C. Simurda

ADDRESS: [redacted]

PHONE: [redacted]
WORK:

CURRENT PROFESSIONAL AFFILIATION: Postdoctoral Research Associate
Department of Microbiology
State University of New York
Buffalo, NY 14214

EDUCATION:

PhD	Microbiology	State University of New York/Buffalo	1988
MS	Physical Sci	Michigan State University, East Lansing	1979
BS	Chemistry	Chestnut Hill College, Phila. PA	1971
AA	Education	Sacred Heart Junior College, Yardley, PA	1969

PROFESSIONAL EXPERIENCE:

1981-1982 Visiting Instructor, D'Youville College, Buffalo, NY
Department of Natural Sciences - Lecturer in General Chemistry with Laboratory, Analytical Chemistry Lab, Organic Chemistry Lab, Intro Chemistry for Nursing.

1978-1981 Laboratory Instructor, D'Youville College, Buffalo, NY
Gen Chemistry, Organic Chem, Analytical Chem Lab

1979-1981 Campus Minister, SUNY/Buffalo
Catholic Diocese of Buffalo Newman Center

1980, 1981 Laboratory Instructor, Canisius College, Buffalo, NY
Gen Chem Lab

1974-1977 Science Dept. Coordinator, Holy Angels Academy H.S.
Buffalo, NY

1971-1977 Chemistry and Physics Teacher, Holy Angels Academy H.S.
Buffalo, NY Instructor for Regent's Chemistry and Physics, Advanced Chemistry, Intro to Chemistry and Physics

PUBLICATIONS:

Simurda, M. and Wilson, J.E. 1980. Localization of hexokinase in neural tissue: Immunofluorescence studies on the developing cerebellum and retina of the rat. Joural of Neurochemistry, 35 (1):58-66.

Simurda, M.C., van Keulen, H., Rekosh, D.M. and LoVerde, P.T. 1988. Schistosoma mansoni: Analysis and characterization of the mRNA and gene encoding superoxide dismutase. Experimental Parasitology 67: 73-84

CONFERENCE PAPERS:

LoVerde, P.T., H. van Keulen, L. Bobek, R. Gregoire, M. Simurda, S. Modavi, L. Spencer, and D. Rekosh. Role of protective antibodies in parasitic infections. In: Antibodies: Protective, Destructive and Regulatory Role. 9th International Convocation on Immunology, F. Milgrom, C.J. Abeyounis, B. Albini (eds.), pp. 90 - 97 (Karger, Basel 1985)

PROFESSIONAL ORGANIZATIONS:

American Society for Microbiology	1984-present
American Association for the Advancement of Science	1979-present
American Chemical Society	1970-1984

GRANTS:

National Science Foundation Summer Research Program for High School Teachers and Students, Roswell Park Memorial Institute, 1974.

AWARDS:

The Erwin Neter Award for Excellence in Graduate Research, February, 1985 at the Spring Meeting of the Western New York Branch of the American Society for Microbiology
Presentation: Identification of Stage and Species Specific in vitro Translation Products of the Blood Fluke Schistosoma. M.C. Simurda, D. Rekosh, P.T. LoVerde.

The Erwin Neter Award for Excellence in Graduate Research, February, 1984 at the Spring Meeting of the Western New York Branch of the American Society for Microbiology
Presentation: Ribonucleic Acid of Schistosoma mansoni: Extraction, Poly A+ Selection, in vitro Translation, and Immunoprecipitation of Translation Products. M.C. Simurda, D. Rekosh, P.T. LoVerde.

Graduate Assistantship from the Nucleic Acid Graduate Group, 1983 - 1984

POSTDOCTORAL RESEARCH

I am presently working as a postdoctoral research associate with Dr. Marie-Louise Hammarskjold and Dr. D.M. Rekosh at SUNY/Bflo. The project concerns the trans- activation of the Long Terminal Redundancy (LTR) of the Human Immunodeficiency Virus (HIV). The tat gene product encoded by HIV is known to stimulate viral mRNA transcription by affecting a region of the viral genome defined as tar. Recent experiments have shown that protein products from the Epstein-Barr virus are also able to affect transcription of HIV. These proteins, EBNA2 and the Epstein-Barr Latent Membrane Protein (LMP), affect regions of the HIV LTR other than tar. A shuttle vector construct containing the LTR of HIV 5' to the chloramphenicaol acetyl transferase (CAT) gene is used to study the control mechanisms for transcription. Using this vector construct and co-transfecting CV-1, Ramos, Raji, or other B cell strains with a vector containing each of the EBV genes, the effect of EBNA2 and LMP on HIV transcription can be measured by assaying for CAT activity. I am determining the region of the HIV LTR that is the site of action of the EBNA2 and LMP. I am using a set of clones, derived from an original HIV LTR-CAT construct, that have specific mutations in the LTR region. Initial findings suggest that the LMP requires the region of the LTR called the core enhancer or kappa B sequences. The EBNA2 does not seem to require any specific region of the LTR for binding.

MASTERS' PROJECT RESEARCH

I also hold a Masters' Degree from Michigan State University for which I did research in both Chemistry and Biochemistry. My work in Biochemistry involved studies on the hexokinase levels in the developing cerebellum and retina of the rat. Indirect immunofluorescence using fluorescein-labeled antibodies localized the hexokinase in cryostat sections of the tissues taken from the neonatal through to the adult stages of the rat. Results of this study, published in the Journal of Neurochemistry, support the concept that the distribution of hexokinase is directly related to the importance of glucose metabolism in supplying the energy required by the cells and that this distribution can be used to identify the cells' functional level during maturation of the cerebellum. I have enclosed a copy of this paper.

I have presented my research work at two local American Society for Microbiology meetings (1984 & 1985), the Lake Ontario Immunology Conference in 1986, the Annual Meeting for Tropical Medicine and Hygiene, Los Angeles, in 1987, and the American Society Microbiology National Meeting, Miami, in May 1988. The abstracts for these presentations are enclosed.

FUTURE RESEARCH PROJECTS

Schistosomiasis, which afflicts 250 million people, is a world-wide health problem. Work on the antigens of S. mansoni and their role in this disease is important in the eventual production of a vaccine. Because of the significance of characterizing these antigens, studies leading to information on the immunology of this disease has a strong potential for extramural funding and I am interested in pursuing that potential.

During my investigations on these antigens, I isolated the cDNA and gene encoding superoxide dismutase. Analysis of the protein sequence showed the potential for this antigen to be the extracellular form of the enzyme. The expression of this enzyme on the surface of the parasite could indicate that the parasite possesses a defense mechanism against attack from activated macrophages and eosinophils. This would be a mode of immune evasion that has not yet been described for this parasite and, therefore, is an advancement in our understanding of the ability of the worm to establish itself in the human host.

While working on the cDNA encoding superoxide dismutase, I isolated three other cDNA clones. These have yet to be identified; however at least one clone shows potential for also encoding an antigen. I am interested in the study of their sequence and expression and their respective genes in a collaboration with Dr. Philip LoVerde and Dr. David Rekosh at SUNY/Bflo. This study is important in the further identification of schistosome antigens since at this time only eight other proteins/antigens are being studied. The work involves the sequencing of the mRNA and the computer characterization of the translated protein. The screening for homology in a protein data bank would identify the protein. Additionally in vitro translation of the selected mRNA and immunoprecipitation of the protein product with sera from patients with chronic schistomiasis would indicate the antigenicity of the protein. The gene encoding this message would be isolated from a S. mansoni genomic library and analyzed for regions controling its transcription. I would also expand this work to include the expression of the antigen in E. coli and studies on the immunological role it plays in schistosomiasis.

Since this work concerns parasitic antigens and involves the characterization of nucleic acids and proteins it does interface biology and chemistry. This research would be applicable for various projects at the undergraduate and Masters' levels in the sequencing and analysis of the cDNA or the gene and the expression of the protein in E. coli.

I am interested in expanding the scope of my work and applying the techniques of molecular biology to other questions of immunological interest.

DOCTORAL THESIS RESEARCH

My thesis research involved the molecular biology of Schistosoma mansoni in the study of the antigens present in the various life cycle stages involved in human infection. When I began my work the protocols for the extraction of the RNA, the in vitro translation of the RNA, and the immunoprecipitation of the translation products were not worked out and optimized for use with S. mansoni. I was able to develop the protocols for the lab and investigate and compare the products of the translation of RNA from the various stages and species of Schistosoma. This work led to the isolation of a cDNA clone encoding an antigen. Consequently, the second part of the thesis concerned the characterization of the mRNA and gene for antigen Cu/Zn superoxide dismutase. I have included the abstract from my dissertation and a copy of the manuscript published in Experimental Parasitology. This work is the first report of the mRNA for superoxide dismutase in the adult worms and is significant because it indicates the potential use of this enzyme by the parasite as an immune evasion mechanism.

Since the structure of this parasite is extremely complex, efforts to study in vivo the antigens involved in eliciting the immune response have been difficult. Molecular biology and recombinant DNA technology offered the potential to study in vitro the proteins that are antigens. To accomplish this, I extracted the ribonucleic acid (RNA) from Schistosoma mansoni cercaria, schistosomula, adult, and egg stages, translated the RNA in vitro in a rabbit reticulocyte lysate system, and then immunoprecipitated the resultant proteins with sera from human patients and hamsters chronically infected with S. mansoni. The results broadly indicated those proteins which were antigenic, as well as indicating which proteins were either stage-specific or common to more tan one stage of the parasite lifecycle. Analyses of these results formed the bases for identifying the proteins translated by the mRNAs that were positively selected by hybridization with the recombinant clones of various bacteriophage and plasmid cDNA libraries.

Following the screening of the cDNA libraries and partial analyses of the various clones selected, I chose one clone for further study. This clone and its mRNA encoded the enzyme superoxide dismutase. The nucleotide sequence of the mRNA, obtained from the sequence of the insert, was analyzed using the Genetics Computer Group Programs to predict the amino acid sequence of the corresponding protein, its structural characteristics, and to identify homologies with known protein sequences in the protein data bank. The proposed amino acid sequence was shown to have structural and functional characteristics similar to both the cytosolic and extracellular form of Cu/Zn superoxide dismutase but not to the Mn-binding enzyme. This result indicated that if S. mansoni expresses superoxide dismutase on its surface, it may possess the ability to defend itself against the onslaught of macrophage and eosinophil damage by the superoxide radical. This would be a novel mechanism for protection in addition to the various others that have been already suggested and studied.

Subsequent screening of a genomic library of S. mansoni, led to the isolation of a lambda bacteriophage clone containing the gene for superoxide dismutase. Dideoxynucleotide sequencing of the clone showed that it was a copy of the functional gene and that this gene contained two introns. Restriction enzyme mapping localized the gene to a 5.1 kb fragment of the kb lambda clone insert.

EXPERIENCE IN EDUCATION

As does the philosophy of Washington and Lee University, I, too, have an intense commitment to excellence in education and firmly believe that an educator in science must tie her teaching with on-going research. My research on schistosomes involved techniques of molecular biology and is quite applicable to undergraduate and graduate research projects.

My interests lie in the areas of immunology and molecular biology, and as required by my degree program, I have been involved in the teaching of Microbiology to nursing students and Microbiology Laboratory to medical students.

Previously, for four years I worked at the Undergraduate level where I taught General Chemistry lecture and laboratory courses, Laboratory Courses in Organic Chemistry and Analytical Chemistry, Biochemistry/Chemistry Lecture Courses for Nursing students, and directed two Chemistry students in their Senior Research Projects.

Additionally, I have six years of experience in secondary education where I taught Chemistry and Physics. I also developed the curriculum for and taught a Ninth Grade Introductory course in Chemistry and Physics and an Advanced Chemistry Course for Twelfth Grade. I was Coordinator of the Science Program for three years.

My previous professional experience, my Masters' program, and my Doctoral program, which included study in Biochemistry, Immunology, Molecular Biology, Microbiology, and Virology, give me a broad background in science and allow me to integrate the various sciences in both research and teaching. I am looking for a position in an academic institution where there is active concern for the overall education of the individual student and where there is support and encouragement for maintenance of research by the educator.

I believe strongly that an educated person in today's technological society needs a solid background in various sciences. Many aspects of any person's life requires comprehension of scientific principles. Consequently, I would be interested in participating in those sciences courses for the non-science major student.

Attachment Three: Radiation Safety Manual

RADIATION SAFETY MANUAL

Washington and Lee University

Lexington, VA 24450

Revised August 1998

In emergencies call the Radiation Safety Officer:

John A. Hufnagel (540) 458-8893 (Office) or [redacted]

TABLE OF CONTENTS

I. THE RADIATION SAFETY COMMITTEE

The Washington and Lee Radiation Safety Committee shall consist of four members to include faculty representatives from the Departments of Biology, Chemistry, and Physics as well as one student member.

It shall be the duty of this committee to:

(a) Establish regulations for the safe operation of any radiation producing equipment on campus.

(b) Establish regulations for the safe handling, use, procurement, transfer, and disposition of all radioactive material on campus.

(c) Establish regulations to insure adequate personnel protection for all people working with or around ionizing radiation.

(d) Review all noncompliance of these and other applicable regulations (NRC etc.) and make recommendations.

The Radiation Safety Committee has been established by the University with the authority to regulate the safe use of radionuclides on the campus. It shall establish and implement the regulations for this purpose. Members of the Committee are selected from personnel of the relevant departments on the basis of their experience in the safe handling of radioactive materials for research and instructional purposes. The Purchasing Agent of the University may be called before the Committee in order to provide guidance in business and financial matters. The Radiation Safety Officer serves as a member and as the Chair of the Committee.

The Chair of the Radiation Safety Committee shall call two meetings yearly and whenever it is otherwise necessary.

The Radiation Safety Committee shall approve all applications for use of radioactive nuclides on the campus and shall review the operations of the users of these radioisotopes. Any modifications or improvements it deems necessary in the interest of radiation safety shall be implemented at the earliest possible date. In case of noncompliance with the codes established in this manual, the Committee has the authority to terminate an experimenter's authorization to use radioactive materials.

II. THE RADIATION SAFETY OFFICER

The Radiation Safety Officer is that person, appointed by the Administration of the University, who by reason of education, training, and experience, is qualified to advise others in the safe handling of ionizing radiations and the supervision of the Health Physics Program. The Radiation Safety Officer shall discharge the duties which are summarized below:

1. The person shall act in a supervisory capacity in all aspects of the University's radiation measurement and protection activities, including responsibility of personnel monitoring, maintenance of exposure records, survey methods, waste disposal, and radiation safety practices, as specified in this manual or as approved by the Radiation Safety Committee.

