NRC FORM 313
(9-88)
10 CFR 30, 32, 33, 34, 35 and 40

U.S. NUCLEAR REGULATORY COMMISSION
APPROVED BY OMB
3150-0120
Expires: 6-30-90

APPLICATION FOR MATERIAL LICENSE

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.

APPLICATIONS FOR DISTRIBUTION OF EXEMPT PRODUCTS FILE APPLICATIONS WITH:

U.S. NUCLEAR REGULATORY COMMISSION
DIVISION OF INDUSTRIAL AND MEDICAL NUCLEAR SAFETY, NMSS
WASHINGTON, DC 20555

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:

U.S. NUCLEAR REGULATORY COMMISSION, REGION I
NUCLEAR MATERIALS SAFETY SECTION B
475 ALLENDALE ROAD
KING OF PRUSSIA, PA 19406

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

U.S. NUCLEAR REGULATORY COMMISSION, REGION II
NUCEAR MATERIALS SAFETY SECTION
101 MARIETTA STREET, SUITE 2900
ATLANTA, GA 30323

IF YOU ARE LOCATED IN:

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

U.S. NUCLEAR REGULATORY COMMISSION, REGION III
MATERIALS LICENSING SECTION
799 ROOSEVELT ROAD
GLEN ELLYN, IL 60137

ARKANSAS, COLORADO, IDAHO, KANSAS, LOUISIANA, MONTANA, NEBRASKA, NEW MEXICO, NORTH DAKOTA, OKLAHOMA, SOUTH DAKOTA, TEXAS, UTAH, OR WYOMING, SEND APPLICATIONS TO:

U.S. NUCLEAR REGULATORY COMMISSION, REGION IV
MATERIAL RADIATION PROTECTION SECTION
611 RYAN PLAZA DRIVE, SUITE 1000
ARLINGTON, TX 76011

ALASKA, ARIZONA, CALIFORNIA, HAWAII, NEVADA, OREGON, WASHINGTON, AND U.S. TERRITORIES AND POSSESSIONS IN THE PACIFIC, SEND APPLICATIONS TO:

U.S. NUCLEAR REGULATORY COMMISSION, REGION V
NUCLEAR MATERIALS SAFETY SECTION
1450 MARIA LANE, SUITE 210
WALNUT CREEK, CA 94596

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 JURISDICTION.

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

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

2. NAME AND MAILING ADDRESS OF APPLICANT *(Include Zip Code)*

East Lansing Laboratory
Michigan Department of State Police
714 South Harrison Road
East Lansing, MI 48823

3. ADDRESS(ES) WHERE LICENSED MATERIAL WILL BE USED OR POSSESSED.

East Lansing Laboratory
Michigan Department of State Police
714 South Harrison Road
East Lansing, MI 48823

9001260039 890505
REG3 LIC30
21-26049-01 PDR

4. NAME OF PERSON TO BE CONTACTED ABOUT THIS APPLICATION

Warren Malchman

TELEPHONE NUMBER: 517/355-0153

SUBMIT ITEMS 5 THROUGH 11 ON 8½ 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.
see enc.

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

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

8. TRAINING FOR INDIVIDUALS WORKING IN OR FREQUENTING RESTRICTED AREAS.
see enc.

9. FACILITIES AND EQUIPMENT.
see enc.

10. RADIATION SAFETY PROGRAM.
see enc.

11. WASTE MANAGEMENT.
see enc.

12. LICENSEE FEES *(See 10 CFR 170 and Section 170.31)*
FEE CATEGORY: exempt
AMOUNT ENCLOSED: $0.00

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

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, 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.

SIGNATURE--CERTIFYING OFFICER	TYPED/PRINTED NAME	TITLE	DATE
Donald L Collins	Donald L. Collins, Lt.	Lab. Director	4-19-89

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TYPE OF FEE	FEE LOG	FEE CATEGORY
APP	APP/A	E 3P

COMMENTS: FEE EXEMPT 170.11(a)(4) OK

AMOUNT RECEIVED	CHECK NUMBER

APPROVED BY: OK 4/25/89

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RECEIVED
APR 24 1989
REGION III

DATE

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ESTIMATED BURDEN PER RESPONSE TO COMPLY WITH THIS INFORMATION COLLECTION REQUEST: 7.5 HRS. FORWARD COMMENTS REGARDING BURDEN ESTIMATE TO THE RECORDS AND REPORTS MANAGEMENT BRANCH (P-530), U.S. NUCLEAR REGULATORY COMMISSION, WASHINGTON, DC 20555, AND TO THE OFFICE OF INFORMATION AND REGULATORY AFFAIRS, OFFICE OF MANAGEMENT AND BUDGET, WASHINGTON, DC 20503.

Item 5 - Radioactive Material Requested

a. Element and Mass Number
1. Sulphur-35 (S-35)
2. Phosphorus-32 (P-32)

b. Chemical and/or Physical Form
S-35 and P-32, Labeled nucleotides as triethylammonium salts in aqueous solution.

c. Maximum amount which will be possessed at any one time.
P-32 40 millicuries
S-35 40 millicuries

Item 6 - Purpose(s) For Which Licensed Material Will Be Used

Forensic DNA typing. See enclosed Item 6a. "Statement of Technical Need". See Item 6b. "Techniques For Labelling Nucleic Acids".See Item 6c "Procedures For The Detection Of Restriction Fragment Length Polymorphisms In Human Use"

Item 7 - Individual(s) Responsible For The Radiation Safety Program And Their Training and Experience.

See item 7 "Radioactive Material Experience". At the present time only Charles Barna and Julie A. Howenstein will be using the radioactive materials. Charles Barna will serve as Radiation Safety Officer. Additional personnel will be added by a license amendment application to the NRC.

Item 8 - Training For Individuals Working In Or Frequenting Restricted Areas

The enclosed items will be used as training materials for individuals working in or frequenting restricted areas. Training will be in accordance with 10 CFR 19.12 requirements.

a. "Michigan State Police Laboratories Radiation Safety Manual"

b. "Radiation Safety Training"

c. "MSU Radiation Safety Study Guide"

Item 9 - Facilities and Equipment

See enclosed Drawings A, B, C, and D and personnel monitoring, portable survey meter and P-32 shielding referenced in Item 10 below. The identified facilities will be secured and locked when not in use.

Item 10 - Radiation Safety Program

See enclosed "Michigan State Police Laboratories Radiation Safety Manual". In particular, note section "Phosphorus-32 Handling Precautions". The radiation safety program will be in accordance with 10 CFR Parts 19 and 20 requirements.

Film Badges -
Whole body film badges will be issued on a monthly basis to personnel utilizing P-32. Additionally, extremity ring badges will be issued on a monthly basis to personnel handling

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P-32 in millicurie quantities. The film badge firm to be used will be R. S.Landauer Jr. & Co.

Portable Survey Meter -
A Ludlum Model 3 with a Ludlum Model 44-9 "pancake" GM detector will be used for S-35 and P-32 monitoring. The unit will employ a standard dial 0-5K CPM with multiplier ranges of x0.1, x1, x10, and x100. The unit will be calibrated at yearly intervals, or after instrument repair, by Warren Malchman, Director and Radiation Safety Officer, Office of Radiation, Chemical & Biological Safety, Michigan State University in the same manner as calibrations are performed for the University NRC broad license No. 21-00021-29. (The percent efficiency of the detector for S-35 and P-32 will determined using NBS traceable beta reference standards from the New England Nuclear Corp.). The approximate detection efficiencies will be 6-8% for S-35 and 30-40% for P-32.

Shielding -
Shielding will be utilized for P-32 work. The shielding will consist of 1/4" thick lucite plastic. For millicurie quantity usage and storage, 1/4' thick lucite plastic plus 1/4" of lead sheeting will be used at the exterior of the lucite shield.

Item 11 - Waste Management

Radioactive waste will be disposed in accordance with 10 CFR Part 20 regulations. Specific procedures will be as follows:

a. Disposal by decay - S-35 and P-32 will be held for ten (10) half lives and then monitored with the portable survey meter described in Item 10. The material will be disposed as ordinary waste if the survey meter readings are no higher than background levels.

b. Disposal by release to the sanitary sewer system in accordance with 10 CFR 20.303. The Department of State Police Building water utilization is precisely known which is evidenced by the enclosed Michigan State University letters dated April 18, 1988, October 20, 1988 and January 23, 1989.

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ITEM 6a

MICHIGAN STATE POLICE

FORENSIC DNA TYPING: STATEMENT OF TECHNICAL NEED

DNA typing is based on the presence in human cells of information stored in linear DNA molecules. In no two individuals, with the exception of identical twins, are the DNA molecules identical. In each individual, however, the DNA molecules in every cell in the body are the same. It is this variation, and its capacity to identify an individual, that is exploited in the forensic DNA typing procedure.

DNA typing is being used worldwide in molecular biology, medical research, and other related fields. The forensic potential for this technique was first realized in the early 1980's by Dr. Alec Jeffreys, a British scientist, when he discovered a unique class of DNA probes that detect "repetitive DNA" in the genome. The Jeffreys' **DNA FINGERPRINTING℠** probes, and other similar probes subsequently developed, are currently being used for forensic identification by both commercial organizations and the Federal Bureau of Investigation.

Personal identification has always been of vital concern to law enforcement. DNA technology now provides the forensic analyst with the ability to identify a particular individual based on a drop of blood or semen, or a single hair. In 1988 the Michigan State Police forensic laboratories analyzed evidence from over 2000 violent crimes. Many of these cases were accompanied by requests from prosecuting attorneys and law enforcement agencies for DNA identifications.

Forensic DNA typing has applications in addition to the identification of evidentiary samples found at a crime scene. The technology can be used in the identification of missing or unidentified persons, including missing children by maternity/paternity testing. DNA typing information can also be entered into a centralized computer data bank thereby creating a suspect file and a source of data for population studies. DNA technology will impact substantially not only on crime lab procedures but also the way certain types of violent crimes are investigated and prosecuted.

Radioisotpic labeling of probes is necessitated in forensic DNA typing by the often small quantities of evidentiary material available. Radiolabeled probes have been found to be ten times more sensitive than currently available non-radioactive labeling systems.

The breakthrough in DNA typing is revolutionizing contemporary forensic approaches to the analysis of biological samples. It is therefore paramount that the Michigan State Police address this new technology.

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CHAPTER 2 Techniques for labelling nucleic acids

ITEM 6B

Introduction

In this chapter the most important nucleic acid labelling techniques are described, discussed and compared. Particular emphasis is placed on their suitability for specific applications.

For convenience the techniques are discussed under five main headings:

1. Nick translation
2. Primer extension
3. Methods based on RNA polymerase
4. End labelling methods
5. Direct labelling methods

1. Nick translation

Although predating many of the methods to be described in the following pages, nick translation remains the commonest means of labelling nucleic acids for hybridization (see figure 2). The technique can be used with a variety of labels to generate probes suitable for most hybridization applications. For example, using [α-^{32}P] deoxynucleoside triphosphates (dNTPs), it is relatively simple to generate probes of a specific activity (>1×10^8dpm/μg) high enough to detect single copy genes on Southern blots of mammalian DNA (see figure 3). Nick translation is also appropriate for use in the generation of biotinylated probes (see page 10).

A major advantage of nick translation over other uniform labelling methods is its flexibility with respect to probe size, specific activity and concentration. It is particularly suitable for the production of large quantities of probe for use in multiple hybridization reactions and/or where a high probe concentration is required.