2. His/her approval is necessary for all activities and procedures which involve actual or potential exposure of personnel to radiation or the release of radioactive materials into the environment. Where such activities are not covered by the established procedures, he/she shall bring these activities before the Committee.

3. The person shall be able to consult with all users of ionizing radiation and give advice in safety practices.

4. He/she shall suspend any operation causing an excessive radiation hazard as quickly as possible. In such an event the person is to notify the Radiation Safety Committee which shall promptly review the incident.

5. The person shall cause routine and special surveys to be performed as deemed necessary in the interest of radiation safety.

6. The person shall prepare a resume of incidents, material received, and an inventory of radioactive material on campus for the Radiation Safety Committee to review at its meetings.

III. HEALTH PHYSICS PROCEDURES

Health physics procedures are established by the Radiation Safety Committee to be followed by all persons working with radioactive materials.

A. General Laboratory Safety Rules and Techniques

1. Eating, drinking, smoking and applying cosmetics are not permitted in laboratories or rooms where radioactive materials are used or stored.

2. All injuries possibly involving radioactive materials, no matter how slight, shall be monitored to determine if the wound is contaminated.

3. Special protection is required for wounds so as to prevent the entry of radioactive materials into the body through the wounds.

4. All equipment which is suspected to have come into contact with loose radioactive material shall be considered potentially contaminated and shall be monitored for contamination before being removed from the laboratory.

5. All persons who are designated to wear personnel monitoring equipment by the Radiation Safety Officer shall wear such devices at all times they work with or near radioactive material.

6. All persons who are permitted to work with radioactive materials shall be fully aware of the procedures specified in this manual and be instructed in matters of radiation safety. A copy of this manual shall be furnished each authorized user of radioactive material and it is his/her responsibility to insure that all personnel in that area be made aware of safe methods of handling radioactive materials.

7. Radioactive material shall be used and stored in such a manner as to restrict unauthorized persons from using such material.

8. All containers for radioactive material shall be properly labeled in conformance with the standards required in Title 10, Code of Federal Regulations, Part 20 (10 CFR 20).

9. Radioactive solutions should not be pipetted by mouth.

10. Protective clothing appropriate to the conditions shall be worn at all times when working with loose radioactive materials. In all cases, disposable gloves shall be the minimum protection required.

11. Radioisotopes shall be used in such a manner that radiation exposure rates to personnel shall be kept as small as possible, in accordance with ALARA (as low as is reasonably achievable) principles. The use of lead bricks and plexiglass shielding, disposable gloves, aprons, "lab mat" and fume hoods will serve to minimize exposure.

12. Remote equipment (long-handled tongs, remote pipets, etc.) shall be used routinely when handling highly radioactive materials when such materials are authorized.

A set of these rules shall be posted in each laboratory.

B. Limits of Exposure to Ionizing Radiation

1. Title 10 of the Code of Federal Regulations Part 20 (10 CFR 20) specifies annual radiation dose limits which vary for (1) professional adults working with radionuclides, (2) minors working with radionuclides, (3) declared pregnant individuals and the embryo/fetus and (4) the general public. No person shall be permitted to receive a radiation dose in one calendar year in excess of listed dose limits as follows:

a) An annual adult occupational dose limit, which is more limiting of-- (i) the total effective dose limit being equal to 5 rems; or (ii) the sum of the deep dose equivalent and the committed dose equivalent to any individual organ or tissue other than the lens of the eye being equal to 50 rems.

b) Annual dose limits to the lens of the eye, the skin, or extremities which are (i) an eye dose equivalent of 15 rems and (ii) a shallow dose equivalent of 50 rems to the skin or each of the extremities.

c) Annual dose limits for minors under the age of 18 are ten percent of the limits described in "a" and "b" above.

N.B. These limits apply to many student research assistants and students in teaching laboratory sessions.

d) Dose limits to an embryo/fetus during the entire gestation period, due to occupational exposure of a

declared pregnant woman, are 0.5 rem. Efforts are to be made to avoid substantial variation above a uniform monthly limit. The dose to an embryo/fetus shall be taken as the sum of (1) the deep-dose equivalent to the pregnant woman and (2) the dose to the embryo/fetus from radionuclides in the embryo/fetus and radionuclides in the declared pregnant woman. This dose limit is interpreted to include radiation from medical as well as occupational sources.

e) Annual dose limits to members of the general public shall not exceed 0.1 rem either from exposure in unrestricted areas or from discharge into the sanitary sewer system; also the dose in any unrestricted area shall not exceed 0.002 rem in any given hour.

2. Radiation exposure is to be monitored either by (1) personal dosimetry or, (2) daily personal monitoring using a survey meter following experimentation. A personal record of the exposure is to be kept after each use of radionuclides as illustrated in Appendix D.

3. As a matter of policy Washington and Lee University does not permit exposures in excess of the above limits, including special situations in which exposures due to medical diagnostic procedures, when summed with the planned occupational exposures, would exceed the annual dose limit. In such cases occupational exposures will be curtailed to bring total exposure to within the prescribed annual limit.

4. The limits established above by 10 CFR 20 are by policy of Washington and Lee University understood as maximal limits; in effect it is the policy of the University to limit its faculty, staff, and all students to 0.5 rem in a given year; protocols requiring exposures in excess of 0.5 rem/year need proportionately greater accrued benefits to be approved by the Radiation Safety Committee.

5. As a matter of policy Washington and Lee University will not allow planned radioactive exposures through ingestion or inhalation; for this reason airborne radioactive substances (e.g. radioactive gasses) or volatile liquid preparations (e.g. radioactive iodide solutions) are not allowed.

6. The use of radioactive materials which emit high energy beta particles (e.g. 32-P) or gamma rays requires the simultaneous use of a dosimeter. Limits for absorbed dosages will be set for each procedure by the Radiation Safety Committee in accord with this section as part of the protocol review process.

C. Marking and Labeling

Rooms, areas, and equipment where radioactive materials are used or stored shall be clearly marked with appropriately worded and designated Health Physics signs whenever required under the conditions set forth in this section.

1. Each area or room where radioactive materials are used or stored in quantities in excess of 10 times the quantities listed in Appendix C of Part 20, or 100 times the quantities listed in the case of natural uranium and thorium, shall be posted with the standard sign and the words CAUTION - RADIOACTIVE MATERIALS. Exceptions to this rule are in cases where:

   a) The radioactive material is in the form of a sealed source such that the radiation level at 12 inches from the surface of the source container does not exceed 5 millirems/hour.

   b) The radioactive material is used in a RESTRICTED AREA and is in use for less than eight hours and is constantly attended during the period by a person trained in radiation safety. (Restricted area means any area, access to which is controlled by Washington and Lee University for purposes of protection of individuals from exposure to radiation and radioactive materials; however, residential quarters cannot be included in a restricted area.)

2. Each container in which radioactive material is used, stored, or transported shall be labeled with the radiation symbol, the words CAUTION - RADIOACTIVE MATERIALS, and the isotope, quantity, and date of measurement if the quantity involved exceeds those listed in Appendix C of Part 20. Exceptions to this rule are in cases wherein:

   a) The concentration of the material in the container is less than the quantities listed in Appendix C of Part 20.

   b) The containers are used transiently in lab work with the user present.

3. Any area will be defined as a radiation area if there exists radiation at such levels that a major portion of the body could receive in any one hour a dose in excess of 5 millirems. Each such area shall be clearly marked with a standard radiation sign bearing the words: CAUTION - RADIATION AREA.

4. Any area will be defined as a high radiation area if there exists radiation at such levels that a major portion of the body could receive in any one hour a dose of 100 millirems. Each such area shall be clearly marked with a standard radiation sign with the words: CAUTION - HIGH RADIATION AREA.

5. Any area will be defined as an airborne radioactivity area under the following conditions:

   a) If airborne radioactive materials exist in the area in concentrations in excess of the amounts specified in Appendix B of Part 20; or

   b) If airborne radioactive materials exist in the area to such a degree that an individual present in the area without protective respiratory equipment could exceed, during the hours present in a week, an intake of 0.6 percent of the annual limit on intake (ALI) or 12 DAC (derived air concentration) hours. Such areas should be clearly labeled with a standard radiation sign bearing the words: CAUTION - AIRBORNE RADIOACTIVITY AREA.

D. Personnel Monitoring

1. All persons who enter an area under such conditions that they may receive a radiation exposure greater than 10 percent of the limits set forth in Part III, B.1 shall wear appropriate monitoring devices. These monitoring devices shall be dosimeters or finger badges as authorized by the Radiation Safety Officer.

   It is the responsibility of the authorized user to notify the Radiation Safety Officer whenever an individual will require personnel monitoring and whenever the need for monitoring is terminated.

2. When finger badges are required (e.g. when assessing absorbed doses), film in the badges shall be

processed/mailed in strict accordance with the manufacturer's instructions by the Radiation Safety Officer. If an exposure in excess of the limits specified is suspected, the Radiation Safety Officer shall be notified immediately so that the film may be processed for rapid analysis.

The Radiation Safety Officer shall supervise the obtaining, distribution, and collection of finger badges.

When not in use badges shall be stored in areas where they will not be exposed to ionizing radiation.

AT NO TIME WILL A BADGE BE EXPOSED TO RADIATION UNLESS IT IS WORN BY THE EXPERIMENTER. Badges shall not be worn during nonoccupational exposure such as medical x-ray.

All personnel monitoring records shall be maintained by the experimenter under the supervision of the Radiation Safety Officer.

E. Radiation Monitoring and Control

1. All laboratories and facilities where radioactive materials are used or stored shall be surveyed periodically in order to detect changes in radiation levels and prevent the spread of radioactive contamination. The frequency of surveys shall be determined by the Radiation Safety Officer after consultation with the authorized user. It shall be the responsibility of the authorized user to inform the Radiation Safety Officer whenever there is a change in working conditions which might necessitate a change in the survey schedule. Failure to do so shall be a sufficient cause for the Radiation Safety Committee to cause operations to be suspended.

2. Laboratories where radioactive materials are used or stored shall be provided with suitable radiation detecting instruments. Laboratory personnel shall use the instruments frequently so as to detect any radiation safety hazards. Whenever survey readings are masked by above-background radiation levels, smear samples shall be taken and checked in a low background area.

3. In the event that the spread of radioactive contamination is suspected, all work shall be halted immediately and the Radiation Safety Officer shall be notified.

Personnel should not expose themselves to the hazard unnecessarily, but should remain in the general area to avoid spreading contamination. When injuries to personnel have occurred, first aid measures take priority over decontamination procedures.

F. Radioactive Waste Disposal

1. No radioactive waste material will be disposed of through the sanitary system except that which is a result of washing laboratory glassware and equipment or in cases wherein the radioactivity can be diluted to background levels in a reasonable volume of running tap water and no toxic chemical is present (e.g., toluene).

2. Radioactive waste shall normally be retained in the laboratory in which it is generated. Liquids shall be stored in capped and labeled plastic bottles. Solid wastes shall be stored in covered plastic or plastic lined containers. The Radiation Safety Officer shall be responsible for the supervision of the disposition of the material.

3. In cases where the above specified system of waste disposal is not satisfactory, the Radiation Safety Officer and authorized user may agree on an alternate method of disposal which satisfies Federal and State regulations and which must be approved by the Radiation Safety Committee.

4. The volume of radioactive waste should be kept to a minimum. Do not use radioactive waste containers for non-radioactive waste.

5. All radioactive waste shall be clearly marked and labeled in accordance with section C, Marking and Labeling.

6. Records of the disposal of all radioactive materials shall be maintained, including information as to isotope, quantity, and ultimate disposal.

G. Authorization To Use Radioactive Material

The following procedure shall be followed by all persons desiring to use radioactive materials.

A person desiring to use radioactive materials for the first time shall submit a "Request for Use of Radioactive Materials" form (a copy of which is attached) to the

Radiation Safety Committee. The Committee will notify the person of its action in writing with the recommendations for procedures, if necessary. A copy of the request will be forwarded to the person's department head. The department head will maintain a file of all authorized users in the department.

At the instruction of the Radiation Safety Officer, the person might also be requested to submit materials for forwarding to the NRC in order to obtain licensing as an authorized user.

H. Procurement of Radioactive Materials

The following procedure shall be followed by all persons desiring to procure radioactive materials.

1. All orders for radioactive materials will be originated by an authorized user, approved and signed by the department head, and then forwarded to the Radiation Safety Officer:

   John A. Hufnagel
   Radiation Safety Officer
   Howe Hall 301 B

2. Purchasing shall not process an order which has not been forwarded by the Radiation Safety Officer.

3. If the radioactive material is to be obtained from an NRC installation, such as Oak Ridge, the authorized user will complete NRC form 391, "Isotope and Service Irradiation Order Form" and submit it with the order.

4. When ordering short half-life material, where time is of the essence, contact the Radiation Safety Officer to make arrangements for the most expeditious delivery. No phone call orders will be made without the Radiation Safety Officer's approval.

5. The Radiation Safety Officer will receive the shipment, perform a safety survey, and notify the authorized user of its availability.

Radiation Safety Committee. The Committee will notify the person of its action in writing with recommendations for procedures, if necessary. A copy of the request will be forwarded to the person's department head. The department head will maintain a file of all authorized users in the department.

At the instruction of the Radiation Safety Officer, the person might also be requested to submit materials for forwarding to the NRC in order to obtain licensing as an authorized user.

H. Procurement of Radioactive Materials

The following procedure shall be followed by all persons desiring to procure radioactive materials.

1. All orders for radioactive materials will be originated by an authorized user, approved and signed by the department head, and then forwarded to the Radiation Safety Officer:

~~John Jay Wielgus~~ John A. Hufnagel
Radiation Safety Officer
~~Parmly Hall 408~~ Howe Hall 301 B

2. Purchasing shall not process an order which has not been forwarded by the Radiation Safety Officer.

3. If the radioactive material is to be obtained from an NRC installation, such as Oak Ridge, the authorized user will complete NRC Form 391, "Isotope and Service Irradiation Order From" and submit it with the order.

4. When ordering short half-life material, where time is of the essence, contact the Radiation Safety Officer to make arrangements for the most expeditious delivery. No phone call orders will be made without the Radiation Safety Officer's approval.

5. The Radiation Safety Officer will receive the shipment, perform a radiation safety survey, and notify the authorized user of its availability.

I. Transfer of Radioactive Material

1. Recommended methods, as approved by the Radiation Safety Committee, will be used to transfer radioactive materials on campus.

2. Transferring of radioactive material off the campus will be handled by the Radiation Safety Officer.

J. Training

The authorized user shall be responsible for the training in the safe handling of radioactive materials of all persons who work on approved projects. A record of preliminary training shall be sent to the Radiation Safety Officer and the Radiation Safety Committee at the time authorization is requested. This training shall include:

1. Knowledge of the pertinent Federal regulations under which the work must be performed, particularly Title 10, CFR, Parts 19 and 20.

2. Familiarity with the general laboratory safety rules and techniques contained in part III A of this manual.

3. Action to be taken in case of a radioactive spill or other accident.

4. Purpose and availability of the Radiation Safety Officer and Committee.

5. Use of radiation detection instruments provided for this purpose.

6. Viewing of the Radiation Safety video series (Radiation Safety Office, Indiana University) in the Leyburn Library (see the Radiation Safety Officer).

7. In the case of students and novices, the use of radioactive materials shall take place only under the direct supervision of an authorized user.

IV. RECORDS

1. The following records shall be established and maintained by the Radiation Safety Officer.

A. Personnel Exposure

The radiation exposure record of every person subject to personnel monitoring as specified in part III D of this manual will be maintained in this file. Information specified on the form in Appendix D shall be included.

B. Radioactive Materials Inventory

The receipt, assignment, and final disposition of all radioactive materials shall be recorded in the Radioactive Materials Inventory.

C. Radiation Survey Log

A complete description of all radiation and contamination surveys performed by the Radiation Safety Officer shall be entered in the Radiation Safety Log.

D. Instrument Calibration Log

The date of calibration of Health Physics survey instruments shall be recorded in the Instrument Calibration Log.

2. Each authorized user will maintain a running log of each radioactive isotope in possession, to include: receipt, quantity on hand, and final disposition. An inventory will be submitted to the Radiation Safety Officer twice yearly or when the authorized user has terminated the work with radioactive materials.

Appendix A: Code of Federal Regulations
Title 10, Chapter 1, Parts 19 and 20

Note: the full texts of Parts 19 and 20 of CFR are available in the office of the Radiation Safety Officer, John A. Hufnagel, Howe 301 B.
Included here in Appendix C of Part 20 which lists "exempt" quantities of radionuclides and is referred to in this manual.

Material	Microcuries
Americium-241	.01
Antimony-122	100
Antimony-124	10
Antimony-125	10
Arsenic-73	100
Arsenic-74	10
Arsenic-76	10
Arsenic-77	100
Barium-131	10
Barium-133	10
Barium-140	10
Bismuth-210	1
Bromine-82	10
Cadmium-109	10
Cadmium-115m	10
Cadmium-115	100
Calcium-45	10
Calcium-47	10
Carbon-14	100
Cerium-141	100
Cerium-143	100
Cerium-144	1
Cesium-131	1,000
Cesium-134m	100
Cesium-134	1
Cesium-135	10
Cesium-136	10
Cesium-137	10
Chlorine-36	10
Chlorine-38	10
Chromium-51	1,000
Cobalt-58m	10
Cobalt-58	10
Cobalt-60	1
Copper-64	100
Dysprosium-165	10
Dysprosium-166	100
Erbium-169	100
Erbium-171	100
Europium-152 9.2 h	100
Europium-152 13 yr	1
Europium-154	1
Europium-155	10
Fluorine-18	1,000
Gadolinium-153	10
Gadolinium-159	100
Gallium-72	10
Germanium-71	100
Gold-198	100
Gold-199	100
Hafnium-181	10
Holmium-166	100
Hydrogen-3	1,000
Indium-113m	100
Indium-114m	10
Indium-115m	100
Indium-115	10
Iodine-125	1
Iodine-126	1
Iodine-129	0.1
Iodine-131	1
Iodine-132	10
Iodine-133	1
Iodine-134	10
Iodine-135	10
Iridium-192	10
Iridium-194	100
Iron-55	100
Iron-59	10
Krypton-85	100
Krypton-87	10
Lanthanum-140	10
Lutetium-177	100
Manganese-52	10
Manganese-54	10
Manganese-56	10
Mercury-197m	100
Mercury-197	100
Mercury-203	10
Molybdenum-99	100
Neodymium-147	100
Neodymium-149	100
Nickel-59	100
Nickel-63	10
Nickel-65	100
Niobium-93m	10
Niobium-95	10
Niobium-97	10
Osmium-185	10
Osmium-191m[1]	100
Osmium-191	100
Osmium-193	100
Palladium-103	100
Palladium-109	100
Phosphorus-32	10
Platinum-191	100
Platinum-193m	100
Platinum-193	100
Platinum-197m	100
Platinum-197	100
Plutonium-239	.01
Polonium-210	0.1
Potassium-42	10
Praseodymium-142	100
Praseodymium-143	100
Promethium-147	10
Promethium-149	10
Radium-226	.01
Rhenium-186	100
Rhenium-188	100
Rhodium-103m	100
Rhodium-105	100
Rubidium-86	10
Rubidium-87	10
Ruthenium-97	100
Ruthenium-103	10
Ruthenium-105	10
Ruthenium-106	1
Samarium-151	10
Samarium-153	100
Scandium-46	10
Scandium-47	100
Scandium-48	10
Selenium-75	10
Silicon-31	100
Silver-105	10
Silver-110m	1
Silver-111	100
Sodium-24	10
Strontium-85	10
Strontium-89	1
Strontium-90	0.1
Strontium-91	10
Strontium-92	10
Sulphur-35	100
Tantalum-182	10
Technetium-96	10
Technetium-97m	100
Technetium-97	100
Technetium-99m	100
Technetium-99	10
Tellurium-125m	10
Tellurium-127m	10
Tellurium-127	100
Tellurium-129m	10
Tellurium-129	100
Tellurium-131m	10
Tellurium-132	10
Terbium-160	10
Thallium-200	100
Thallium-201	100
Thallium-202	100
Thallium-204	10
Thorium (natural)[2]	100
Thulium-170	10
Thulium-171	10
Tin-113	10
Tin-125	10
Tungsten-181	10
Tungsten-185	10
Tungsten-187	100
Uranium (natural)[3]	100
Uranium-233	.01
Uranium-234-Uranium-238	.01
Vanadium-48	10
Xenon-131m	1,000
Xenon-133	100
Xenon-135	100
Ytterbium-175	100
Yttrium-90	10
Yttrium-91	10
Yttrium-92	100
Yttrium-93	100
Zinc-65	10
Zinc-69m	100
Zinc-69	1,000
Zirconium-93	10
Zirconium-95	10
Zirconium-97	10

58 FR 67657
58 FR 67657
58 FR 67657

Appendix B: Laboratory Rules and Procedures

Laboratory Radiation Safety Rules and Procedures

1) Radioactive materials are used or stored in this lab; no eating, drinking, or smoking is permitted.

2) Radioactive solutions are not to be pipetted by mouth.

3) Protective clothing appropriate to the conditions shall be worn at all times when working with radioactive materials. In all cases, disposable gloves shall be the minimum protection.

4) Radiation exposure rates shall be kept to a minimum by shielding, maintenance of distance (by use of tongs, etc.), and limits of the time of exposure.

5) All persons who are designated to wear or have in their possession personnel monitoring equipment by the Radiation Safety Officer shall use such devices at all times they work with or near radioactive material.

6) Spills of radioactive material which are not easily contained and cleaned up shall be reported immediately to the Radiation Safety Officer.

7) All injuries possibly involving radioactive material, no matter how slight, shall be monitored to determine if the wound is contaminated. Decontamination procedures are to be performed under the supervision of the Radiation Safety Officer.

8) Special protection is required for wounds so as to prevent the entry of radioactive materials into the body.

9) All equipment which is suspected to have come into contact with loose radioactive material shall be considered potentially contaminated and shall be monitored for contamination before being removed from the laboratory.

10) All radioactive material shall be used and stored in such a manner as to prevent unauthorized persons from using or being exposed to radioactive materials.

11) All containers for radioactive material shall be clearly labeled in conformance with NRC regulations and the Radiation Safety Officer.

12) All persons who are permitted to work with radioactive materials shall be fully aware of the relevant procedures specified in the Radiation Safety Manual or be directly supervised by an authorized user. All shall be instructed in

matters of radiation safety and emergency procedures. A copy of the Radiation Safety Manual is available in your department's main office.

Appendix C: Application to Use Radioactive Materials

Application for Authorization to Use Radioactive Materials

Washington and Lee University
Office for Radiation Safety

1. Applicant(s)_____

2. Campus address(es)_____

3. Who will be the participating users under the applicant's supervision?

4.

Radioisotope (Element and Mass Number)	Chemical and/or Physical Form	Total amount of Inventory (Ci)

5. Room(s) and building(s) where radioactive material is to be used.

6. Room(s) and building)s) where radioactive material is to be stored.

7. On a separate sheet describe briefly the experimental procedure which will involve use of the radioactive material.

_____ _____

Applicant's Signature Date

Appendix D: Sample Exposure Record Sheet

Washington and Lee University
Office of Radiation Safety
Exposure Record

Name:

Date	Procedure	DPM/μCi	Dose

Note: On this form please denote a "procedure" by abbreviating an approved protocol (e.g. 35-S DNA sequencing); this record should be complete enough, when considered with approved protocol records already in the hands of the Radiation Safety Officer, to allow a summary as shown on the following page.

Washington and Lee University
Office of Radiation Safety
Exposure Record

Name: [redacted]

Date: 3/25/92

Procedure: Training excercise; transfer of ^{14}C-cholesterol in ethanol:acetone (1:1/v:v) solution from a stock vial to a scintillation vial using a Hamilton syringe.

Total radioactivity used: 100 μL solution with a total activity of 30,000dpm ($1.4x10^{-2}$ μCi or $8.5x10^{-7}$ rem if absorbed).

Absorbed dose: None; disposable gloves and aprons were used; there was no contamination of personnel or of lab surfaces.

Sample summary exposure record sent by the Radiation Safety Office to an employer requesting total exposure records.

Washington and Lee University
Office of Radiation Safety
Exposure Record

Name:

Date	Procedure	DPM/μCi	Dose

WASHINGTON & LEE UNIV
ATTN JOHN HUFNAGEL
ATTN BIOLOGY DEPT
LEXINGTON VA 24450

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www.landauerinc.com

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RADIATION DOSIMETRY REPORT

ACCOUNT NO.	SERIES CODE	ANALYTICAL WORK ORDER	REPORT DATE	DOSIMETER RECEIVED	REPORT TIME IN WORK DAYS	PAGE NO.
150614		0502630365	02/07/05	01/26/05	8	1 OF 1

PARTICIPANT NUMBER	NAME / ID NUMBER / BIRTH DATE / SEX	DOSIMETER	USE	RADIATION QUALITY	DOSE EQUIVALENT (MREM) FOR PERIODS SHOWN BELOW: DEEP DDE	EYE LDE	SHALLOW SDE	YEAR TO DATE DOSE EQUIVALENT (MREM): DEEP DDE	EYE LDE	SHALLOW SDE	LIFETIME DOSE EQUIVALENT (MREM): DEEP DDE	EYE LDE	SHALLOW SDE	RECORDS FOR YEAR	INCEPTION DATE (MM/YY)
FOR MONITORING PERIOD:					10/15/04 - 01/14/05			2004							
00000	CONTROL	Pa	CNTRL		M	M	M							4	07/96
	CONTROL	U	CNTRL				M								07/96
00002	AREA MONITOR	Pa	AREA		M	M	M	M	M	M	14	18	22	4	07/96
00003	[redacted]	Pa	WHBODY		M	M	M	2	2	4	2	2	4	4	04/03
	[redacted]	U	RFINGR				M			410			410		04/03

M: MINIMAL REPORTING SERVICE OF 1 MREM

QUALITY CONTROL RELEASE: RCH 1 - PR 8169 - RPT1318- N1 - 24947

PERSONAL INFORMATION WAS REMOVED BY NRC. NO COPY OF THIS INFORMATION WAS RETAINED BY THE NRC.

NVLAP

NVLAP LAB CODE 100516-0

Attachment Four: Room Plans

Scale drawings of all areas where licensed materials are used and/or stored.

Rooms in Howe Hall Science Center: Departments of Biology and Physics

Howe 102: Modern Physics Lab/Position Lifetime Studies
Authorized User: Prof. Kenneth E. Van Ness

Howe 309: Biology Darkroom
Authorized Users: Prof. John Jay Wielgus and Maryanne Simurda

Howe 312: Cell and Molecular Biology Lab
Authorized User: Prof. John Jay Wielgus

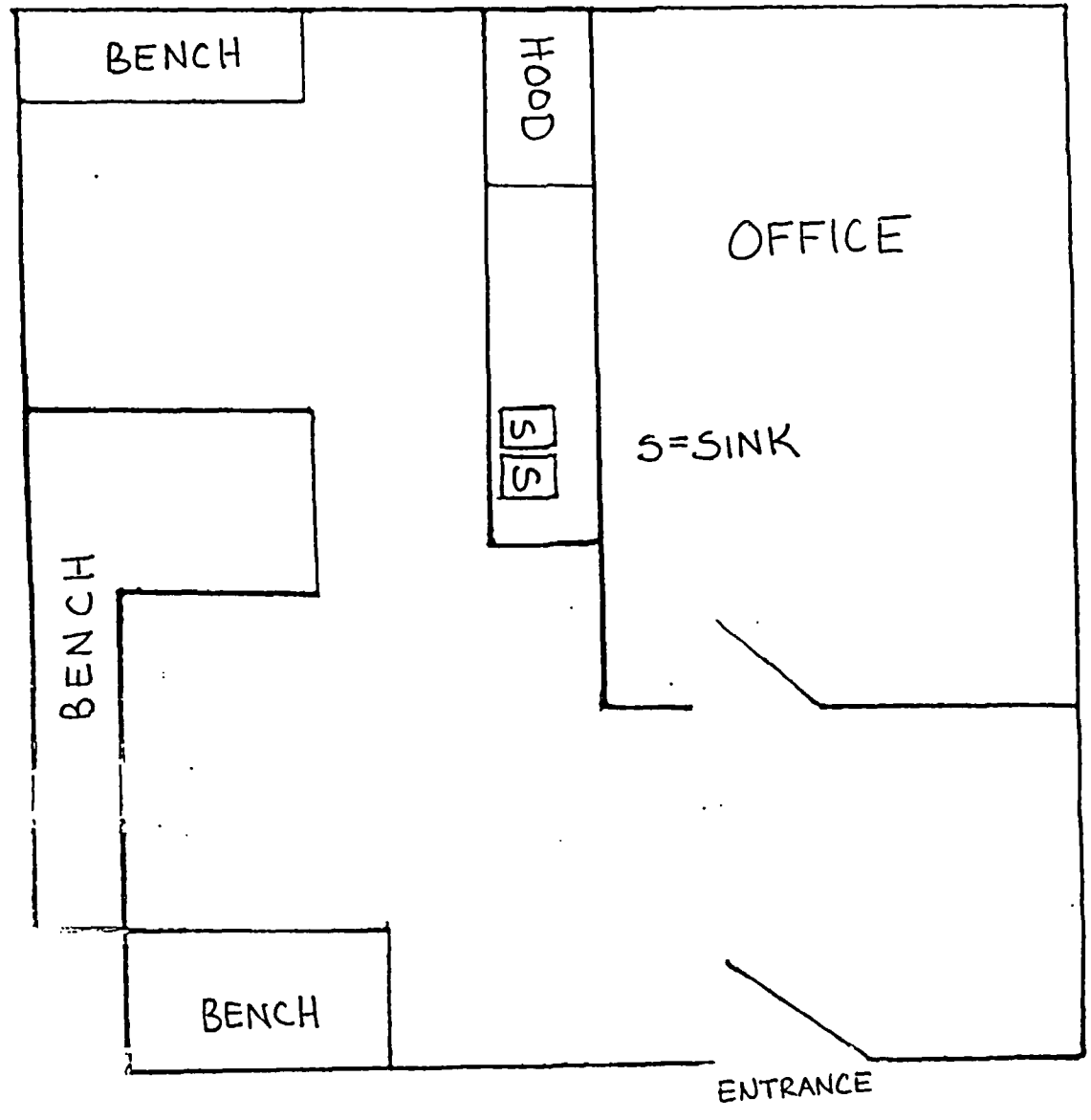
Howe 315: Insect Biochemistry Research Lab
Authorized User: Prof. John Jay Wielgus