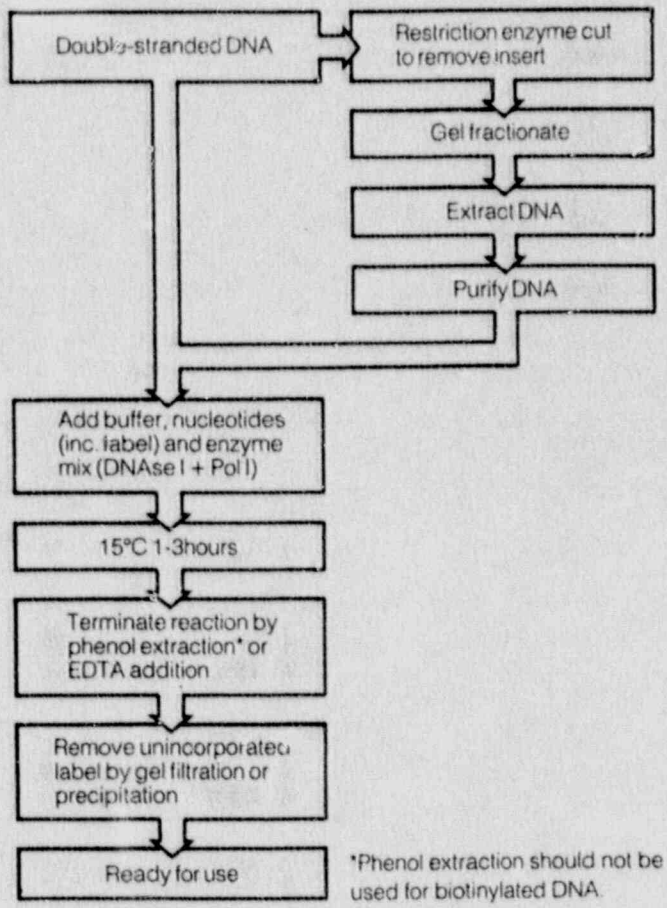
The nick translation reaction

The nick translation reaction[73, 87] involves the simultaneous action of two enzymes, pancreatic deoxyribonuclease I (DNase I)[63] and *E. coli* DNA polymerase I (DNA pol I)[51].

DNase I introduces nicks randomly in each strand along the length of a DNA molecule, resulting in both free 3'-hydroxyl and free 5'-phosphate groups. DNA pol I has two major activities which operate at the site of the nick. A 5'-3' exonuclease activity progressively removes nucleotides from double-stranded DNA working from the free 5'-end. Simultaneously, its 5'-3' polymerase activity successively adds nucleotides working from the free 3'-hydroxyl end, using the complementary DNA strand as a template. Thus, the initial nick is actually 'translated' along the DNA molecule in a 5'-3' direction.

As the DNase I introduces nicks randomly, the net effect is the production of a uniformly labelled population of

Flowchart of the nick translation method



*Phenol extraction should not be used for biotinylated DNA.

Figure 2

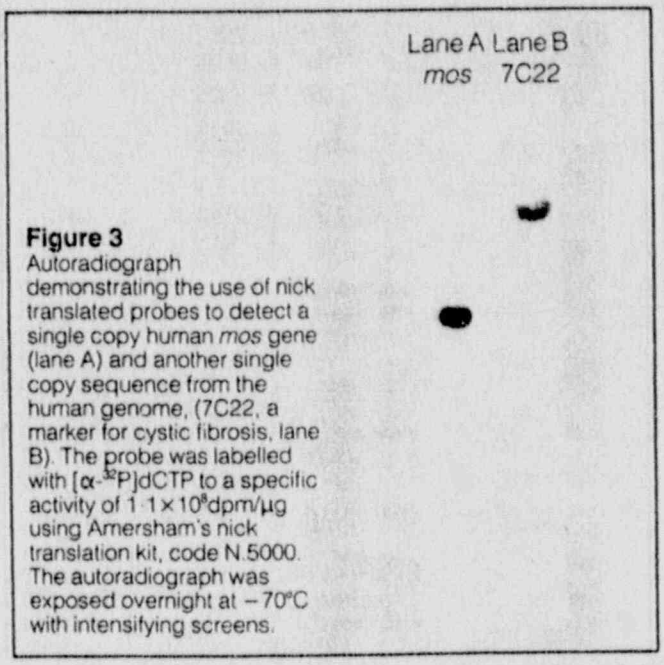


Figure 3
Autoradiograph demonstrating the use of nick translated probes to detect a single copy human *mos* gene (lane A) and another single copy sequence from the human genome, (7C22, a marker for cystic fibrosis, lane B). The probe was labelled with [α-^{32}P]dCTP to a specific activity of 1.1×10^8dpm/μg using Amersham's nick translation kit, code N.5000. The autoradiograph was exposed overnight at −70°C with intensifying screens.

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molecules. The process is illustrated diagrammatically in figure 4. By the inclusion of one or more radiolabelled nucleoside triphosphates in the reaction, the DNA can be labelled to very high specific activity.

Factors affecting the nick translation reaction

Nature of the substrate

Using standard protocols, ~1µg of relatively pure double-stranded DNA is required and the reaction is effective with both linear and circular molecules. The relatively large quantity of DNA in the reaction leads to high input of any contaminating material, which can lead to poor efficiency when labelling impure DNA substrates. This is a particular problem if it is necessary to prepare a probe free of vector sequences by gel purification (see page 11) since there is some evidence that DNase I is sensitive to inhibitors in agarose[25]. This problem can be offset to a large extent by using less DNA in the reaction (<100ng), concomitantly reducing the level of added contaminants.

Nature of the probe

A wide variety of protocols are in general use for nick translation. Since their details will determine the quantity, size and specific activity of labelled probe produced, the parameters involved will be discussed in some depth. The examples quoted are for the preparation of ^{32}P-labelled probes, but similar considerations apply to other radiolabels and to non-radioactive labels.

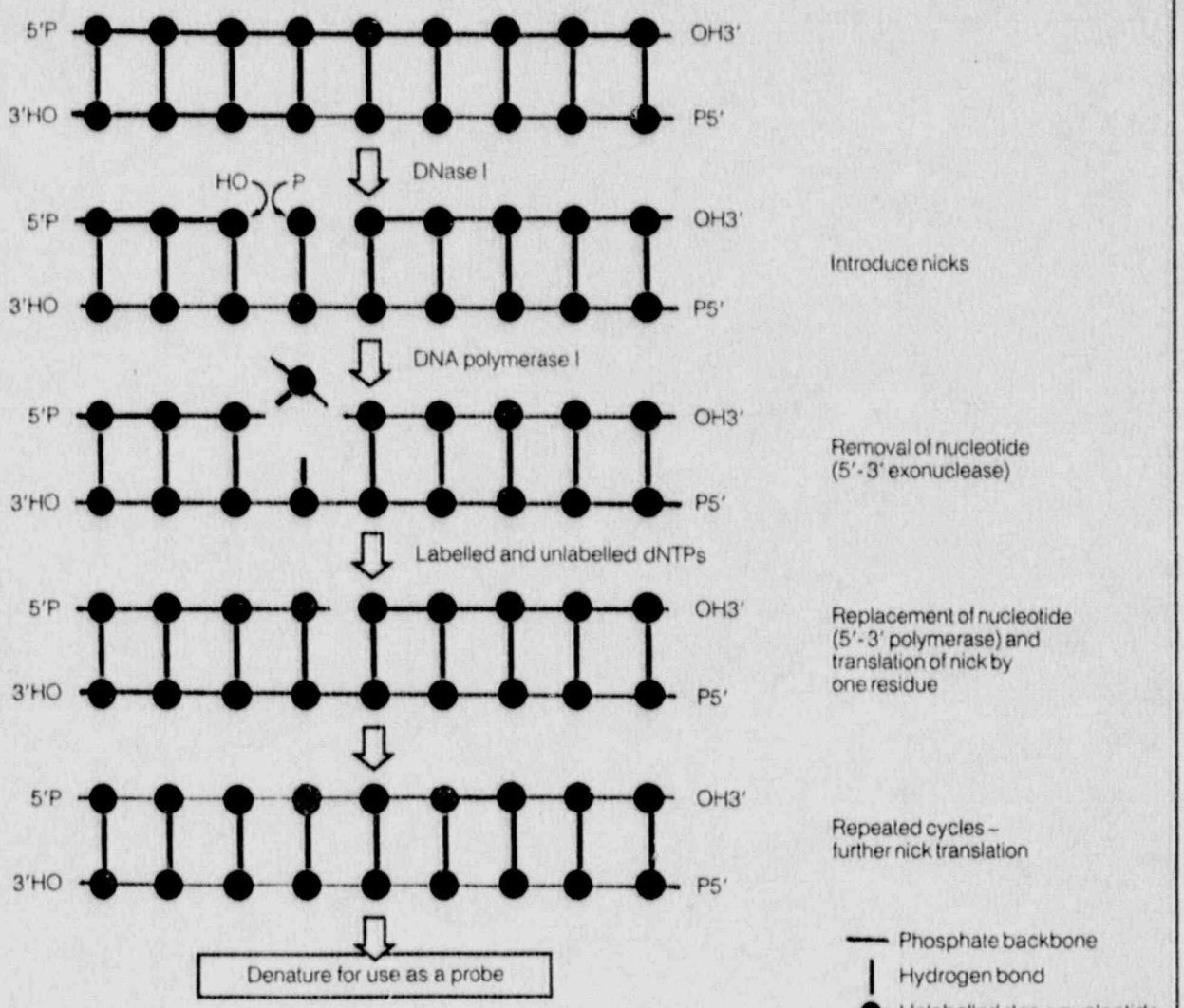

Figure 4
The nick translation reaction.

DNase I concentration

The concentration of DNase I determines the frequency of nicks and hence the final single strand length of the labelled products. Most of the standard protocols result in a single strand probe length of approximately 500 nucleotides. Lower concentrations can be used to produce longer probes and higher concentrations to produce shorter probes (for example, for *in situ* hybridization).

It should also be noted that the use of higher concentrations of DNase I allows shorter reaction times as more ends become rapidly available for the DNA pol I. A short pre-treatment with a high concentration of DNase I forms the basis of a recently described method for rapid nick translation which also leads to highly efficient incorporation of labelled nucleotides[52].

Reaction temperature

Nick translation reactions are normally carried out at low temperature (~15°C), as the use of higher temperatures can lead to rapid degradation of the probe due to increased activity of DNase I and the 5'-3' exonuclease activity of DNA pol I. Investigations of the reaction mechanism at higher temperatures[73] have demonstrated that nick translation is to some extent replaced by strand displacement. In this case an elongating strand displaces residues from the duplex instead of excising them by the 5'-3' exonuclease activity. Under these conditions it is possible for template switching to occur, so that the polymerase begins to copy a displaced strand. This leads to the production of regions of intrastrand complementarity. These reactions are illustrated in figure 5.

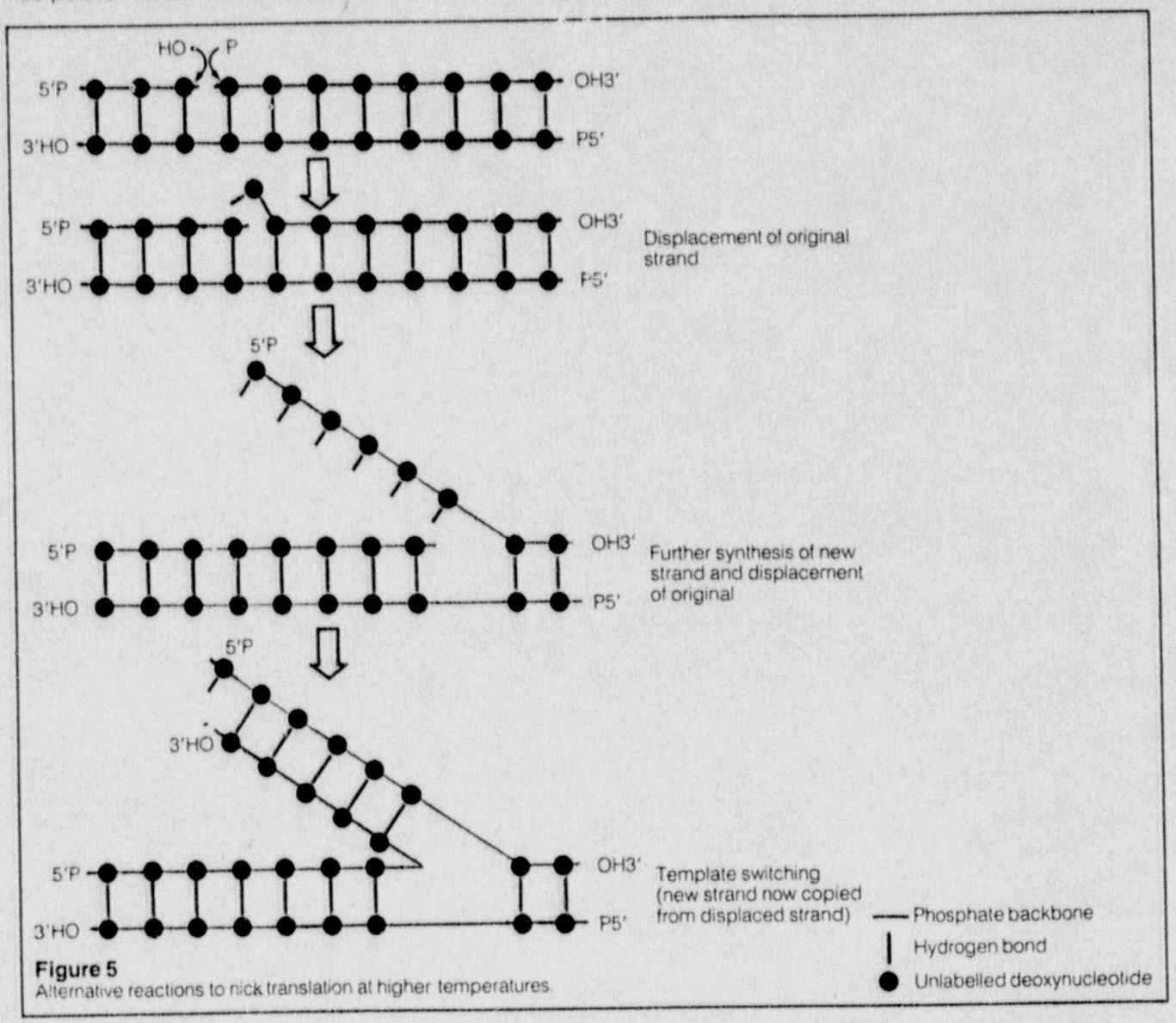

Figure 5
Alternative reactions to nick translation at higher temperatures.

Time course

Maximum incorporation can be obtained at incubation times varying between 30 minutes and 5 hours (see figure 6) depending on the precise protocol used. Apparent reaction rate, as measured by incorporation of labelled precursor, increases with addition of more enzyme (DNA pol I and /or DNase I), or when the concentration of labelled nucleotide is reduced, for example by changing from low to high specific activity at the same level of added radioactivity (see page 10).

Nick translation reactions should not be left for longer than is necessary (usually ~3 hours) as both DNase I and the 5'-3' exonuclease activity of DNA pol I will continue to degrade the DNA causing loss of incorporated label. Both enzymes should be inactivated if the reaction mix is to be stored before use. Use of chelating agent (EDTA) and/or detergent (SDS) is recommended in preference to heat denaturation, as this can cause a transient increase in nuclease activity.

Specific activity

The specific activity of a probe prepared by nick translation depends both on the extent of replacement of existing nucleotides in the DNA substrate and on the specific activity of the input labelled nucleotides[87]. In theory, complete replacement can be obtained if the labelled nucleotide is present at the same level as its equivalent in the unlabelled DNA template. For example, 1µg DNA contains approximately 750 picomoles dCMP (assuming equimolar amounts of the four constituent deoxynucleotides). If 750 picomoles [α-^{32}P]dCTP (approximately 300µCi at 400Ci/mmol) are added to the reaction, then complete replacement is theoretically possible. In practice, a level of ~1000 picomoles [α-^{32}P]dCTP (400µCi at 400Ci/mmol) is necessary, because levels of incorporation of labelled nucleotide higher than ~75% rarely occur using standard protocols. The specific activity of probe produced under these conditions is $6{\cdot}6\times10^{8}$dpm/µg.

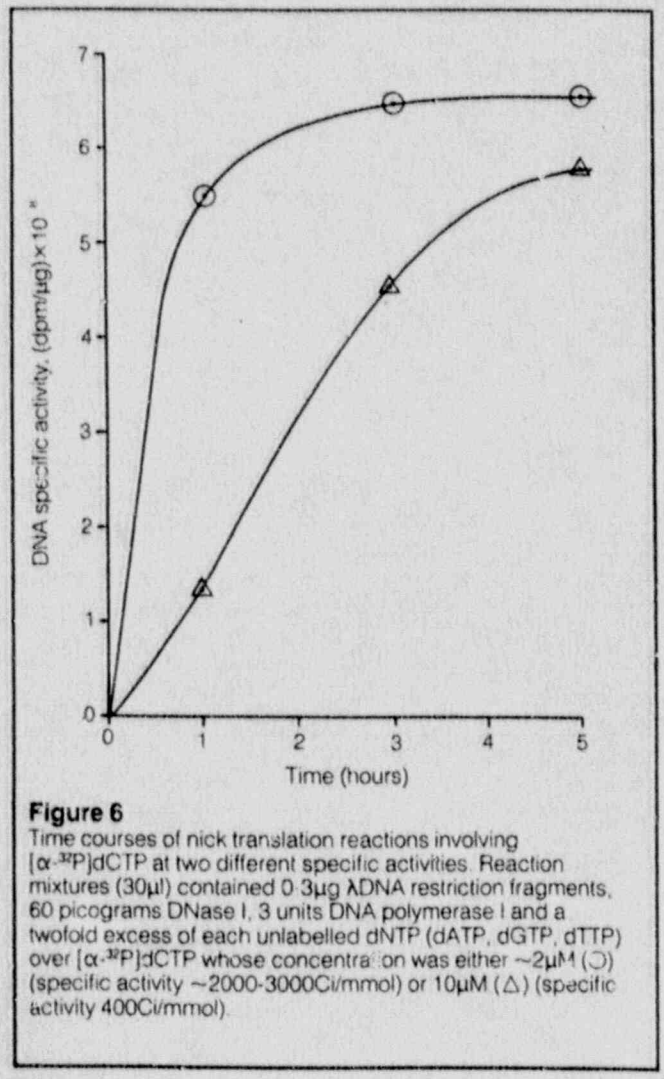

Figure 6
Time courses of nick translation reactions involving [α-^{32}P]dCTP at two different specific activities. Reaction mixtures (30µl) contained 0·3µg λDNA restriction fragments, 60 picograms DNase I, 3 units DNA polymerase I and a twofold excess of each unlabelled dNTP (dATP, dGTP, dTTP) over [α-^{32}P]dCTP whose concentration was either ~2µM (○) (specific activity ~2000-3000Ci/mmol) or 10µM (△) (specific activity 400Ci/mmol).

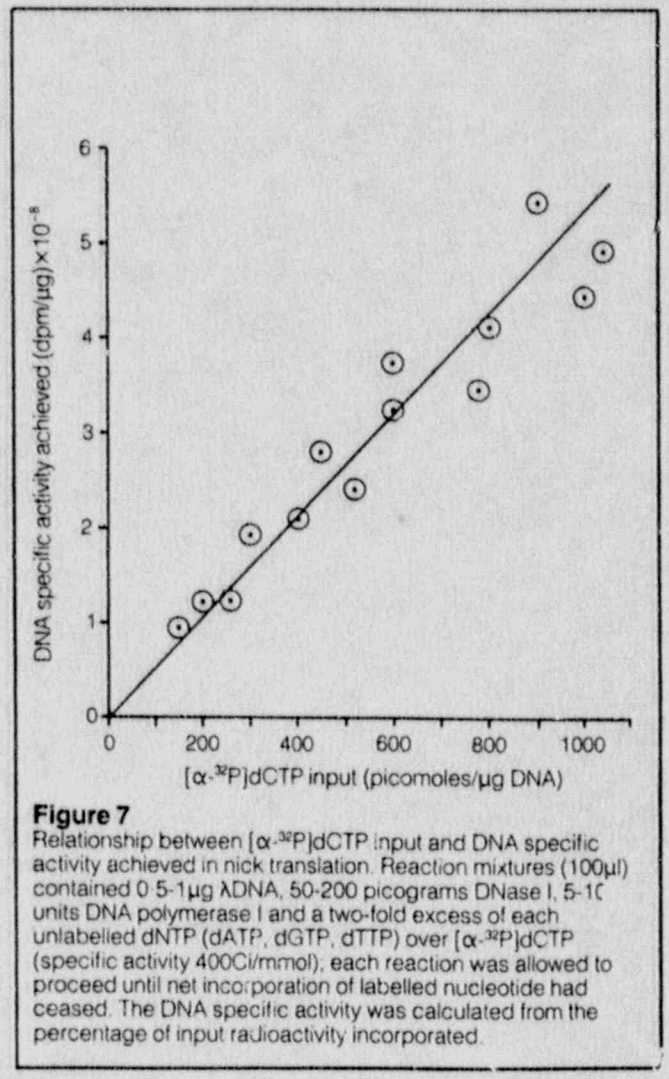

Figure 7
Relationship between [α-^{32}P]dCTP input and DNA specific activity achieved in nick translation. Reaction mixtures (100µl) contained 0·5-1µg λDNA, 50-200 picograms DNase I, 5-10 units DNA polymerase I and a two-fold excess of each unlabelled dNTP (dATP, dGTP, dTTP) over [α-^{32}P]dCTP (specific activity 400Ci/mmol), each reaction was allowed to proceed until net incorporation of labelled nucleotide had ceased. The DNA specific activity was calculated from the percentage of input radioactivity incorporated.

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At levels of labelled nucleotide below ~1000 picomoles/µg DNA, the specific activity of the final product is generally proportional to the amount of label added (see figure 7). Changing to nucleotides of higher specific activity (for example 3000Ci/mmol), without altering the total microcuries added, will not significantly affect the final specific activity. However, since the effective labelled nucleotide concentration will be lower, the rate of reaction, as monitored by incorporation of radiolabel into DNA, will appear to be faster (figure 7). This is simply due to a lower final level of replacement in the template.

It is, nevertheless, possible to produce a higher specific activity probe using label of higher specific activity, because a greater amount of label can potentially be added to the reaction. For example, it is possible to use up to 3000µCi/µg DNA for label at 3000Ci/mmol giving a final probe specific activity of approximately 5×10^9dpm/µg. Although it is not generally economical to use such high levels of label in a reaction containing microgram amounts of DNA, it is possible to achieve such specific activities by using less DNA (<100ng).

Work carried out at Amersham has demonstrated that higher specific activities can also be obtained by the simultaneous use of more than one labelled nucleotide, but the efficiency of incorporation tends to fall if more than one nucleotide is limiting. When designing a nick translation protocol, the optimal specific activity and concentration of probe required for hybridization (see page 36) should be considered, and the labelling parameters changed as necessary.

Note on the use of biotin with nick translation
Nick translation is the preferred method for incorporation of biotinylated nucleotides. This is because microgram amounts of biotinylated probe can be produced and stored for several months. Furthermore, in contrast to radioactive probes, biotinylated probes do not lead to elevated non-specific binding when used at high concentrations.

Applications

The major advantages and disadvantages of nick translation are outlined in table 2. Nick translated probes are appropriate for a variety of applications such as the detection of single copy genes or low abundance mRNA in filter hybridization, and for *in situ* hybridization (see chapters 3 and 4).

Associated techniques

Reactions analogous to nick translation have been used in other procedures. For example, by substituting RNase H for DNase I, it is possible to carry out nick translation on an RNA:DNA hybrid, leading to replacement of the RNA strand. This is used for second strand synthesis in the cDNA synthesis procedure of Gubler and Hoffman[31].

Table 2. Advantages and disadvantages of nick translation.

Advantages
1. Allows control over a variety of parameters, for example, probe size, yield, specific activity, substrate concentration and reaction time.
2. Subcloning is not required.
3. Medium (10^8-10^9dpm/µg) and high (>10^9dpm/µg) specific activity probes can be produced.
4. Good utilization of label (60-70% incorporation).
5. DNA probes allow standard hybridization temperatures to be used (see page 36).
6. Large amount of probe produced in standard protocols (~1µg), and so a single labelling reaction usually produces sufficient probe for several hybridizations.
7. Ideal for generating microgram quantities of biotinylated probe.
8. Labels circular and linear DNA.
9. Short reaction times possible (60-90 minutes).
10. Leads to very uniform labelling.
11. A variety of radiolabels (^{3}H, ^{35}S and ^{32}P) can be used.
12. More than one labelled nucleotide can be used simultaneously, for example [α-^{32}P]dCTP and [α-^{32}P]dATP.