Howe 304: Biology Hot Lab
Authorized Users: Prof. John Jay Wielgus and Maryanne Simurda

Howe 306: Biology Instrumentation Lab
Authorized Users: Prof. John Jay Wielgus and Maryanne Simurda

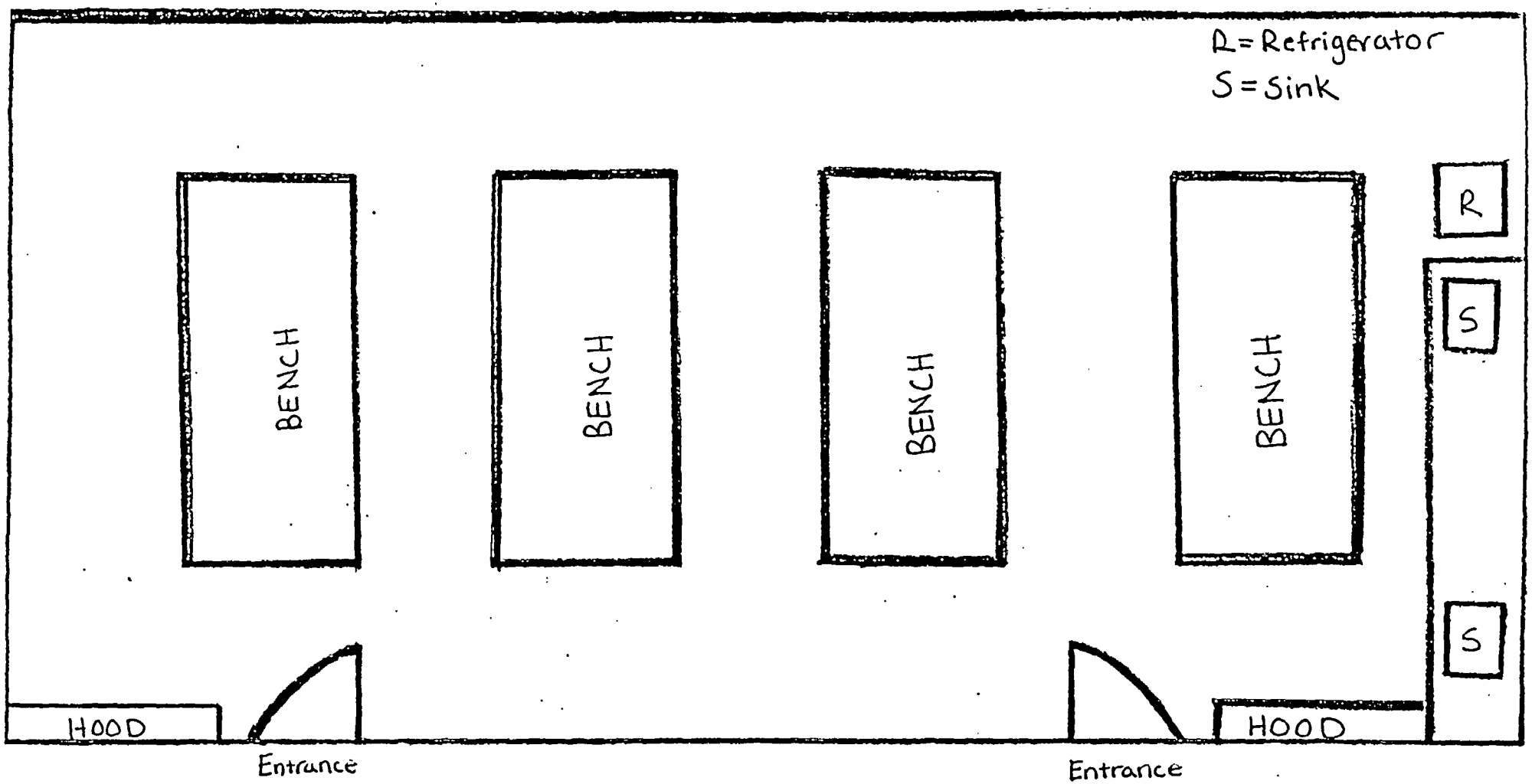
Please see Attachment 4 for scale drawings of all areas where licensed materials are used and/or stored.

Howe 315: Personal Research Laboratory, Insect Biochemistry Research
Authorized User: Prof. Jack Wielgus



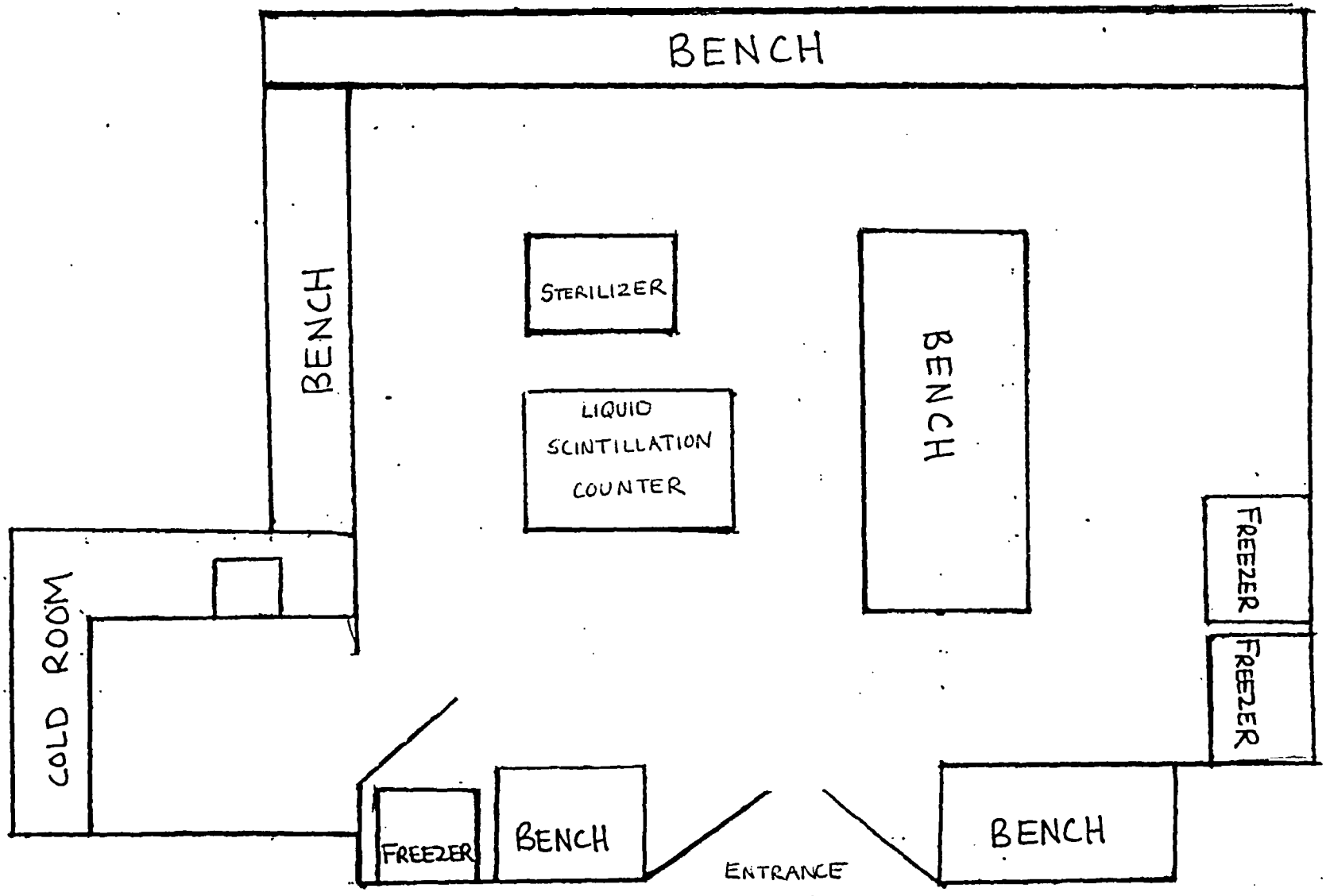
H315 - FACULTY RESEARCH

Scale 1/4" = 1'



Howe 312: Cell and Molecular Biology Lab
Authorized User: Prof. Jack Wielgus

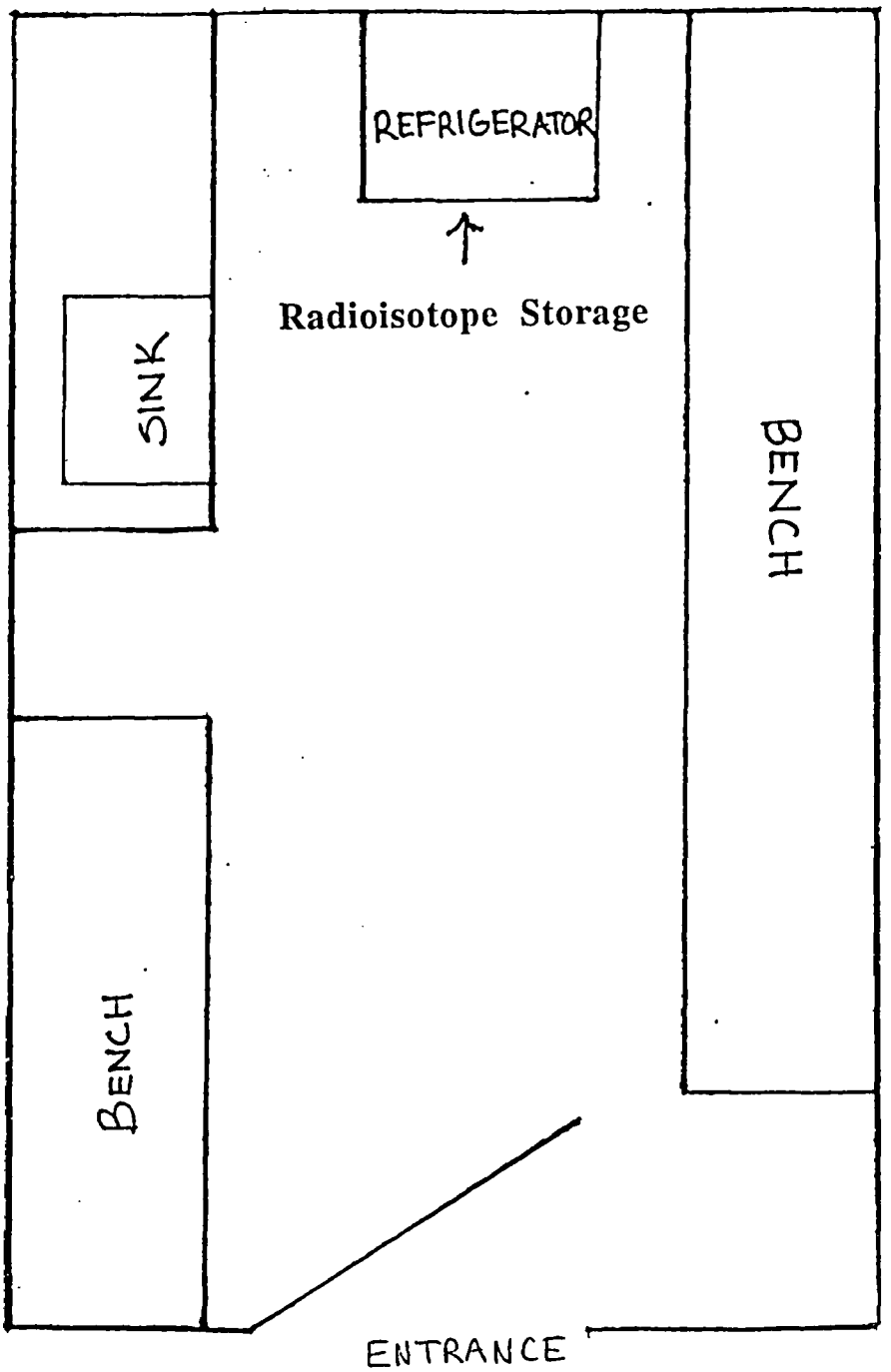
Scale ¼" = 1'



Howe 306: Biology Instrumentation Laboratory
Authorized Users: John Jay Wielgus and Maryanne Simurda

Scale 1/4" = 1'