Particularly suitable for purified DNA available in relatively large amounts, (for example, a plasmid preparation for which the presence of vector sequence in hybridization is unimportant).

Disadvantages
1. Unpredictable results with impure DNA substrates (particularly DNA in agarose).
2. Uniform labelling method – not limited to insert sequence.
3. Large amount of substrate (~1µg) usually required.
4. Temperature and time of reaction require careful control to avoid strand displacement and excision of incorporated label.
5. Requires control of two enzyme activities.
6. DNA:DNA hybrids have lower stability than DNA:RNA and RNA:RNA hybrids (see page 17).
7. Probe denaturation required.
8. Probe not strand-specific. Strand reannealing of probes may occur.
9. Will not label single-stranded DNA.

Products for the nick translation method
Nick translation kits see page 45
^{32}P, ^{35}S, ^{3}H and ^{125}I labelled deoxynucleotides see pages 46, 47
DNA polymerase I see page 47
DNase I see page 47

2. Primer extension methods

In common with nick translation, primer extension methods utilize the ability of DNA polymerases to synthesize a new DNA strand complementary to a template strand, starting from a free 3'-hydroxyl. In this case the latter is provided by a short oligonucleotide primer annealed to the template.

Two general approaches are possible. In the first, a mixture of primers of random sequence is used in order to produce a uniformly labelled DNA copy of any sequence. The second method uses a unique primer to restrict labelling to a particular sequence of interest.

It is essential to use a polymerase lacking a 5'-3' exonuclease activity. Otherwise degradation of the primer will occur. Both the Klenow fragment of *E. coli* DNA polymerase I, which lacks the 5'-3' exonuclease activity, and reverse transcriptase have been used successfully in this way. Klenow fragment was originally produced as a protease cleavage fragment of *E.coli* DNA polymerase I [43, 40] but is now widely available as a cloned enzyme.

Flowchart of the random primer labelling method

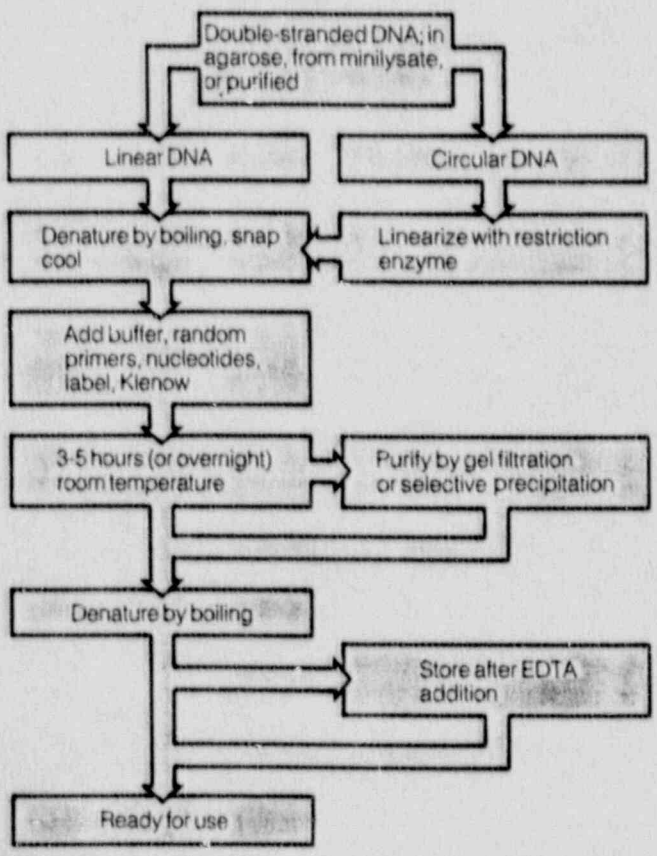

Figure 8

For convenience the approaches will be considered as follows:

A. Random primers used with Klenow polymerase.
B. Unique primers used with Klenow polymerase
C. Unique primers used with reverse transcriptase
D. Random primers used with reverse transcriptase

A. Random primers used with Klenow polymerase

Hexanucleotides of random sequence, either derived from DNase I digestion of calf thymus DNA or produced by oligonucleotide synthesis, have been used to prepare labelled copies of both DNA and RNA. Feinberg and Vogelstein[25, 26] first described this approach for the labelling of DNA fragments to very high specific activity (see figure 8). The reaction is illustrated in figure 9.

Factors affecting the random priming reaction

Nature of the substrate

Random priming requires a single-stranded template which may be either derived from a suitable single-stranded bacteriophage (for example M13) or more frequently produced by brief heat denaturation of a double-stranded molecule. If the latter is used, a linear molecule is preferable to avoid rapid renaturation of complementary DNA circles. Random primers, Klenow polymerase, labelled and unlabelled nucleotide precursors are then added in a suitable buffer and the reaction is allowed to proceed.

Synthesis has been found to occur very efficiently with nanogram amounts of DNA (generally 20-30ng). Using amounts of labelling nucleotide approximately equimolar to the equivalent nucleotide in the DNA template, levels of incorporation as high as 70-80% are routinely achievable within 3 hours.

Random priming has been used successfully to label DNA which is relatively impure. For example, it is often necessary to label specific DNA fragments which have been purified by agarose gel electrophoresis. This procedure is frequently used to separate a cloned insert from vector sequence which may show some cross-hybridization with the target DNA. Feinberg and Vogelstein[26] have demonstrated that it is possible to label DNA fragments in the presence of low gelling temperature agarose without prior purification. Work at Amersham has shown that the procedure also efficiently labels DNA from minilysates prepared by either the alkaline[7] or boiling lysis[36] methods.

Reaction temperature and time

As the 5'-3' exonuclease activity of DNA pol I is absent from Klenow polymerase, a wide range of temperatures may be adopted for random primer labelling. Room temperature is frequently employed, but 37°C may be

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used for more rapid labelling, thus increasing the flexibility in choice of reaction times. As there is no significant excision of incorporated nucleotides (see page 9), reactions may be left to proceed overnight if required. The reaction temperatures commonly used permit significant strand displacement (see page 8) so that, for example, starting from 25ng template, a further 30-50ng labelled DNA may be synthesized. Both this and the presence of unlabelled template DNA should be taken into account when calculating probe specific activity.

Specific activity

Because net synthesis occurs in this reaction, it is possible to obtain good incorporation of labelled nucleotide added at a concentration higher than that of equivalent nucleotide in the template. As the level of input DNA is low, the labelled nucleotide can be of high specific activity. Therefore a probe of high specific activity will be produced. For example, using 200µCi of an [α-^{32}P]dNTP at 6000Ci/mmol, probe specific activities in excess of 5×10^8dpm/µg are achievable.

In a standard nick translation reaction, using 1µg of DNA, it would be necessary to use >1mCi of labelled dNTP to achieve an equivalent probe specific activity. However, it should be remembered that the amount of probe produced in a typical random primer labelling reaction is substantially lower than in a nick translation reaction, because of the lower amount of template generally used. Thus random primer labelling is ideal for situations such as single copy gene detection on a Southern blot, where high specific activity and low probe concentration are desirable (see figure 10). In general, the addition of more label to a random primer reaction leads to the production of a probe of higher specific activity, but the efficiency of utilization of the label is reduced.

The use of more than one labelled nucleotide species has been investigated in some detail at Amersham. At the same level of incorporated label there has been found to be little advantage in using more than one ^{32}P-labelled nucleotide because, although a slightly higher specific activity may be achieved, yield of probe and efficiency of utilization of label are both reduced. With ^{35}S, a slight advantage is obtained, but with ^{3}H, where limiting nucleotide concentrations are not used, a significant improvement in specific activity is possible. A maximum of $\sim1.2\times10^8$dpm/µg with three labels and $\sim2\times10^8$dpm/µg with four labels can be obtained, as compared to $\sim7.5\times10^7$dpm/µg with one label only. In general, incorporation rates are reduced when using two or more labels, and it is advisable to leave the reaction for at least five hours for maximum incorporation.

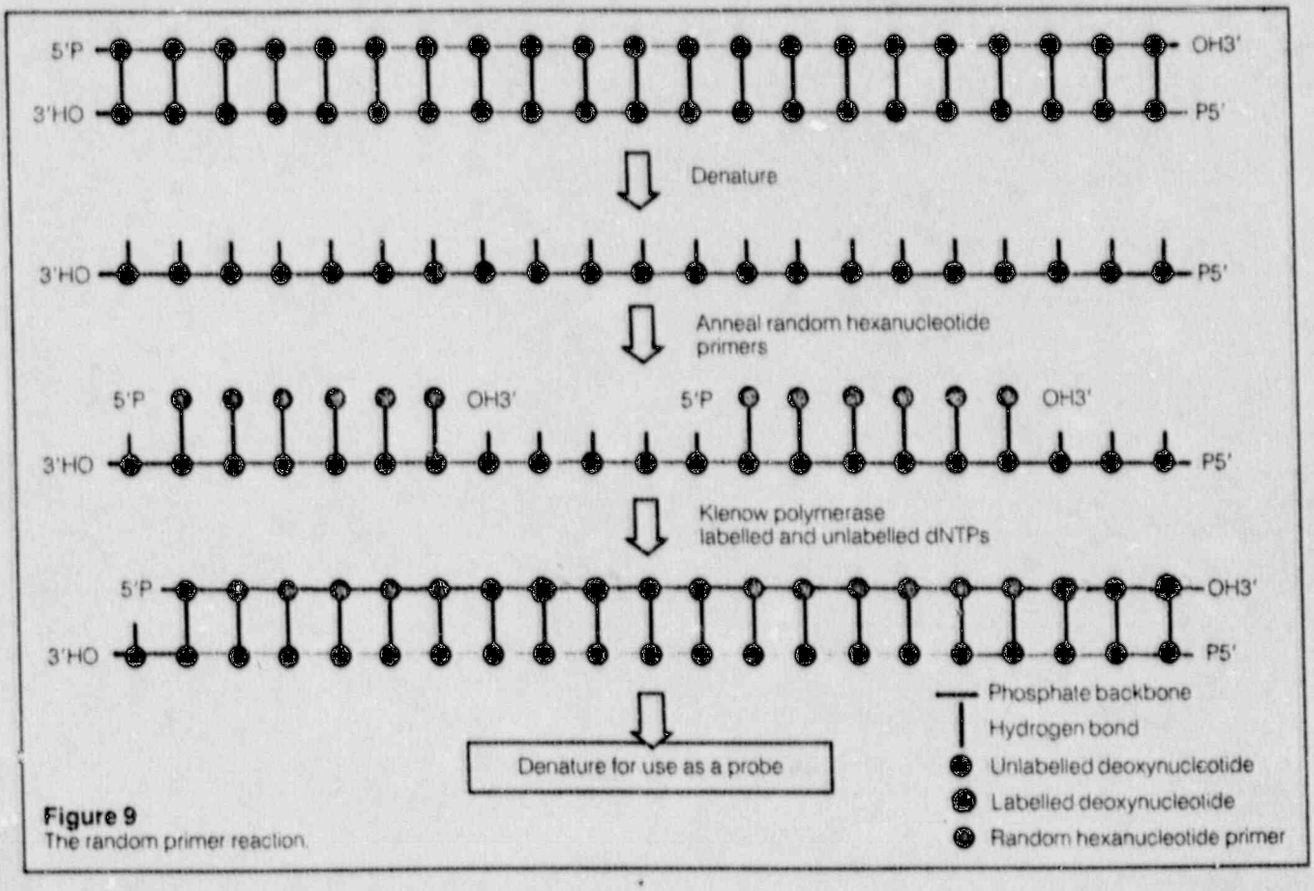

Figure 9
The random primer reaction.