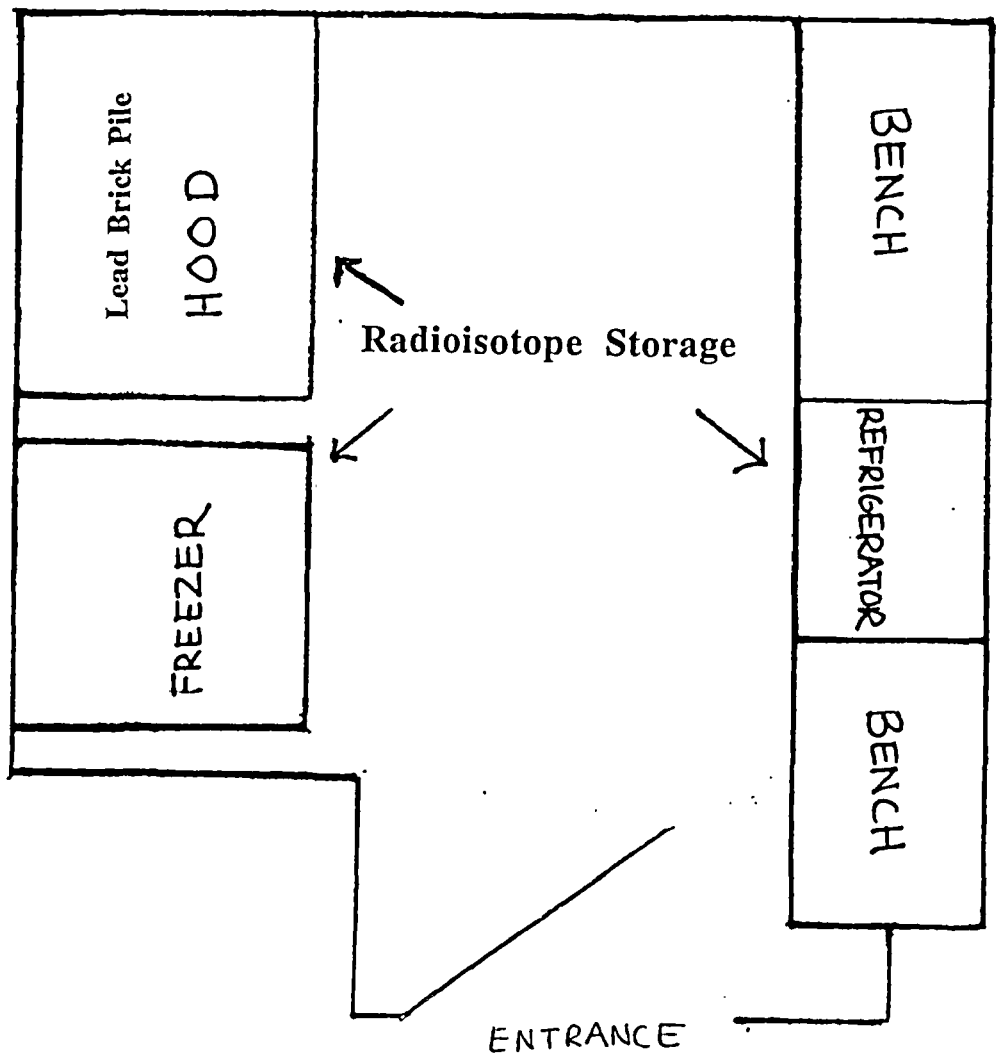
Howe 309: Biology Darkroom*
Authorized Users: John Jay Wielgus and Maryanne Simurda



*** General student access allowed only under direct supervision of authorized user. Otherwise room is kept locked.**

SCALE ½" = 1'

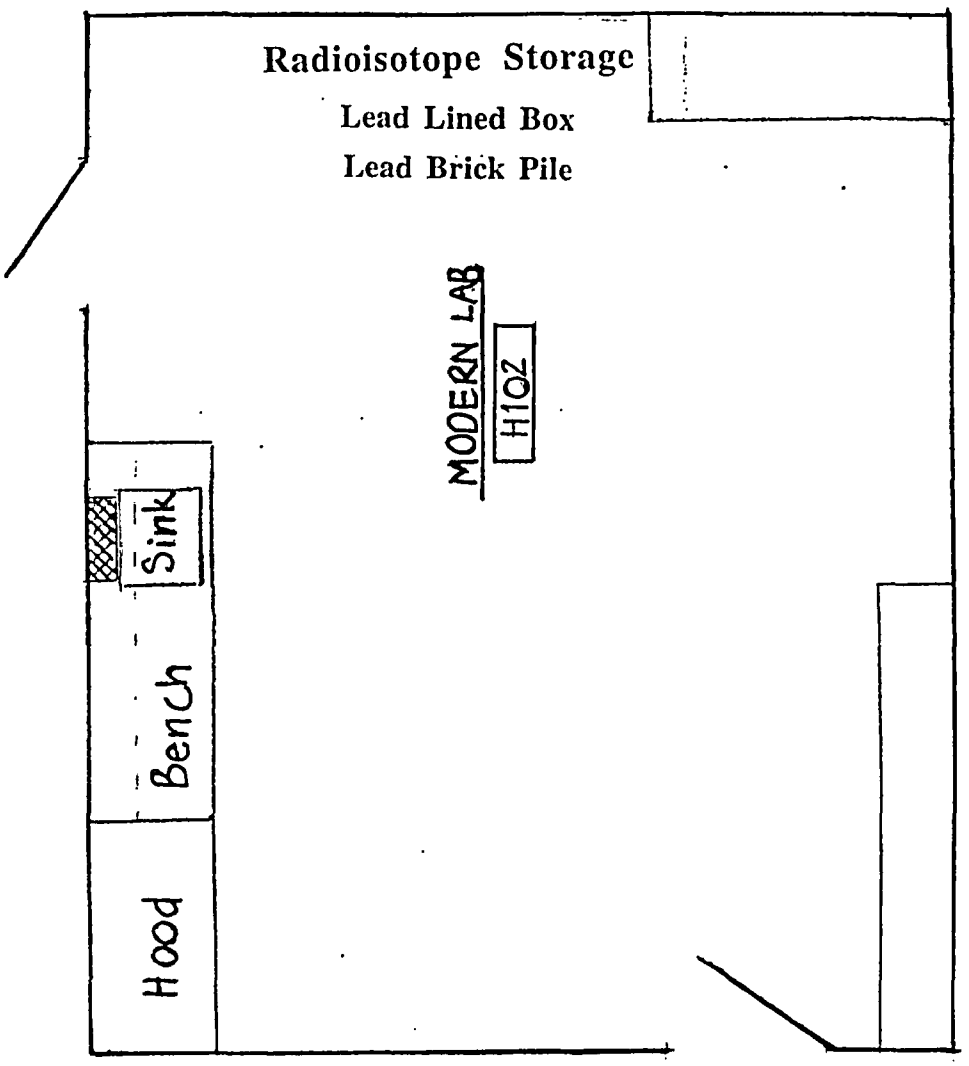
Howe 304: Biology Hot Laboratory*
Authorized Users John Jay Wielgus and Maryanne Simurda



*** General student access allowed only under direct supervision of authorized user. Otherwise room is kept locked.**

SCALE ½" = 1'

Howe 102: Physics Modern Laboratory
Authorized User: Kenneth E. Van Ness



Scale 1/4" = 1'

Attachment Five: Training and Safety Procedures

Washington and Lee Univeristy
Office of Radiation Safety
Initial Training Program: Phase I Lecture Outline

I. The Purposes of Radiotracers in Experimentation
- a) History of scientific use of radionuclides
- b) Analytical radionuclide techniques

II. Radioactive isotopes used in science; nuclear disintegration products; half lives
- a) alpha particles (atomic radiation, thorium)
- b) beta particles (^{3}H, ^{14}C, ^{45}Ca, ^{32}P)
- c) gamma rays (^{125}I, ^{131}I, ^{60}Co)

III. Liquid Scintillation Counting
- a) the general principle
- b) scintillation cocktails
  - 1) aromatic solvents
  - 2) solutes (PPO, POPOP)

IV. Units of Measurement
- a) the curie (2.2×10^{12} DPM)
- b) the dpm v. cpm
- c) specific activities
- d) units of absorbed dose; the rem

V. Counting Efficiency
- a) the problem of quenching
  - 1) color quenching
  - 2) chemical quenching
- b) AES ratios, their meaning and importance
- c) correction for quench

VI. Health Physics
- a) Somatic dangers (stochastic and non-stochastic)
- b) Hereditary and reproductive dangers
- c) Dose limits
- d) Protection procedures
  - 1) contamination control
  - 2) time limits
  - 3) distancing
  - 4) shielding

VII. Legal Enforcement
- a) the Nuclear Regulatory Commission
- b) Radiation Safety Officer and Committee
- c) Laboratory regulations

The Training Program

Training falls into two categories: (1) initial training and (2) refresher training. The extent of initial training varies according to the documented formal background of the individual, whether experienced or novice, and (if experienced) the relevance of the experiences for the proposed protocol.

Initial training is composed of four phases as follows:

Phase I : Ninety minute lecture on nuclear physics, routine laboratory procedures, emergency procedures, and health physics. This lecture is conducted by the Radiation Safety Officer and is offered annually in March, or when otherwise required. Attached is an outline of the lecture.

Phase II : Viewing of three video cassettes produced by the Radiation Safety Office of Indiana University.

Indiana University Radiation Safety Series

TITLE: Radiation Safety, introduction [videorecording] / a production of Indiana University Environmental Health and Safety in cooperation with Indiana University Instructional Television.
PUBLISHER: Bloomington, Ind. : Indiana University, Audio-Visual Center, c. 1982
DESCRIPT: 1 cassette (15 min.) : sd., col. ; ¾ in.
SERIES: Radiation safety series ; #1
ALT SERIES : Radiation safety series [videorecording] ; no. 1.
ADD AUTHOR : Indiana University. Audio-Visual Center.

TITLE: Radiation safety, Laboratory techniques [videorecording] / a production of Indiana University Environmental Health and Safety in cooperation with Indiana University Instructional Television.
PUBLISHER: Bloomington, Ind. : Indiana University, Audio-Visual Center, c1982.
DESCRIPT: 1 cassette (15 min) : sd., col. ; ¾ in.
SERIES: Radiation safety series; #2
ALT SERIES: Radiation safety series [videorecording] ; no. 2.
ADD AUTHOR : Indiana University. Audio-Visual Center.

TITLE: Radiation safety, emergency procedures[videorecording] / a production of Indiana University Environmental Health and Safety in cooperation with Indiana University Instructional Television.
PUBLISHER: Bloomington, Ind. : Indiana University, Audio-Visual Center, c1982.
DESCRIPT: 1 cassette (15 min) : sd., col. ; ¾ in.
SERIES: Radiation safety series; #3
ALT SERIES: Radiation safety series [videorecording] ; no. 3.
ADD AUTHOR : Indiana University. Audio-Visual Center.

Phase III: Discussion of the Radiation Safety Manual, with emphasis on Part II, Health Physics Procedures. Each user (with the exception of student who perform a single procedure under direct supervision) perceives a copy of the manual.

Phase IV: Supervised "dry-run" of all experimental procedures for researchers, or for students, one of the radioisotope dilution exercises detailed in "Attachment One" on the Materials Application.

These four phases combined address at least once each item of the Regulatory Guide 10.7, item 15d (1-14) and 10 CFR 19.12.

The minimal training given (for example, in the case of a Ph.D. in nuclear physics who has documented expertise in the handling of radionuclides) is a combination of phases II, III, and IV.

Refresher Training consists of annual viewing of the video tapes described above. All users much sign a form indicating that they have viewed all three tapes. This form of refresher training parallels the U.S. Public Health Service OPRR required training for all those working with animals under the aegis of the Institutional Animal Care and Use Committee, which requires as refresher training the annual viewing of an animal care tape.

In addition, all users receive copies of relevant NRC publications (Regulatory Guides, Policy Statements, and Information Notices) in order to stay abreast of regulatory concerns.

Ancillary personnel are advised of the presence of radioactive material and waste locations, levels of concern, meaning of radioactive warning labels and instructions. In the case of permanent custodians this instruction is given by the authorized users as part of job orientation. In the case of maintenance personnel who might have cause to work in a licensed room, the instruction is given as part of the description of the required maintenance before the work actually commences. On a yearly basis key maintenance personnel are given as part of an overall hazardous materials training program an introduction to the radioisotope program employed by the science departments. The training included the types of radiation and their dangers; safety precautions and emergency procedures; warning signs and places of storage; and necessary procedures of coordination with the Radiation Safety Officer when maintenance must be performed in areas of isotope storage. Annually custodial and security supervisors are given a tour of the rooms designated for isotope storage and use. This in particular is done to emphasize posted warnings and emergency instructions, and the necessity of alerting the RSO in the case of their personnel discovering an emergency of any type in these designated areas.

Establishment and posting of emergency procedures.

At present each authorized room has a list of regulations posted which read as follows:

This room is authorized by the Nuclear Regulatory Commission for the use and/or storage of radioactive materials. Federal law requires adherence to these laboratory safety rules and techniques. If you are uncertain about safety procedures in this room, see (authorized user) in room _____ for instructions.

1. Eating, drinking, smoking and applying cosmetics are not permitted in laboratories or rooms where radioactive materials are used or stored.

2. All injuries possibly involving radioactive materials, no matter how slights, shall be monitored by the Radiation Safety Officer to determine if the wound is contaminated.

3. Special protection is required for wounds so as to prevent the entry of radioactive materials into the body through the wounds.

4. All equipment which is suspected to have come into contact with loose radioactive material shall be considered potentially contaminated and shall be monitored for contamination before being removed from the laboratory.

5. All persons who are designated to wear personnel monitoring equipment by the Radiation Safety Officer shall wear such devices at all times they work with or near radioactive materials.

6. All persons who are permitted to work with radioactive materials shall be fully aware of the procedures specified in the Radiation Safety Manual and be instructed in matter of radiation safety.

7. Radioactive material shall be used and stored (locked) in such a manner as to restrict unauthorized persons from using such material.

8. All containers for radioactive material shall be properly labeled in conformance with the standards required in Title 10, Code of Federal Regulations, Part 20 (10 CFR 20).

9. Radioactive solutions should not be pipetted by mouth.

10. Protective clothing appropriate to the conditions shall be worn at all times when working with loose radioactive materials. In all cases, disposable gloves and labor coats or aprons shall be the minimum protection required.

11. Radioisotopes shall be used in such a manner that radiation exposure rates to personnel shall be kept as small as possible, in accordance with ALARA (as low as is reasonably achievable) principles. The use of lead bricks and Plexiglas shielding, disposable gloves, aprons, "lab mat" and fume hoods will serve to minimize exposure.