Probe size

At Amersham it has been found that, with linear wild type lambda DNA as substrate and standard reaction conditions, probe length is determined largely by the concentration of radiolabelled nucleotide used as precursor. Over a range of total input label between 10 and 100 picomoles (20-200 picomoles for ^{3}H) with between one and three labelling nucleotide species (one and four for ^{3}H), average probe size varies between 200 and 300 bases for ^{32}P, 100 and 150 bases for ^{35}S, and 400 and 500 bases for ^{3}H.

Although probe length obtained with tritium-labelled nucleotides may be too great for some *in situ* applications, this can be remedied by prior sonication of the substrate. Exhaustive sonication of DNA yields a population of molecules with a mean size of about 200 base pairs. Probes produced from such a template using >10 picomoles of two [^{3}H]dNTPs average ~95 bases in length.

In general it has been found that the variance of sizes obtained by random primer labelling is somewhat wider than with nick translation.

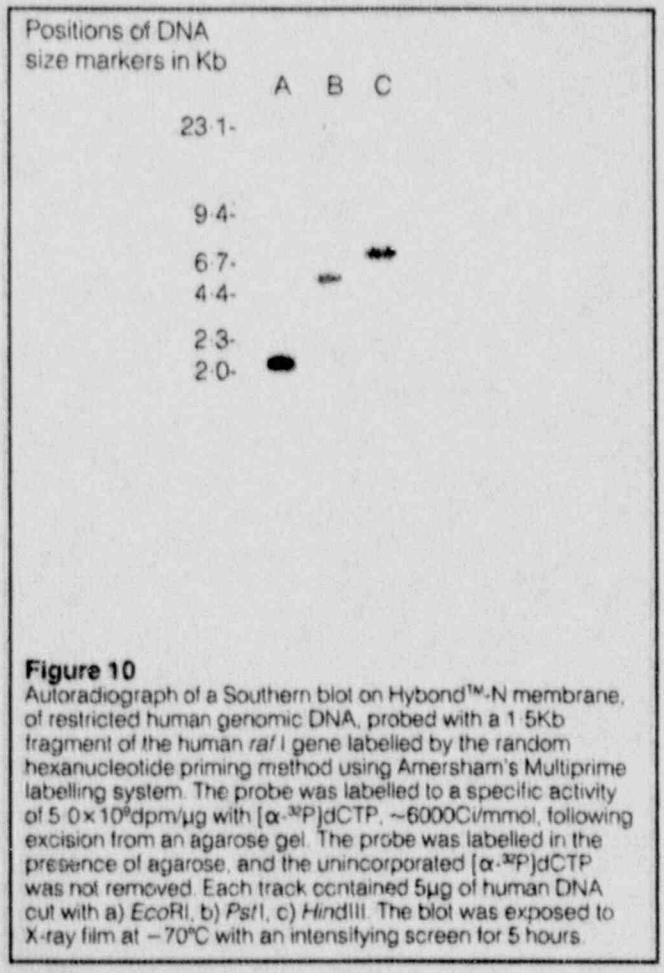

Figure 10
Autoradiograph of a Southern blot on Hybond™-N membrane, of restricted human genomic DNA, probed with a 1·5Kb fragment of the human *raf* I gene labelled by the random hexanucleotide priming method using Amersham's Multiprime labelling system. The probe was labelled to a specific activity of 5·0×10^{9}dpm/µg with [α-^{32}P]dCTP, ~6000Ci/mmol, following excision from an agarose gel. The probe was labelled in the presence of agarose, and the unincorporated [α-^{32}P]dCTP was not removed. Each track contained 5µg of human DNA cut with a) *Eco*RI, b) *Pst*I, c) *Hin*dIII. The blot was exposed to X-ray film at −70°C with an intensifying screen for 5 hours.

Applications

The major advantages and disadvantages of random primer labelling are outlined in table 3. In general, random priming is appropriate for those applications detailed for nick translation, in particular the detection of single copy genes or low abundance mRNA on membrane blots. Although not widely used for *in situ* hybridization, the results quoted above suggest that random priming can be used for this application, particularly when using ^{3}H probes. At Amersham random priming has also been successfully carried out with biotin-11-dUTP as label.

Table 3. Advantages and disadvantages of random primer labelling with Klenow polymerase

Advantages

1. High specific activity probes (>10^{9}dpm/µg).
2. Efficient utilization of label (70-80%).
3. Flexible reaction temperature and time (up to overnight).
4. Can label small amounts of DNA.
5. Labels single-stranded DNA (and double-stranded after denaturation).
6. Labels impure DNA efficiently (minilysate or DNA in agarose).
7. Several labelled nucleotides can be used simultaneously.
8. Subcloning is not required.
9. DNA probes allow standard hybridization temperatures to be used (see page 36).
10. Leads to uniform labelling.
11. Incorporated label not excised during reaction.
12. Reaction parameters can be controlled (for example, input label, primer concentration) to influence probe size, yield, etc.

Ideal for probe sequence available in small amounts in impure form (for example, gel-purified insert).

Disadvantages

1. Quantity of probe produced (~70ng); generally sufficient for a single hybridization only.
2. Relatively inefficient with circular DNA substrates.
3. Uniform labelling method – not limited to insert sequence.
4. DNA:DNA hybrids have lower stability than DNA:RNA and RNA:RNA hybrids (see page 17).
5. Denaturation of input DNA required.
6. Probe denaturation required.
7. Probe not strand-specific. Strand reannealing of probes may occur.

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B. Unique primers used with Klenow polymerase

The use of Klenow polymerase with unique primers to label DNA was developed out of the M13 dideoxy sequencing methodology[62, 74]. Two major approaches have been used. In the first of these, the oligonucleotide primer is annealed to the region 5′ to the multiple cloning site of an M13 vector and, in the second, the primer is annealed to the region 3′ to the multiple cloning site.

Nature of the substrate

For both approaches (that is, primer annealing to region 3′ or 5′ to the M13 multiple cloning site) a single-stranded substrate is used. This is normally the (+) strand of any of the M13 vectors, that is, the strand packaged and extruded into the culture medium. A typical reaction requires 50ng of M13 (+) strand template and 2ng of primer.

Although it is theoretically possible to carry out unique primer labelling with denatured duplex DNA, in practice the degree of specificity of labelled probe is usually low, most probably due to partial renaturation of the template.

(i) Primer annealed to region 5′ to the multiple cloning site

The reaction

This approach, originally described by Hu and Messing[37], uses a hybridization primer which anneals to the region 5′ to the multiple cloning site on the template. DNA synthesis is then initiated and the vector rather than the insert sequence is copied (see figure 11). To avoid read-through into the insert sequence the reaction is limited by a low concentration of labelled nucleotide. The reaction is terminated by the addition of chelating agent (EDTA) to remove Mg^{2+} and the probe is not denatured before use.

Nature of the probe and application

The single-stranded insert acts as a strand-specific probe while the labelled strand complementary to the vector sequence acts as a 'tag'. This method is particularly suited to screening M13 clones for sequences complementary to the insert either by plaque screening or dot blot.

Specific activity

Very high specific activities are theoretically achievable, that is, up to 4.5×10^9dpm/µg DNA using [α-^{32}P]dNTP at 6000Ci/mmol. This assumes complete copying of vector sequence and excludes the minor contribution of insert sequence. As the vector is generally much larger than the insert in M13, the ability to copy the vector in this way allows a significant amplification of labelling over that achievable were the insert itself to be labelled by more

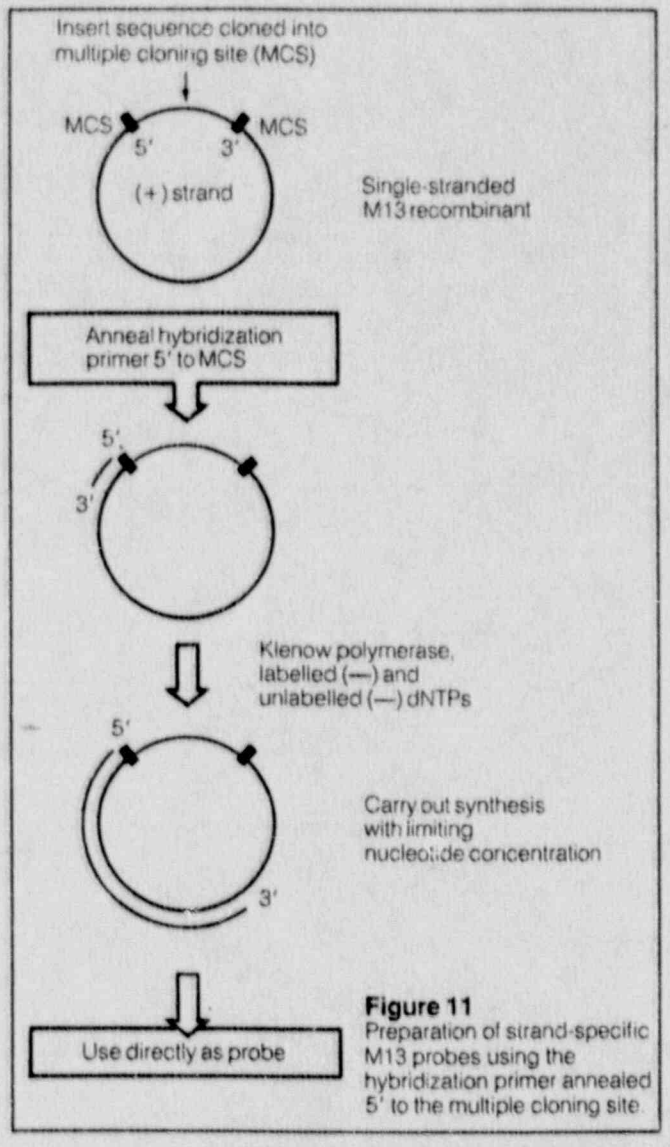

Figure 11
Preparation of strand-specific M13 probes using the hybridization primer annealed 5′ to the multiple cloning site.

conventional methods. For example, with an insert of approximately 500 bases, the effective maximum specific activity is in excess of 5×10^{10}dpm/µg insert, assuming complete copying of the vector.

(ii) Primer annealed to region 3′ to the multiple cloning site

The reaction

The second approach uses the universal sequencing primer which anneals to the region 3′ to the multiple cloning site on the (+) strand of M13 (see figure 12). Thus, primer extension produces a labelled copy of any insert sequence.

Nature of the probe

In this case the reaction is not nucleotide-limited, so that a complete copy of the insert is made. As this region is then

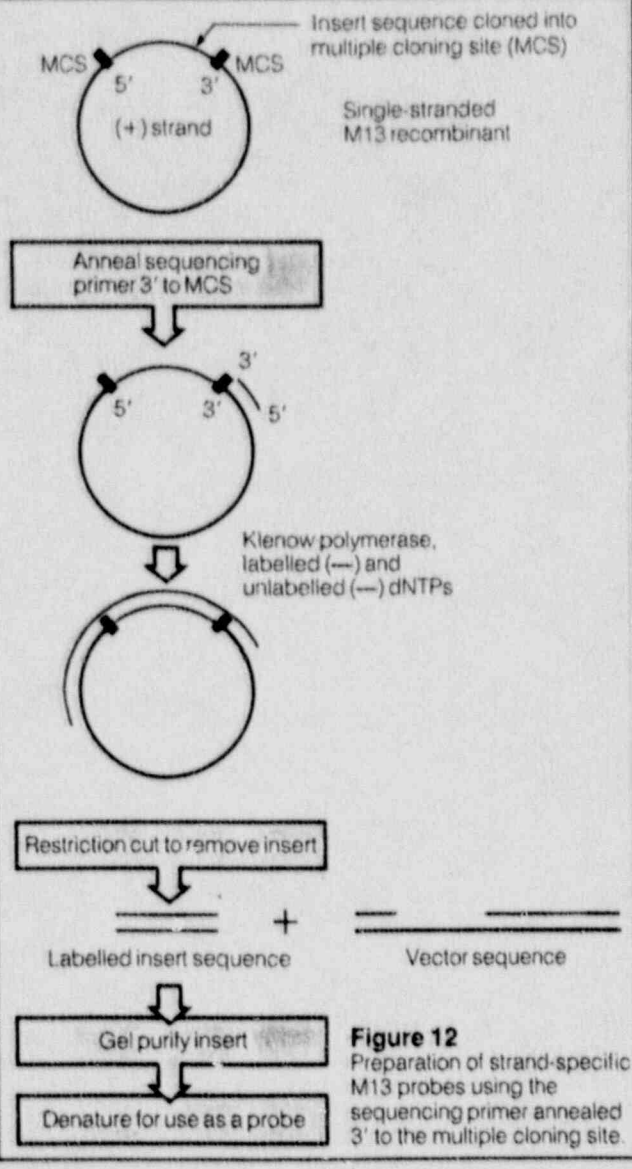

Figure 12
Preparation of strand-specific M13 probes using the sequencing primer annealed 3' to the multiple cloning site.

rendered double-stranded, it is possible to excise the insert using suitable restriction enzymes which cut in the multiple cloning site either side of the insert. The labelled insert fragment can then be gel-purified and denatured prior to use as a probe.