12. Remote equipment (long-handled tongs, remote pipettes, etc.) shall be used routinely when handling highly radioactive materials (energies higher than 180 keV) when such materials are authorized.

To this will be added the following emergency notice:

Radioactive Spill Emergency Procedures

Minor spills (100μCi or less), which are easily contained, are to be cleaned up immediately using the "spill kit" located (location specified here). Lab mat and the paper toweling used to contaminated are to be placed in a new plastic bag and labeled with radioisotope and approximate amount of radioactivity and have a yellow radioactive sticker attached. The waste should be kept secured (locked) until disposition by the Radiation Safety Officer. A survey meter should be used to monitor the efficacy of the decontamination. However, the room is not to be used further until the Radiation Safety Officer has completed a wipe survey and performed further decontamination, if necessary.

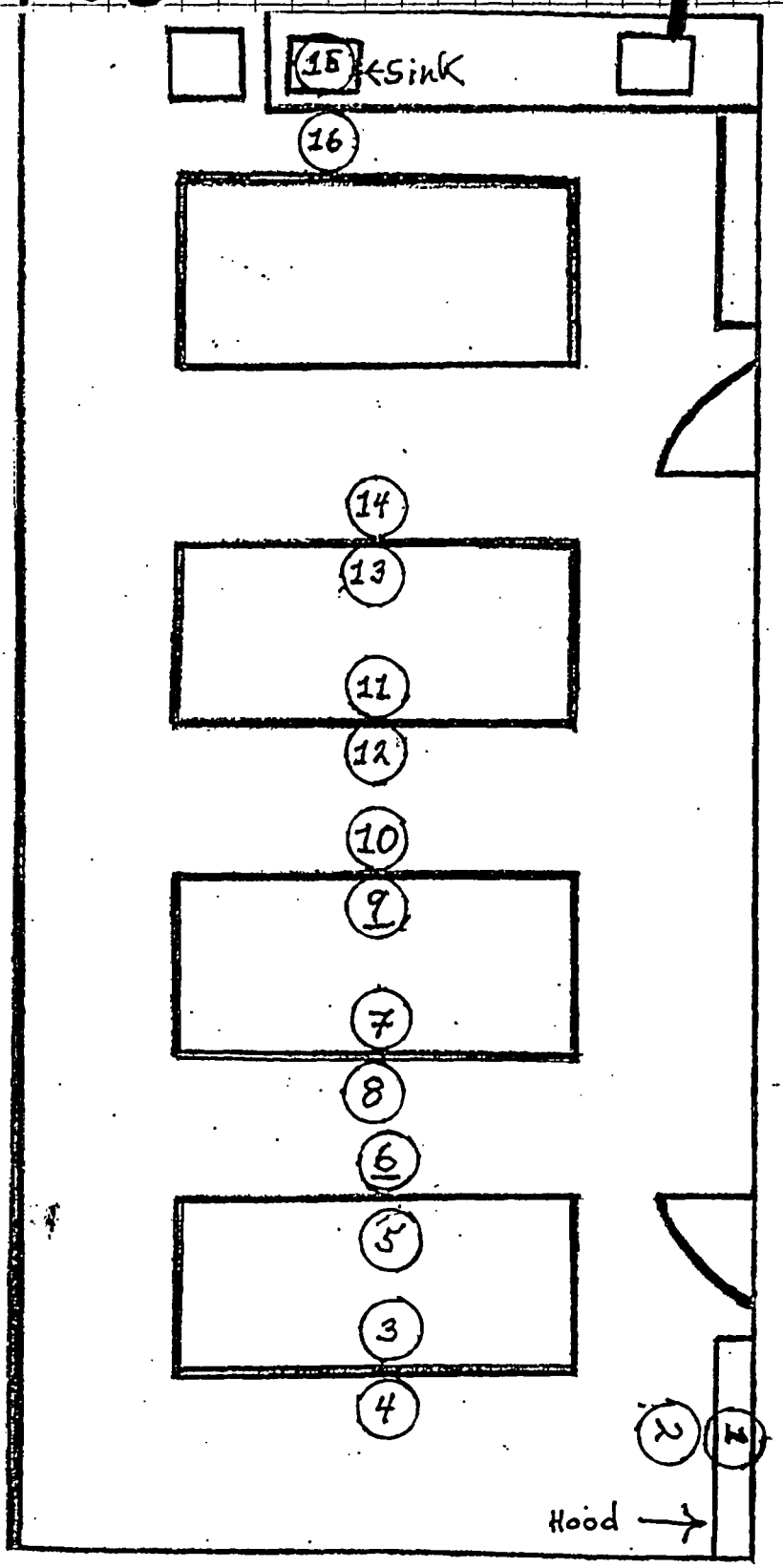
Major spills (more than 100mCi , or spillage not easily contained, such as on the floor) require immediate attention of the Radiation Safety Officer. Contain the spill as best possible with paper toweling and sodium bicarbonate from the spill kit. Secure the room by locking and posting a sign saying "Do not enter. Room contaminated with (name of Isotope). Call (number of authorized user) for information." Monitor all personnel, including shoes, for contamination before leaving the area.

Call: John A. Hufnagel
Radiation Safety Officer
458-8893 (days)
[redacted]**(evenings)**

These procedures will be posted in a prominent place and will be of large enough form to be easily noticeable. They will be laminated in plastic for durability.

Wipe Test Example

Please See Page Following



Howe 312 (Molecular Biology)

Howe, Room 312 Wipe Test. The following proceedure will be used on an annual basis at the completion of the "Nuclear Safety Laboratory" taught in Biology 215, Cell and Molecular Biology

1. **Front center of hood floor located at east end of laboratory**
2. **Floor in front of same hood**
3. **Student bench 1st from East wall, front center of bench, east side**
4. **Floor directly below #3**
5. **West side of same bench, front center of bench**
6. **Floor directly below #5**
7. **Student bench 2nd from east wall, front center of bench, east side**
8. **Floor directly below #7**
9. **West side of same bench, front center of bench**
10. **Floor directly below #9**
11. **Student bench 3rd from east wall, front center of bench, east side**
12. **Floor directly below #11**
13. **West side of same bench, front center of bench**
14. **Floor directly below #13**
15. **Double sink at south end of prep bench, sink bottoms**
16. **Floor directly below #15**

This ammended protocol will be standard operating practice as of 2003

```
Protocol #:12          Name:C-14 DPM                    26-Mar-05   10:57
Region A: LL-UL= 0.0-156.   Lcr=    0  Bkg= 0.00  %2 Sigma=0.00
Region B: LL-UL= 4.0-156.   Lcr=    0  Bkg= 0.00  %2 Sigma=0.00
Region C: LL-UL= 0.0- 0.0   Lcr=    0  Bkg= 0.00  %2 Sigma=0.00
Time =  5.00      QIP = tSIE/AEC      ES Terminator = Count
Conventional DPM
Nuclide 1 = 128000

  S#    TIME     CPMA     DPM1  tSIE  A:2S%  FLAG
   1    5.00    21.00    22.40  437.  19.51
   2    5.00    22.40    23.85  449.  18.89
   3    5.00    23.40    24.89  457.  18.49
   4    5.00    22.20    23.83  400.  18.98
   5    5.00    21.80    23.19  458.  19.15
   6    5.00    20.40    21.80  425.  19.80
   7    5.00    22.40    23.83  456.  18.89
   8    5.00    24.20    26.00  397.  18.18
   9    5.00    22.20    23.61  460.  18.98
  10    5.00    21.60    23.05  435.  19.24
  11    5.00    23.00    24.47  457.  18.65
  12    5.00    22.60    24.18  420.  18.81
  13    5.00    22.80    24.26  456.  18.73
  14    5.00    20.80    22.24  422.  19.61
  15    5.00    32.20    34.44  421.  15.76
  16    5.00    24.20    25.92  414.  18.18
```

"Wipe Test" survey of Howe 312 following Laboratory 12, Radiation Safety Workshop. 3/26/05

John D. Hufnagel R.S.O.

Attachment Six: Certification Documents for Sources, Dosimetry Program and Instrument Calibration

Duratek

CALIBRATION CERTIFICATE

Duratek Instrument Services
628 Gallaher Road
Kingston, TN 37763
Phone: (865) 376-8337
Fax: (865) 376-8331

This Certificate will be accompanied by Calibration Charts or Readings where applicable

CUSTOMER INFORMATION		INSTRUMENT INFORMATION	
Customer Name: Washington and Lee University-Dept. of Biology		Manufacturer: Bicron	
Address: 116 N. Main Street Lexington, VA 24450		Model: Surveyor M	Serial Number: A619M
Contact Name: John Hufnagel		Probe: G1LE	Serial Number: A879P
Customer Purchase Order Number: 131061	Work Order Number: 2004-01621	Calibration Method: Electronic and Source	

INSTRUMENT CALIBRATION INFORMATION

Instrument Range	Calibration Standard Value	Instrument Response		Comments
		Before Calibration	After Calibration	Calibrated in accordance with OEM Technical Manual
X1	200	200	200	Pulser: 120935 Cal Due: 04/13/05
X1	500	500	500	D-814: 2525 Cal Due: 10/22/04
X1	800	805	805	Psychron: 7480 Cal Due: 02/10/05
X10	2,000	2,000	2,000	DVM: 6565015 Cal Due: 10/14/05
X10	5,000	5,000	5,000	
X10	8,000	8,100	8,100	Temperature: 21.3°C
X100	20,000	20,000	20,000	Pressure: 744mmHg
X100	50,000	50,000	50,000	Humidity: 71%
X100	80,000	81,000	81,000	
X1000	200,000	190,000	190,000	
X1000	500,000	510,000	510,000	Audio: SAT Batt. Check: SAT
X1000	800,000	880,000	880,000	Fast/Slow: SAT Overange: SAT

EFFICIENCY DETERMINATION

Threshold: 110mV

Scale	Source ID and Value	Net CPM	Efficiency
EFF X10	I-129#040202 95,682dpm Cert date 04/13/99	15,750	16.5%
High Voltage	N/A	800V	800V

Background: 250cpm

Special Remarks: HV: 800V
I-129 Efficiency on contact: 18.4%

STATEMENT OF CERTIFICATION

We Certify that the instrument listed above was calibrated and inspected prior to shipment and that it met all the Manufacturers published operating specifications. We further certify that our Calibration Measurements are traceable to the National Institute of Standards and Technology. (We are not responsible for damage incurred during shipment or use of this instrument).

Instrument Calibrated By: [signature]

Reviewed By: [signature] Date: 6-25-04

Calibration Date: 06/25/04

Calibration Due: 06/25/05

ISOTOPE PRODUCTS LABORATORIES 1800 NORTH KEYSTONE ST. BURBANK, CA. 91504
(818) 843-7000 FAX (818) 843-6168

Nominal Source Data Sheet

Customer: WASHINGTON AND LEE UNIVERSITY P.O. No. P0063763 Date: JUNE 8 1990

Catalog No. GF-060 Quantity: 1

Capsule Type: M

Nature of Active Deposit: EVAPORATED METALLIC SALTS

Active Diameter/Weight: 5mm

Backing: 9.23 mg/cm² KAPTON

Cover: 0.254mm ALUMINIZED MYLAR

Isotope	Source No.	Activity	Date
Co60	359-47-1	20 μCi	JUNE 1, 1990

Remarks: LEAK TEST CERTIFICATE ATTACHED

LEAK TEST CERTIFICATE

CUSTOMER Washington And Lee University P.O.# P0063763

CATALOG # GF-060 CAPSULE TYPE M

RADIONUCLIDE Co 60 NOMINAL ACTIVITY 20 μCi

SERIAL NUMBER 359-47-1 NUMBER OF UNITS 1

THE LEAK TESTS INDICATED BY THE CHECKED BOXES WERE APPLIED TO DETERMINE THE INTEGRITY OF THE SOURCE(S) IN THIS SHIPMENT.

☑ **1. STANDARD WIPE TEST**

The source is swabbed over its entire surface with a moistened paper disc. After being allowed to dry, the swab is checked for activity using a windowless proportional counter or end-window G.M. tube. Activity levels exceeding 0.001 microcuries beta-gamma or 0.0001 microcuries alpha will be cause for rejection.

Measured Activity: < 0.001 μCi beta-gamma ~~μCi alpha~~

☐ **2. SOAK TEST**

The source is immersed in distilled water and maintained at 50°C ± 10°C for a minimum of four hours. After removal of the source the liquid is evaporated in a planchet and the residue is checked for activity using a windowless proportional counter or end window G.M. tube. Activity levels exceeding 0.001 microcuries beta-gamma or 0.0001 microcuries alpha will be cause for rejection.

Measured Activity: μCi beta-gamma μCi alpha

☐ **3. GAS SOURCE TEST (Radioactive Gasses)**

The source is placed in a vacuum desiccator and is maintained at less than 1 mm Hg for not less than 12 hours. Air is introduced into the desiccator and is monitored with an end window G.M. tube. Readings exceeding 1000 CPM will be cause for rejection of the source.

☐ **4. OTHER LEAK TEST**

_____

_____

_____

☐ **5. LEAK TEST NOT APPLICABLE**

The active area of this source is uncovered or is protected by a very thin coating. Although the deposit is adherent, it is not designed or certified to pass a standard leak test. The inactive portions of the source have been checked using the standard wipe test and found not to exceed 0.001 microcuries of removable activity at time of shipment.

June 8, 1990
Date

Signature

ISOTOPE PRODUCTS LABORATORIES
Burbank, California 91504
(818) 843-7000

Attachment Seven: Exposure of Unmonitored Individuals

This attachment details the protocols of Laboratories # 11 & 12 of Biol 215 – Biochemistry of the Cell. This lab is taught once a year and is the only time that students and unmonitored personnel of any kind are subjected to radioactivity from isotopes under this license.