Specific activity
As with the previous procedure, very high specific activity probes can be produced (up to $4{\cdot}5\times10^9$dpm/µg DNA with [α-^{32}P]dNTP at 6000Ci/mmol).

Applications
This method of labelling clones in M13 has been used in the DNA fingerprint methodology of Jeffreys[41]. Probes produced in this way are also suitable for mapping studies (see page 38).

C. Unique primers used with reverse transcriptase

Unique primers have been used most frequently in conjunction with reverse transcriptase in two ways. The first is in the technique of primer extension mapping[93], by which a labelled primer is annealed to an RNA molecule and is then extended in order to map the 5'-end of the RNA (see page 39). The second major approach is more strictly a method of probe production. By annealing an oligo-dT primer to the 3'poly(A) tail of an mRNA molecule, it is possible to synthesize a labelled cDNA in the presence of reverse transcriptase and appropriate nucleotides[1, 75]. Such probes can be used for any of the applications outlined for nick translated and random primer labelled probes, but are particularly appropriate for the specific application of subtractive cDNA cloning[26].

D. Random primers used with reverse transcriptase

Although very high specific activities are achievable using an oligo-dT primer and reverse transcriptase, it is difficult to ensure complete copying of a long message, due to problems of nucleotide limitation. In most cases it is possible to opt for a higher nucleotide concentration and consequent lower probe specific activity if a complete copy is required. For those cases where this is not adequate, or where the RNA to be copied is poly $(A)^-$, the use of random hexanucleotides with reverse transcriptase is a possible alternative. The main disadvantage of this approach is that it will lead to the copying of all RNA molecules present and, if no prior purification of mRNA has been carried out, ribosomal and transfer RNA will be present in vast excess over message.

Random hexanucleotides have also been used in cDNA synthesis and cloning[35], either with reverse transcriptase for first strand cDNA synthesis or with Klenow polymerase for second strand synthesis. In some cases, DNA ligase may be required for ligation of the fragments produced in order to obtain full length copies, because unlike Klenow polymerase, reverse transcriptase does not cause strand displacement[83].

Products for primer extension methods

Multiprime DNA labelling system see page 45
^{32}P, ^{35}S, ^{3}H and ^{125}I labelled deoxynucleotides see pages 46, 47
Klenow fragment see page 47
Reverse transcriptase see page 47
M13 hybridization primer see page 48
M13 sequencing primer see page 48

3. Methods based on RNA polymerase

Introduction

The procedures so far described all utilize the ability of DNA polymerase to incorporate labelled deoxynucleotides into new DNA strands. RNA polymerases catalyze the synthesis of RNA from ribonucleoside triphosphates using a DNA template. They thus have the ability to incorporate labelled ribonucleotides into RNA molecules which can be used in all the applications appropriate for uniformly labelled DNA probes. Historically, two main approaches to the production of RNA probes have been adopted. The first involved the use of *E.coli* RNA polymerase[9]. This enzyme when used *in vitro*, shows very little template and promoter specificity and therefore produces transcripts which have been initiated and terminated more or less at random[75]. This approach has now been largely superseded by the more elegant use of bacteriophage RNA polymerases (see figure 13).

Flowchart of the RNA polymerase based method with double-stranded DNA template.

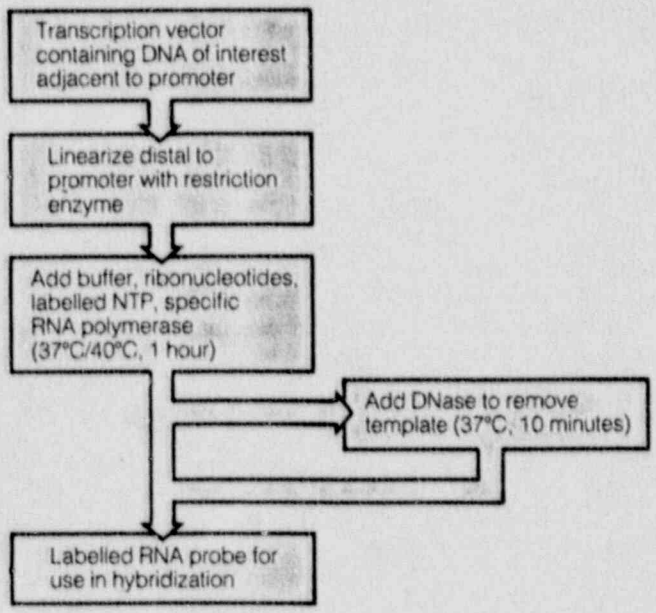

Figure 13

The reaction

The RNA polymerases from a number of bacteriophages (for example, *Salmonella* phage SP6 and coliphages T3, T5 and T7)[11, 17, 64], possess a high degree of specificity for their own promoters *in vitro*. Thus, transcription can be limited to sequences cloned downstream of an appropriate promoter. The basic reaction is illustrated in figure 14.

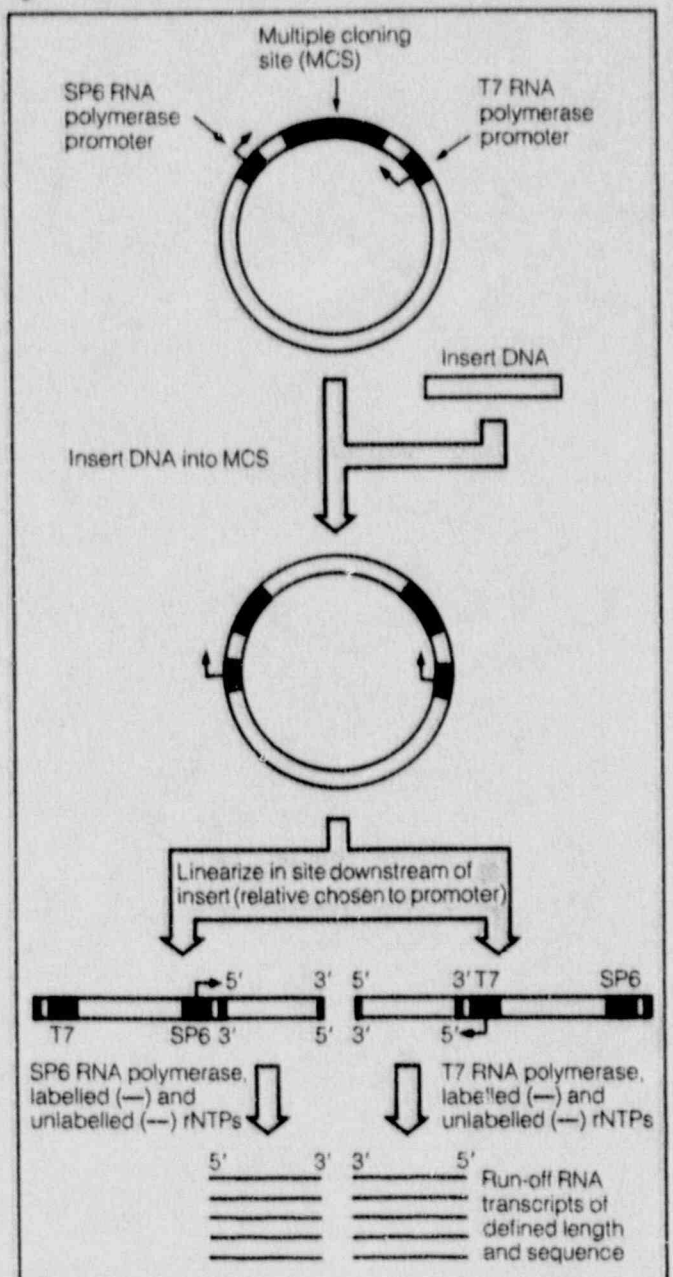

Figure 14
Preparation of RNA probes using a plasmid containing SP6 and T7 polymerase promoters.

Factors affecting the reaction

Nature of substrate

The need for a cloning step has led to the development of a variety of vectors in which a phage promoter is present upstream from a multiple cloning site, thus allowing transcription from any cloned insert. One of the most frequently used types of vector contains two phage promoters in opposite orientation separated by a multiple cloning site. Transcription from vector sequence is avoided by linearization of the vector downstream of the insert so that run-off transcripts are produced. The use of two promoters allows transcription from either strand to be chosen (see figure 14), so that, for example, sense or anti-sense RNA may be produced[59]. In the presence of high concentrations of precursor nucleotides it is possible to produce large amounts of transcript, up to 10µg from 1µg template, useful for a variety of applications such as splicing studies and *in vitro* translation[44].

Nucleotide concentration

When using the system to produce high specific activity labelled probes there are several factors to be considered, including economical use of label, desired probe specific activity and requirement for full length transcripts. In practice it is usual to include the labelled nucleotide at a concentration of 12·5µM (approximating to the apparent Km of SP6 RNA polymerase for CTP, GTP and UTP). This is equivalent to 100µCi of a nucleotide at 400Ci/mmol in a 20µl reaction. For most labelled nucleotides, an incorporation level of 80% can be routinely obtained within 60 minutes using 2µg template. This is equivalent to the synthesis of about 250ng of transcript. The level of incorporated nucleotide is sufficient to produce a transcript of approximately 450 bases assuming one initiation per template molecule of ~3Kb size. However, Melton *et al*[59] have reported >80% full length yield of an SP6 transcript of 1850 bases with either CTP, ATP or GTP at 10µM. This suggests that, in practice, initiation is limited to only a fraction of template molecules, most of which are then elongated to full length. Results obtained at Amersham support this finding with inserts up to 2·5Kb.

With a single limiting nucleotide at as low as 2µM, Melton *et al*[59] report the best yield of full length copy (~50%) to be obtained with limiting GTP. A possible explanation of this is that in the vector used, as in most other vector constructs, GTP is the initiating nucleotide. As the apparent Km for initiation is higher than that for elongation, when GTP is limiting, the rate of initiation will be decreased relative to elongation. This will increase the probability of full length transcription occurring before nucleotide exhaustion. A further observation is that, with some preparations of SP6 RNA polymerase, probe synthesis occurs inefficiently with limiting GTP, suggesting that these preparations may be deficient in initiation activity. In contrast, both CTP and UTP as limiting nucleotide routinely give high levels of incorporation with SP6 and T7 RNA polymerases. While ATP gives good levels of incorporation with T7 RNA polymerase, it is less efficient with SP6 RNA polymerase which has a higher apparent Km (40µM) for this nucleotide.

Probe specific activity

Using a radiolabelled nucleotide at 400Ci/mmol, the specific activity of probe produced is $6·7\times10^8$dpm/µg RNA, while at 800Ci/mmol (200µCi per 20µl reaction) a specific activity of $1·3\times10^9$dpm/µg RNA is obtained. A maximum specific activity of 5×10^9dpm/µg is possible using nucleotide at 3000Ci/mmol but, since this requires the addition of 750µCi per 20µl reaction the nucleotide solution must be evaporated to dryness.

In general, it has been found to be inadvisable to use more than one labelled species in bacteriophage RNA polymerase reactions. This is because the efficiency of the reaction falls if more than one nucleotide is present at limiting concentration, and the proportion of full length transcript is also significantly decreased.