Biol 215 - Biochemistry of the Cell
Laboratory # 11 & 12: Radiation Safety Workshop

Session One: Nuclear and Health Physics

I. The Purpose of radiotracers in biology

II. Radioactive isotopes of use; nuclear disintegration products; half lives
- a) alpha particles (atomic radiation; thorium)
- b) beta particles (^{3}H [tritium], ^{14}C, ^{45}Ca, ^{32}P)
- c) gamma rays (^{125}I, ^{131}I)

III. Liquid scintillation counting
- a) the general principle
- b) scintillation cocktails
  - 1) aromatic solvent
  - 2) solutes (PPO, POPOP)

IV. Units of measurement
- a) the curie (2.2×10^{12} dpm)
- b) the dpm vs. the cpm
- c) specific activities (dpm/unit material)
- d) the unit of dosage (the rem)

V. Counting efficiencies
- a) the problem of quenching
  - 1) color quenching
  - 2) chemical quenching
- b) external standardization

VI. Radioisotope dilution calculations

A scientist needs to study the rate of chitin (poly-N-acetyl glucosamine) synthesis in crayfish from the Mississippi delta. The crayfish are in polluted waters and are not growing at expected rates; it might be that some pollutant is affecting exoskeletal synthesis which, in turn, affects the total growth of the animal. Each young captured crayfish is staged (age is estimated), weighed, and, during a period when a new exoskeleton is being synthesized just before molting), injected into the hemocoel with 1000 dpm of N-acetyl glucosamine/gram weight. After allowing 48 hours for incorporation of the radiolabeled precursor into the new exoskeleton, the newly synthesized chitin is extracted and its specific activity is determined. Assuming the experimental design is valid, answer the following questions and bring your calculation with you to radiation lab next week.

1) How many μCi of ^{14}C are present in the vial? (See label on the next page for required information)
2) How many μCi will be injected into a 10gram crayfish?

3) How many μmol of N-acetyl glucosamine are being injected into a 10 gram crayfish?
4) What would be a proper control(s) for this experiment?
5) If the adult, non-pregnant scientist accidentally drank all of the contents of the vial, would the annual limit of intake be exceeded? (Note: I becquerel (Bq) equals 1 dps)

Session Two: Radioisotope Dilution Lab Exercise stock = 7.5 μCi

This week you will use a solution of ^{14}C – N-acetylglucosamine to practice the commonly used technique of serial dilution as well as the proper handling of radionuclides; in addition, you will be able to determine the level of pipetting skill that you have gained during our laboratories in cell & molec.

1) Using a micropipeter, measure out 100 μL of stock ^{14}C into a scintillation vial; set up 4 other vials, Do not write on the vials; the caps may be written upon, however. (100 μL stock = 2.2×10^4 dpm)

2) Add 900 μL 5% ethanol (5:95/v:v) to each of the vials; cap and vortex carefully!

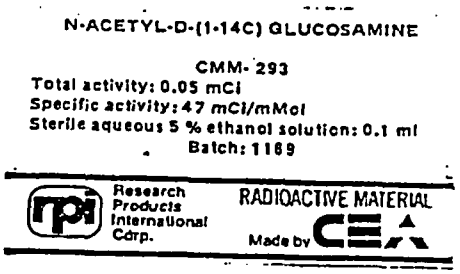
3) Initiate a "serial dilution" by transferring 100 μL from vial 1 to vial 2; cap and vortex.

4) Transfer 100 μL from vial 2 to vial 3, cap and vortex, then repeat the procedure until all the vials are done. What is the dilution ratio in this series?

5) Add 5.0 mL of scintillation fluid to each vial using the autopipeter at the public bench; cap and vortex until there is no phase separation. The instructor will show you how to count the samples and you will soon receive your counting printouts.

6) Your report should be abbreviated; answer: (a) what was the original (stock) dpm/mL (specific activity)? (b) what are the number of curies in 1.0 ml of stock solution? (c) what is the 'r' value of your pipetting? Your report should have an introduction, the present sheets (as Materials and Methods), the scintillation counter printout (as data), short calculations for specific activity and a plot of dpm vs. vial # as Analysis, and a one (1) sentence discussion followed by the Reference section. **Please attach your "homework" calculations from the first week of this exercise to the end of the report.**

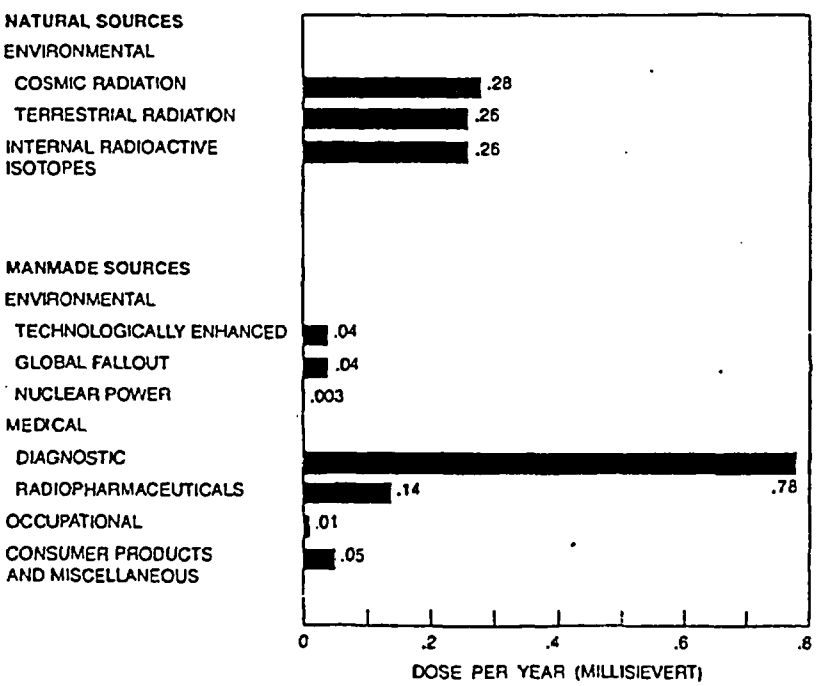
Technique is critical: use gloves and aprons; follow contamination avoidance procedures as specified by the NRC regulations!!!



The Unit of Radiation Dosage

The curie is the most often used unit of radiation. It is equal to 2.2×10^{12} disintegrations per minute (dpm). This unit measures the quantity of the radioactivity, but does not tell of the relative danger of each particle or ray to a human.

Different units are used to indicate the biological effects of different radiation, called the absorbed dose. The SI unit for absorbed dose is the gray (Gy) and it is equal to 1J/kg; this unit replaces the rad; 1 rad = 100 erg/g. Another modification takes into account the severity of the absorbed dose by factoring in the "Q" factor, which varies for radiation type. Most radiation a normal person contacts has a Q of 1, so it doesn't figure into assessment of radiation dose. This leads to the old system of dose, the rem (for roentgen equivalent man), or rads x Q. A newer unit is the sievert (Sv) wherein 1 Sv = 100 rem. The recommended dose limit for the general public is 5 mSv/year or 0.5 rem/year. Professionals, miners, etc. working around radioactivity routinely are limited to ten times that amount. The medical community still uses the rem unit; for example, the typical X-ray is about 78 mrem, or .78 Sv. See the table below for typical radiation exposures in the US.



RADIATION EXPOSURE of a typical person in the U.S. from natural and manmade sources is represented in millisieverts. One sievert is the amount of any radiation equal in biological effect to 100 rads (a rad is 100 ergs per gram of tissue) of gamma rays. The total average dose rate per year is .8 millisievert from natural sources and 1.06 millisieverts from manmade sources. A typical dental X ray delivers about one millisievert to the center of the cheek.

From: Chapman & Aspey. The Use of Radioactive Isotopes in the Life Sciences. Allen & Unwin (Brotan) 1981.

3 Radiation protection and safe handling of radioactive materials

3.1 Nature of the hazards

When ionizing radiations pass through living tissue they can cause extensive damage. The hazard is particularly insidious since man has no sense capable of detecting radiation even at very high intensities. Madame Curie's husband was probably the first person who knowingly received a 'radiation burn', and the early experimenters with X-rays soon realized the harmful nature of such ionizing radiations. It has been estimated that by 1922 around 100 X-radiologists had died as a direct result of their work and it was around that time that the first moves to specify safety recommendations were made. Much other evidence concerning the harmful nature of radioactive materials has since been collected. For example: from girls working with luminous paint who licked their brushes and subsequently developed mouth and bone cancer; from miners digging radioactive ores and working in high levels of radon gas who subsequently developed lung cancers; from patients given large doses of X-rays for therapeutic purposes who subsequently developed leukaemia and other forms of cancer; and of course from the survivors of the two atomic bombs which ended World War II.

Somatic effects (i.e. damage to the exposed individual) are well documented and range from minor burns, dermatitis, ulcers, diarrhoea, hair loss and blood changes to most forms of cancer and eventual death. Some somatic effects such as carcinogenesis (production of cancer) are known as **stochastic effects** since the probability of the effect occurring is regarded as a function of dose without threshold (i.e. there is no evidence at present of a 'minimum dose'). **Non-stochastic effects** are those such as cataract of the eye lens, non-malignant damage to the skin, blood deficiencies, etc., for which the severity of the effect varies with dose and for which there is a threshold below which no detrimental effects are seen.

Hereditary effects are those which become manifest in the descendants of the exposed person. Studies with irradiated insects (*Drosophila*) and mammals (mice and rats) have shown that

radiation-induced gene and chromosome mutations can cause a wide range of malformations of organs and tissues in the offspring. In man, the available evidence is very limited but, in general, studies on the children of groups of people who have been subjected to radiation have revealed no obvious genetic effects. Nevertheless, the results with animals suggest that at present it would be wise to view hereditary effects as stochastic and to accept that there is no threshold dose.

3.2 The development of radiation protection

Once the hazards had been clearly recognized, radiation workers organized themselves to formulate safeguards and have consistently led the field with regard to the safety of individuals and populations. The International Commission on Radiological Protection (ICRP) was formed in 1928 and was making specific recommendations long before national governments became involved in radiation health and safety legislation. Health physicists and radiation protection officers are respected members of the scientific community and their vigilance has ensured that the 'nuclear and isotope industry' and medical and biological uses of radiation and isotopes are amongst the safest fields of endeavour in which to be employed.

The current philosophy of the ICRP is set out in their Publication 26 (1977). They state that: 'The aim of radiation protection should be to prevent detrimental non-stochastic effects and to limit the probability of stochastic effects to levels deemed to be acceptable.' To this end various units and concepts have been defined and specific recommendations made.

3.3 The concept of radiation dose

In pharmacology the word 'dose' is used to indicate the amount of a substance per unit body weight which will produce a certain biological effect. As radiation produces biological effects, the term 'dose' has been adopted by analogy, but there the similarity ends, since radiation dose is defined in purely physical terms as the amount of energy absorbed per unit weight of tissue. The SI unit of absorbed dose is the gray:

$$1\,\text{Gy} = 1\,\text{J kg}^{-1}$$

The unit replaces the older unit the rad (1 rad = 100 erg g^{-1}). Since many instruments calibrated in rads will continue to be used for a long time, the relationship between the two units should be noted:

$$1\text{ rad} = 10\text{ mGy}$$

100 rad = 1 Gy

In practice it has been found that the absorbed dose alone is insufficient to predict either the severity or the probability of the deleterious effects. Some radiations are more damaging than others and hence ICRP recommends introduction of a quality factor, Q, as set out in Table 3.1.

Table 3.1 Values of quality factor used in defining dose equivalents.

Particles	Q
X-rays, γ-rays and electrons	1
neutrons, protons, etc., of unknown energy	10
α-particles and other multiply-charged particles of unknown energy	20

Q is used to calculate the dose equivalent:

$$\text{dose equivalent} = \text{absorbed dose} \times Q$$

Under the old system of units, the roentgen equivalent man or rem was defined as the number of rads $\times Q$. The SI unit of dose equivalent is the sievert (Sv), where

$$1 \text{ rem} = 10 \text{ mSv (or } 1 \text{ Sv} = 100 \text{ rem)}$$

When attempting to assess a radiation hazard it is essential to know the rate at which radiation is being absorbed, and hence we have the concept of dose rate, which is usually expressed as dose per hour, i.e. microsieverts per hour, micrograys per hour, rads per hour, etc.

3.4 Recommended dose equivalent limits

To prevent non-stochastic effects and to limit stochastic effects to acceptable levels, the ICRP recommends, for radiation workers, an annual dose equivalent limit for uniform irradiation of the whole body of 50 mSv (5 rem), although individual parts of the body may receive higher doses. The recommended dose limit for members of the general public is 5 mSv (0.5 rem) per year. Bearing in mind the very limited opportunities for individual exposure the average dose to the population as a whole will be extremely small. Despite these upper limits, there is an over-riding philosophy that doses should be kept as low as is readily achievable, and in practice very few radiation workers ever receive anything like the permissible dose.

3.5 Secondary limits and derived limits

Sources of radiation external to the body can readily be monitored with suitable instruments and the potential dose equivalent assessed.

However, when open sources of radioisotopes are used there is the possibility of ingestion into the body. The subsequent assessment of dose is greatly complicated by the uneven distribution and metabolism of the material. To meet these difficulties the ICRP publishes a list of **annual limits of intake** (ALI) by inhalation or ingestion. These are secondary limits designed to ensure that the internal dose does not exceed the recommended dose equivalent limit. Some examples are given in the list of nuclides in Appendix B.

Finally, by taking account of the water consumed, or the air breathed, in a 'working year' it is possible to calculate derived limits such as the maximum permissible concentrations in air or in drinking water. By making certain other assumptions regarding the physical transfer of activity from, for example, bench surfaces into the air or on to the hands and hence into the mouth, it is possible to calculate maximum permissible levels of surface contamination in a radioisotope laboratory such that the dose equivalent limit cannot be exceeded. The current British National Radiological Protection Board (NRPB) recommendations are set out in Table 3.2 overleaf.

3.6 Radiation protection and student experiments

ICRP Publication 13 (1968) deals specifically with radiation protection in schools for pupils up to the age of 18 years. The recommendations are entirely suitable for adoption for undergraduate class experiments in universities. For tracer experiments it is recommended that the amount of activity used per experiment should never exceed the limit for ingestion or inhalation, whichever is the less, by a member of the public in a year. Some typical examples are given in Appendix B. Almost all suitable experiments can be carried out with activities much smaller than those listed, and if activities are kept to the practical minimum then the hazards are virtually negligible. Nevertheless, good working practice will further ensure safety and this is dealt with in the final section of this chapter.

3.7 Radiation monitors

For assessing the radiation emanating from a source or for measuring surface contamination, portable versions of the ionization chambers, Geiger–Müller counters or scintillation counters described in Chapter 4 are used. For weak β-particle emitters such as ^{14}C or ^{35}S a Geiger–Müller counter with a very thin mica window is required. Surface contamination by tritium can only be detected by wiping the surface with tissue paper moistened with solvent. The 'wipe' is then

Biochemistry of the Cell 215: Sample Scintillation Counter Printout

The printout of your data from the Packard CA 2200 scintillation counter will appear as below. **It is necessary to understand what is meant by "2S%" or two sigma percent, so that one can calculate the standard deviation of the dpm of each sample.** Notice that limits of confidence become larger (one is LESS certain) as the dpm drop.