It should be noted that, in contrast to nick translation or random priming, the specific activity of the probe produced is unrelated to the efficiency of incorporation of label. This is because the RNA probe does not need to be denatured prior to hybridization. Thus, the excess of double-stranded DNA does not participate in the hybridization and there is no dilution with unlabelled template. Probe specific activity is therefore determined solely by the specific activity of the labelled nucleotide, the efficiency of incorporation giving a measure of the yield of probe from the reaction.

In some cases it may be found necessary to remove the DNA template before hybridization. This can be readily achieved by treatment with RNase free DNase.

Applications

RNA probes have been used in all the applications previously outlined for DNA probes prepared by the nick translation and random priming methods (see figure 15).

Advantages of RNA probes

RNA probes have been reported to show higher sensitivity than equivalent nick translated probes in both Northern blots[59] and *in situ* hybridization[14]. This is most probably due to the avoidance of probe reannealing during hybridization and because RNA:RNA hybrids are more stable than DNA:DNA or DNA:RNA hybrids. A further advantage of RNA probes is that non-specifically bound probe can be removed by treatment with RNase A, which is very specific for single-stranded RNA[59]. This specificity also enables transcripts labelled to somewhat lower specific activity to be used in an alternative

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technique to S1 mapping, termed RNase mapping, for quantifying rare RNAs or mapping exons, introns and ends of genes (see page 38). Finally, there is some evidence that avoiding probe denaturation before hybridization leads to lower backgrounds on nitrocellulose. The advantages and disadvantages of phage polymerase based methods of labelling are outlined in table 4.

Table 4. Advantages and disadvantages of using phage polymerase based methods

Advantages

1. Transcription limited to insert.
2. Efficient utilization of label (80%).
3. High specific activity probes (>10^9dpm/µg).
4. Short reaction time (60 minutes).
5. RNA:DNA and RNA:RNA hybrids have a higher stability than DNA:DNA hybrids.
6. No requirement for probe denaturation leading to improved background on nitrocellulose.
7. Probe can be specific for either strand.
8. No strand reannealing of probe during hybridization.
9. RNase clean-up possible.
10. Template removal possible.
11. Variety of labels (^{32}P, ^{35}S, ^{3}H, biotin) can be used.

Ideal for multiple use probes; avoids contamination with vector sequences.

Disadvantages

1. Subcloning required.
2. Restriction digestion to completion required (except mICE system).
3. RNA probe must be stored correctly to avoid RNase activity.
4. Narrow temperature optima for hybridization for many RNA probes.
5. Difficult to control probe size without limiting transcription.
6. Large amount of template required (1-2µg) (except mICE system).
7. Small amount of probe produced (~300ng).
8. Premature termination may occur.
9. Template removal may be required.
10. Performance depends on labelling nucleotide.
11. Only a single labelling nucleotide can be used efficiently (except for ^{3}H, where concentration is not limiting).

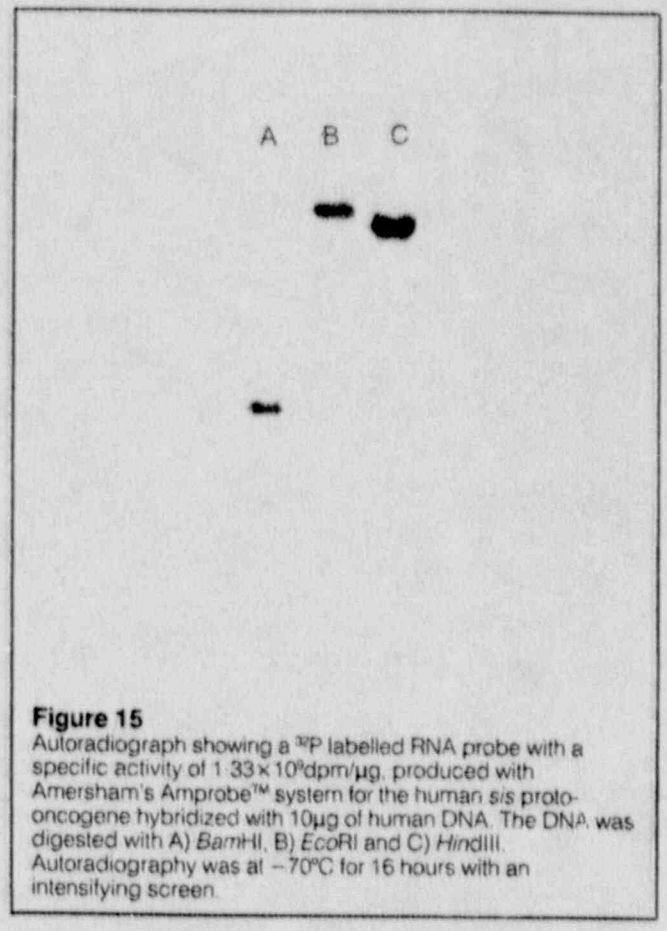

Figure 15
Autoradiograph showing a ^{32}P labelled RNA probe with a specific activity of 1·33×10^9dpm/µg, produced with Amersham's Amprobe™ system for the human *sis* proto-oncogene hybridized with 10µg of human DNA. The DNA was digested with A) *Bam*HI, B) *Eco*RI and C) *Hin*dIII. Autoradiography was at −70°C for 16 hours with an intensifying screen.

Recent developments in transcription vectors

Probably the most significant disadvantage of RNA polymerase based labelling is the requirement to subclone an insert into a vector containing an appropriate promoter. However, once an insert has been cloned into such a vector, DNA probes may still be prepared using nick translation or random primer labelling. A variety of transcription vectors have been developed that can be used as primary cloning vehicles. Two general approaches are discernable. Plasmid vectors are available that, in addition to the presence of one or two RNA polymerase promoters, have some of the following features:

(i) they contain antibiotic resistance markers
(ii) they allow recombinant selection by α-complementation of ß-galactosidase[62]
(iii) they contain a single strand bacteriophage-derived origin of replication. This allows production of single-stranded DNA in the presence of an appropriate helper phage, thus facilitating sequencing by the dideoxy method[20]. An alternative approach is to use 3'-dNTPs as chain terminators during transcription by SP6, T7 or T3 RNA polymerases[3,66].

A different route to vector construction has been to clone one or more phage polymerase promoters into a single-stranded bacteriophage such as M13. While retaining colour selection and readily allowing dideoxy sequencing, such vectors provide a means of avoiding a further disadvantage of plasmid-based systems: the requirement to linearize the template in order to limit transcription to the sequence of interest. This is achieved by using the single-stranded form of the phage as the initial substrate for the transcription reaction, as outlined in figure 16 for the mICE series of vectors originated by Eperon[23].

Flowchart of the RNA polymerase based method with single-stranded mICE DNA

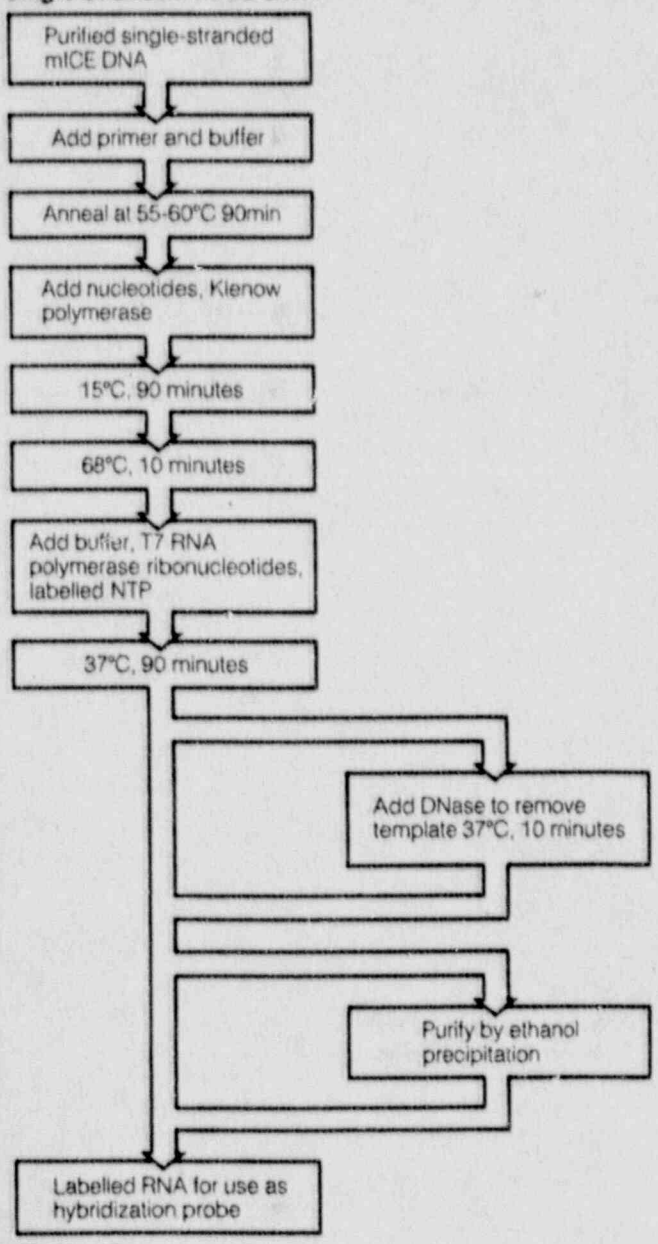

Figure 16

Use of mICE vectors

The reaction

By annealing an appropriate oligonucleotide primer and copying the strand using Klenow polymerase in the presence of dNTPs, a partial duplex is formed (see figure 17). This serves as an efficient substrate for T7 RNA polymerase which recognises the double-stranded form of the T7 promoter and terminates synthesis at the end of the double-stranded region. The use of an oligonucleotide as a transcription terminator allows increased flexibility in choice of run-off point, which is no longer confined to a known restriction enzyme site.

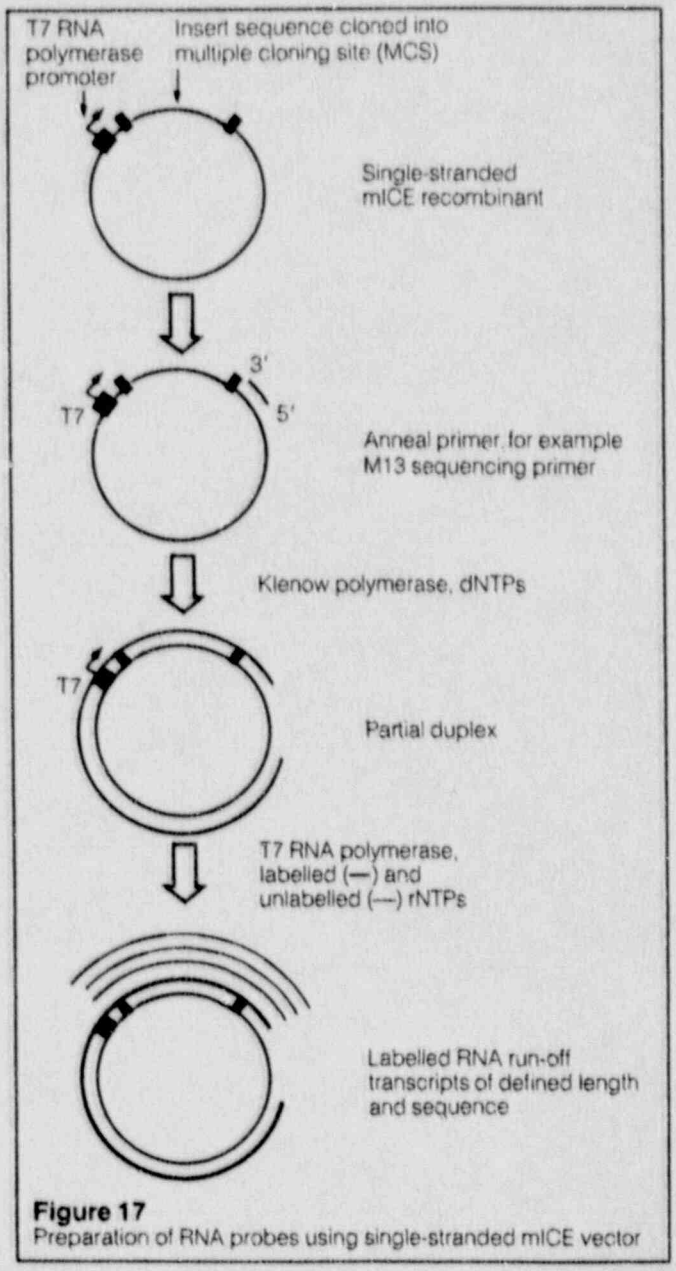

Figure 17
Preparation of RNA probes using single-stranded mICE vector

Nature of substrate
The single-stranded starting material is more readily prepared than is a double-stranded phage or plasmid. Furthermore, as phage RNA polymerases appear to work more efficiently with circular than with linear DNA substrates, it is possible to obtain high levels of incorporation at approximately five-fold lower DNA concentrations than those used in standard transcription reactions. Transcription may also be carried out in the traditional manner by using the double-stranded replicative form of the phage.