Sample scintillation counter printout

```
Protocol #: 3        Name:Cell Biology              20-Mar-90   10:06
Region A: LL-UL= 0.0-156.   Lcr=    0  Bkg= 0.00  %2 Sigma=0.00
Region B: LL-UL= 4.0-156.   Lcr=    0  Bkg= 0.00  %2 Sigma=0.00
Region C: LL-UL= 0.0- 0.0   Lcr=    0  Bkg= 0.00  %2 Sigma=0.00
Time =  5.00      QIP = tSIE/AEC      ES Terminator = Count
1989 Laboratories
Conventional DPM
Nuclide 1 = 127700

  S#   TIME     CPMA     DPM1      SIS tSIE A:2S%
   1   5.00 104341. 107695. 160.72 985.   0.27
```

2 standard deviations are 0.27% of the dpm.

$\therefore \frac{(107695)(.0027)}{2}$ = standard deviation

$\therefore$ dpm = 107695 ± 145 (SD)

EXPOSURE RECORD INFO
LABORATORIES 11 & 12, RADIATION SAFETY WORKSHOP
BIO. 215: BIOCHEMISTRY OF CELL

- LARGEST QUANTITIY HANDLED IS
  75 mL of ^{14}C with 2.2×10^5 dpm/mL
- $(2.2 \times 10^5)\ (75) = 1.65 \times 10^7$ dpm
- $\left(\frac{1.65 \times 10^7}{2.2 \times 10^2}\right)$ Ci $= 7.5 \times 10^{-6}$ Ci $= 7.5\ \mu$Ci

or

convert to dpm/mL

$$\left(\frac{2.2 \times 10^4 \text{ dpm}}{100\mu\text{L}}\right) = \frac{x}{1000\mu\text{L}}$$

$$100\,x = 2.2 \times 10^7 \text{ dpm}$$

$$x = 2.2 \times 10^5 \text{ dpm}/1000\mu\text{L} \quad (\text{or mL})$$

calculate amt. in 75 mL

$$(2.2 \times 10^5 \text{ dpm/mL})\ (75\text{mL}) = 1.65 \times 10^7 \text{ dpm}/75\text{mL}$$

$$\left(\frac{1.65 \times 10^7 \text{ dpm}}{2.2 \times 10^{12} \text{ dpm}}\right) \text{Ci} = \underline{7.5 \times 10^{-6} \text{ Ci}}$$

or <u>7.5 μCi</u>

<u>This is 1.5% of the 500 μCi limit.</u> -- This is the maximum student exposure in this laboratory, occurring once annually.

Appendix B

Radiation protection data

Radio-isotope	*Toxicity class*†	*Annual limit of intake*‡ *(Bq)*		*γ-dose rate at 1 m* § *(μGyh⁻¹ MBq⁻¹)*	*Maximum activity per student experiment*‖	
		Oral	*Inhalation*		*(Bq)*	*(μCi) (approx.)*
^{3}H	4	3×10^{9}¶	3×10^{9}¶	—	3×10^{8}¶	8×10^{3}¶
^{14}C	3	(2×10^{8})	(4×10^{8})	—	2×10^{7}	500
^{22}Na	2	2×10^{7}	2×10^{7}	0.30	2×10^{6}	50
^{24}Na	3	1×10^{8}	2×10^{8}	0.47	1×10^{7}	250
^{32}P	3	2×10^{7}	1×10^{7}	—	1×10^{6}	25
^{35}S	3	2×10^{8}	8×10^{7}	—	8×10^{6}	200
^{36}Cl	2	6×10^{7}	9×10^{6}	—	9×10^{5}	25
^{42}K	3	2×10^{8}	2×10^{8}	0.034	2×10^{7}	500
^{45}Ca	2	6×10^{7}	3×10^{7}	—	3×10^{6}	80
^{51}Cr	3	1×10^{9}	7×10^{8}	0.004	7×10^{6}	150
^{54}Mn	2	7×10^{7}	3×10^{7}	0.118	3×10^{6}	80
^{55}Fe	3	3×10^{8}	7×10^{7}	—	7×10^{6}	150
^{59}Fe	3	3×10^{7}	1×10^{7}	0.160	1×10^{6}	25
^{57}Co	3	2×10^{8}	2×10^{7}	0.024	2×10^{6}	50
^{60}Co	2	7×10^{6}	1×10^{6}	0.334	1×10^{5}	2.5
^{64}Cu	3	4×10^{8}	8×10^{8}	0.030	4×10^{7}	1000
^{65}Zn	3	1×10^{7}	1×10^{7}	0.078	1×10^{6}	25
^{82}Br	3	1×10^{8}	1×10^{8}	0.367	1×10^{7}	250
^{90}Sr	1	1×10^{6}	1×10^{5}	—	1×10^{4}	0.25
^{113}Sn	3	(2×10^{7})	(4×10^{7})	0.067	2×10^{6}	50
^{125}I	2	1×10^{6}	2×10^{6}	0.034	1×10^{5}	2.5
^{131}I	2	1×10^{6}	2×10^{6}	0.051	1×10^{5}	2.5
^{137}Cs	2	4×10^{6}	6×10^{6}	0.083	4×10^{5}	10
^{198}Au	3	4×10^{7}	4×10^{7}	0.061	4×10^{6}	100

†Taken from *A basic toxicity classification of radionuclides*. Vienna: International Atomic Energy Agency, 1963.

‡Taken from *Limits for intakes of radionuclides by workers*, ICRP Publication 30, Part 1, *Annals of the ICRP* 2(3/4). 1979; Part 2, *Annals of the ICRP* 4 (3/4), 1980. Figures in brackets calculated from the data in *Handling, storage, use and disposal of unsealed radionuclides in hospitals and medical research establishments*, ICRP Publication 25, *Annals of the ICRP*, 1(2), 1977: these figures are subject to reappraisal by the ICRP and revised recommendations are to be published.

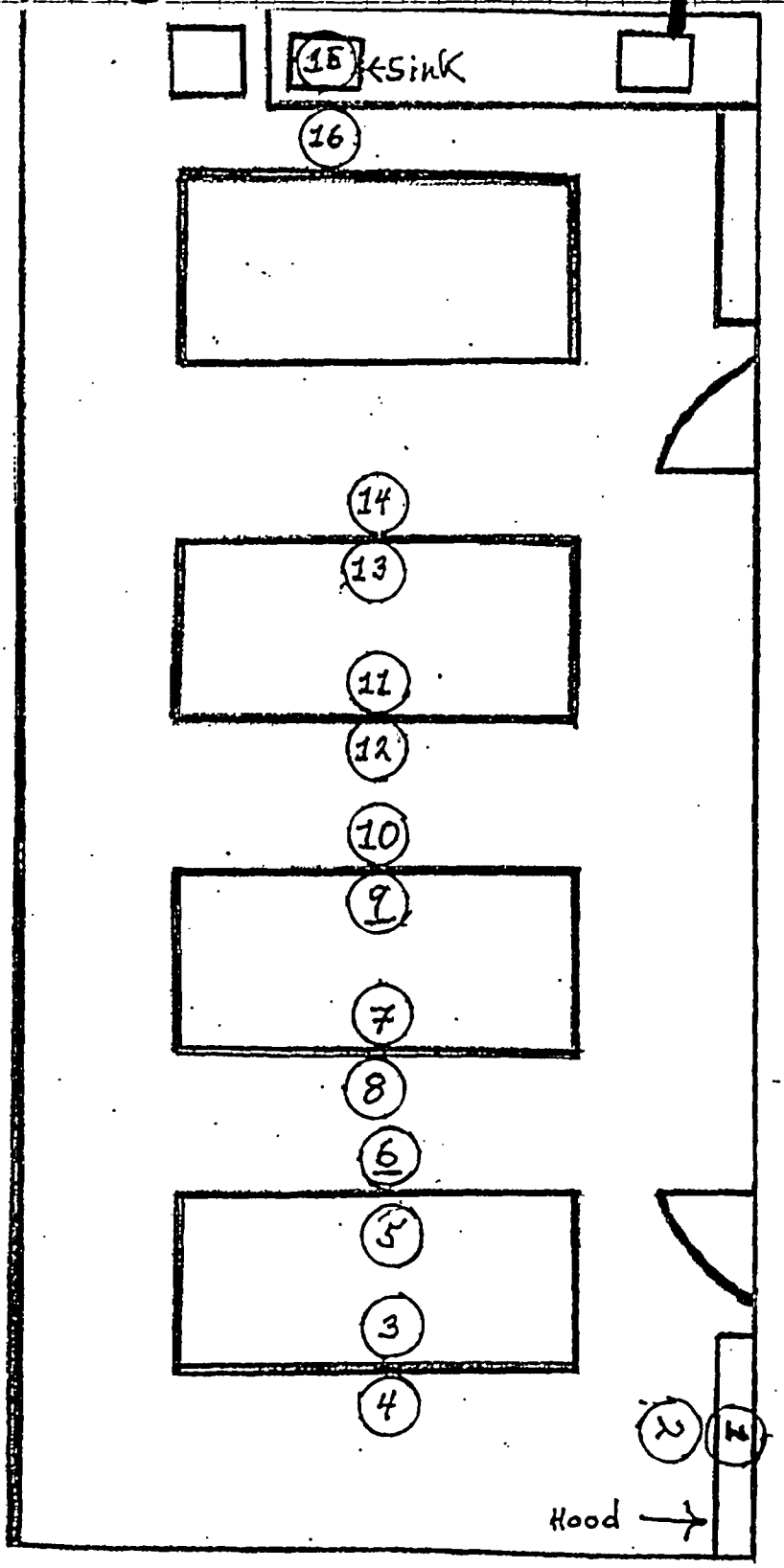
§Calculated from data in ICRP Publication 25 (see above).

‖Calculated according to the recommendations in *Radiation protection in schools*. ICRP Publication 13. Oxford: Pergamon Press, 1970.

¶This value is for tritiated water. For organic compounds such as tritiated thymidine the value should be reduced to one fiftieth of the quoted value.

Wipe Test Example

Please See Page Following



Howe 312 (Molecular Biology)

Howe, Room 312 Wipe Test. The following proceedure will be used on an annual basis at the completion of the "Nuclear Safety Laboratory" taught in Biology 215, Cell and Molecular Biology

1. **Front center of hood floor located at east end of laboratory**
2. **Floor in front of same hood**
3. **Student bench 1st from East wall, front center of bench, east side**
4. **Floor directly below #3**
5. **West side of same bench, front center of bench**
6. **Floor directly below #5**
7. **Student bench 2nd from east wall, front center of bench, east side**
8. **Floor directly below #7**
9. **West side of same bench, front center of bench**
10. **Floor directly below #9**
11. **Student bench 3rd from east wall, front center of bench, east side**
12. **Floor directly below #11**
13. **West side of same bench, front center of bench**
14. **Floor directly below #13**
15. **Double sink at south end of prep bench, sink bottoms**
16. **Floor directly below #15**

This ammended protocol will be standard operating practice as of 2003

Protocol #:12 Name:C-14 DPM 26-Mar-05 10:57
Region A: LL-UL= 0.0-156. Lcr= 0 Bkg= 0.00 %2 Sigma=0.00
Region B: LL-UL= 4.0-156. Lcr= 0 Bkg= 0.00 %2 Sigma=0.00
Region C: LL-UL= 0.0- 0.0 Lcr= 0 Bkg= 0.00 %2 Sigma=0.00
Time = 5.00 QIP = tSIE/AEC ES Terminator = Count
Conventional DPM
Nuclide 1 = 128000

S#	TIME	CPMA	DPM1	tSIE	A:2S%	FLAG
1	5.00	21.00	22.40	437.	19.51	
2	5.00	22.40	23.85	449.	18.89	
3	5.00	23.40	24.89	457.	18.47	
4	5.00	22.20	23.83	400.	18.98	
5	5.00	21.80	23.19	458.	19.15	
6	5.00	20.40	21.80	425.	19.80	
7	5.00	22.40	23.83	456.	18.09	
8	5.00	24.20	26.00	397.	18.18	
9	5.00	22.20	23.61	460.	18.98	
10	5.00	21.60	23.05	435.	19.24	
11	5.00	23.00	24.47	457.	18.65	
12	5.00	22.60	24.18	420.	18.81	
13	5.00	22.80	24.26	456.	18.73	
14	5.00	20.80	22.24	422.	19.61	
15	5.00	32.20	34.44	421.	15.76	
16	5.00	24.20	25.92	414.	18.18	

"Wipe Test" survey of Howe 312 following Laboratory 12, Radiation Safety Workshop. 3/26/05
John D. Hufnagel R.S.O.

This is to acknowledge the receipt of your letter/application dated

3/29/2005, and to inform you that the initial processing which includes an administrative review has been performed.

Renew 45-25034-01

☒ There were no administrative omissions. Your application was assigned to a technical reviewer. Please note that the technical review may identify additional omissions or require additional information.

☐ Please provide to this office within 30 days of your receipt of this card

---

A copy of your action has been forwarded to our License Fee & Accounts Receivable Branch, who will contact you separately if there is a fee issue involved.

Your action has been assigned **Mail Control Number** 136921.
When calling to inquire about this action, please refer to this control number.
You may call us on (610) 337-5398, or 337-5260.

NRC FORM 532 (RI)
(6-96)

Sincerely,
Licensing Assistance Team Leader

BETWEEN:

License Fee Management Branch, ARM
and
Regional Licensing Sections

(FOR LFMS USE)
INFORMATION FROM LTS

Program Code: 03620
Status Code: 2
Fee Category: EX 3M
Exp. Date: 20050531
Fee Comments: 170.11(A)(4)
Decom Fin Assur Reqd: N

LICENSE FEE TRANSMITTAL

A. REGION I

1. APPLICATION ATTACHED
   Applicant/Licensee: WASHINGTON AND LEE UNIVERSITY
   Received Date: 20050421
   Docket No: 3030971
   Control No.: 136921
   License No.: 45-25034-01
   Action Type: Renewal

2. FEE ATTACHED
   Amount: /
   Check No.: /

3. COMMENTS

Signed Rebecca Jurcord
Date 4/28/2005

B. LICENSE FEE MANAGEMENT BRANCH (Check when milestone 03 is entered /__/)

1. Fee Category and Amount: _____

2. Correct Fee Paid. Application may be processed for:
   Amendment _____
   Renewal _____
   License _____

3. OTHER _____
   _____

Signed _____
Date _____