Nature of probe
It has been observed at Amersham that, when compared with conventional systems, a higher proportion of full length transcripts is usually produced in the mICE system in the presence of limiting nucleotide concentrations. Although this has not been fully explained, it may be partially due to the lower DNA:nucleotide ratio in this system. Some of the advantages of the mICE system over standard plasmid systems are summarized in table 5.

Table 5. Advantages of mICE vectors over standard phage polymerase plasmid vectors.

1. No restriction digestion (and subsequent purification) is required.
2. Transcription termination not limited to known restriction site.
3. Use of oligonucleotides allows a series of overlapping transcripts to be produced.
4. Efficient transcription from circular template allows reduced concentration of input DNA to be used.
5. Higher proportion of full-length transcripts.
6. Single-stranded template more readily prepared than plasmid.
7. Flexibility of reaction possible – may use single or double-stranded template.
8. Colour selection for recombinants.
9. Can be used directly for sequencing and mutagenesis.

Products for RNA polymerase based methods

SP6 system see page 45
Paired promoter SP6 system see page 45
Paired promoter T7 system see page 45
M13 T7 transcription system see page 46
^{32}P, ^{35}S and ^{3}H labelled ribonucleotides see pages 46, 47
SP6/T7 Grade ^{32}P- and ^{35}S-labelled ribonucleotides see page 46
SP6 RNA polymerase see page 47
T7 RNA polymerase see page 47

4. End labelling of nucleic acids

A wide variety of techniques is available for introducing label at either the 3'- or 5'-ends of linear DNA or RNA. Usually only a single label is introduced, so that the specific activities achievable by such techniques are significantly lower than those obtained by the uniform labelling methods discussed in the previous sections. An exception to this is T4 DNA polymerase, which can also be used to introduce a uniform label.

Some of the more commonly used methods for end labelling both DNA and RNA will now be described, followed by a general discussion of their applications. These are considered under the categories, 5'-end labelling, 3'-end labelling and end repair, although the latter is simply a subset of 3'-end labelling.

A. 5'-end labelling method
T4 polynucleotide kinase based

B. 3'-end labelling method
(1) Terminal deoxynucleotidyl transferase based
(2) Other 3'-end labelling methods

C. End repair methods
(1) Klenow based
(2) DNA polymerase I based
(3) T4 DNA polymerase based

A. 5'-end labelling method

T4 polynucleotide kinase (PNK) based method

RNA and DNA may be 5'-end labelled using T4 polynucleotide kinase. Probably the most common use for this reaction is to label restriction fragments for Maxam and Gilbert sequencing[56]. The enzyme catalyzes a reversible reaction outlined below.

The reaction
T4 polynucleotide kinase catalyzes the transfer of the γ-phosphate of a ribonucleoside 5'-triphosphate donor to the 5'-hydroxyl group of a polynucleotide, oligonucleotide or nucleoside 3'-phosphate[72]. For end labelling, [γ-^{32}P]ATP is most frequently used as donor. The radiolabelled γ-phosphate group is transferred to DNA or RNA containing a 5'-hydroxyl terminus. This is termed the 'forward' reaction. However, most polynucleotides have a 5'-phosphate group which must be removed using alkaline phosphatase before they can be used as substrates for this reaction. This step may be avoided by

carrying out an 'exchange' reaction[5] with T4 polynucleotide kinase (see figure 18). In this case the reaction is driven by excess ADP which causes the enzyme to transfer the terminal 5'-phosphate from DNA to ADP. The DNA is then rephosphorylated by transfer of the labelled γ-phosphate from [γ-^{32}P]ATP. Although this reaction is more convenient than dephosphorylation followed by the forward reaction, it usually occurs at a lower efficiency.

Nature of the substrate

Both single and double-stranded nucleic acids can be used as substrates. Generally the labelling of blunt or recessed 5'-ends in duplex DNA is less efficient than that of protruding ends, particularly in the case of the exchange reaction, while 5'-hydroxyl ends at nicks in duplex DNA are usually very poor acceptors. Removal of a 5'-cap from RNA is essential for 5'-end labelling[78]. [γ-^{35}S]ATPγS can also be used as donor, although higher enzyme concentrations are required for efficient reaction rates.

Specific activities

Specific activities of 5×10^5dpm/picomole ends and 8×10^5dpm/picomole ends can be achieved with the exchange and forward reactions respectively using blunt-ended DNA fragments.

Advantages and disadvantages of 5'-end labelling

The major advantages of 5'-end labelling are that:
(i) both DNA and RNA can be labelled
(ii) oligonucleotides can be conveniently labelled
(iii) the location of labelled group is known
(iv) very small fragments of DNA/RNA can be labelled
(v) restriction digest fragments may be labelled so that several probes can be prepared at once.

A major practical disadvantage of this method is the necessity for prior dephosphorylation of substrate if the forward reaction is to be used. As with all end labelling methods, the achievable specific activity depends solely on the specific activity of labelled nucleotide used and cannot be adjusted by adding more enzyme (assuming all available ends are labelled).

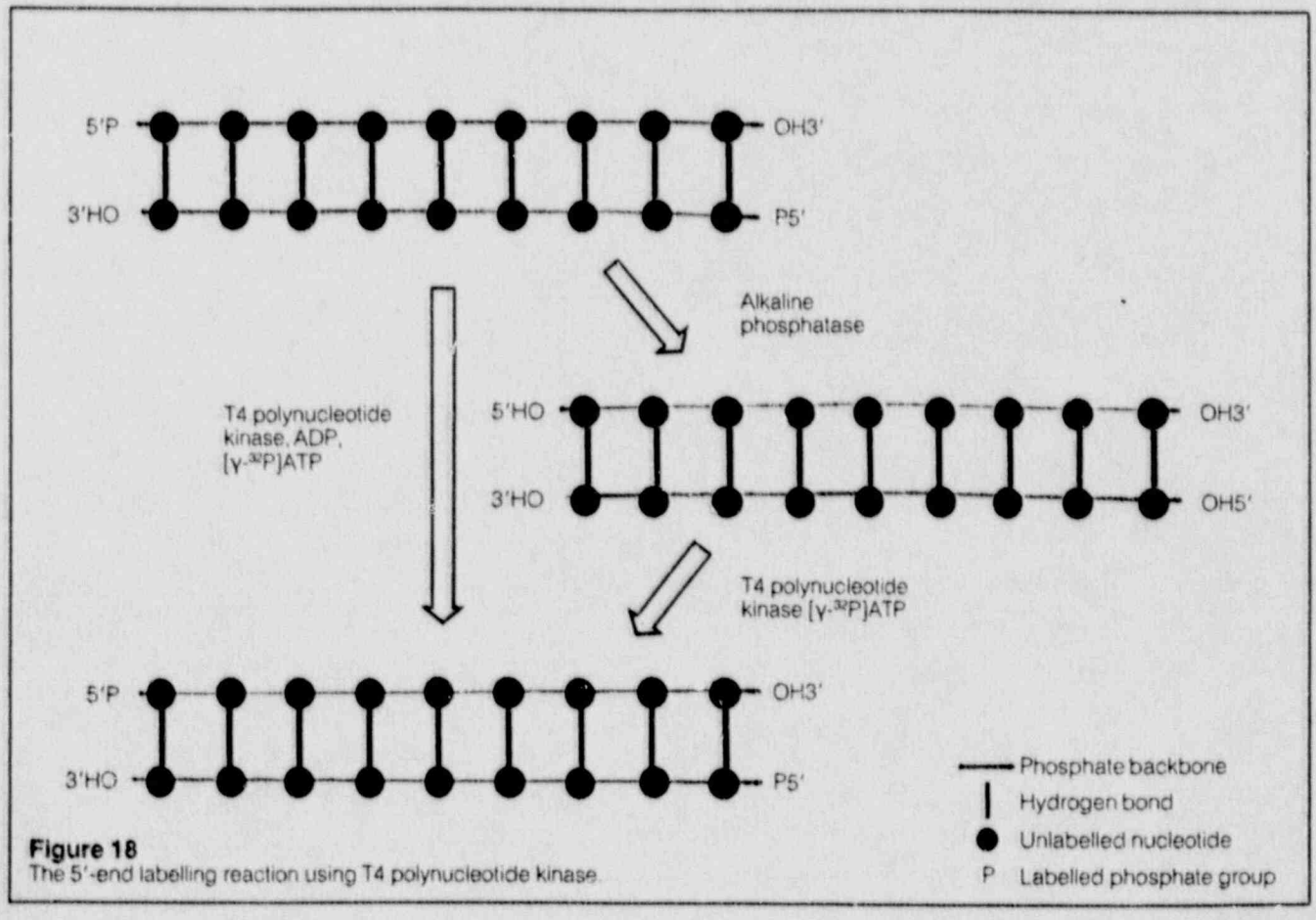

Figure 18
The 5'-end labelling reaction using T4 polynucleotide kinase.

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B. 3′-end labelling methods

(1) Terminal deoxynucleotidyl transferase (TdT) based method

Terminal deoxynucleotidyl transferase adds deoxyribonucleotides onto the 3′-ends of DNA fragments. It can be used in conjunction with ^{32}P, ^{35}S or ^{3}H-labelled nucleotides to 3′-end label DNA for a variety of applications.

The reaction

This enzyme does not require a template, but will simply add a series of supplied deoxynucleotides onto the 3′-end of (preferably) single-stranded DNA[6]. When employing this enzyme to incorporate a single end label, the most reliable approach, developed at Amersham, is to use a dideoxynucleoside 5′-triphosphate (usually [α-^{32}P]ddATP) as precursor (see figure 19). As this lacks a 3′-hydroxyl group, polymerization stops when one residue has been added[94]. The reaction is illustrated in figure 20.

Before the adoption of a dideoxynucleotide terminator, cordycepin 5′-[α-^{32}P]triphosphate ([α-^{32}P]KTP) (see figure 19) was frequently used as a labelled terminator with this enzyme. KTP, although also lacking a 3′-hydroxyl group, does have a hydroxyl group at the 2′-position and is a rather poor substrate for terminal transferase. [α-^{32}P]ddATP (see figure 19), with a 2′-deoxy as well as a 3′-deoxy group, is a much more efficient substrate. Table 6 illustrates some efficiencies obtained using terminal transferase with [α-^{32}P]KTP and [α-^{32}P]ddATP to label 3′-ends, and using T4 polynucleotide kinase and [γ-^{32}P]ATP to label 5′-ends. The results demonstrate that, for all three categories of ends, the labelling efficiency using [α-^{32}P]ddATP is three to four times greater than with [α-^{32}P]KTP. They also show that for 3′-protruding and blunt ends, 3′-end labelling with [α-^{32}P]ddATP is significantly more efficient than 5′-end labelling of the complementary strand with [γ-^{32}P]ATP. Figure 21 shows an autoradiograph of 3′-end labelled restriction fragments using [α-^{32}P]ddATP.

Nature of substrate

Single-stranded and double-stranded DNA are substrates for TdT, although the end labelling reaction is most efficient with single-stranded DNA of at least three residues. Recessed 3′-ends can be labelled if a cobalt ion cofactor is supplied[19].

(2) Other 3′-end labelling methods

Both poly(A) polymerase[80] and T4 RNA ligase[22] are used to label the 3′-end of RNA molecules, usually in preparation for chemical[67] or enzymatic[21] sequencing.

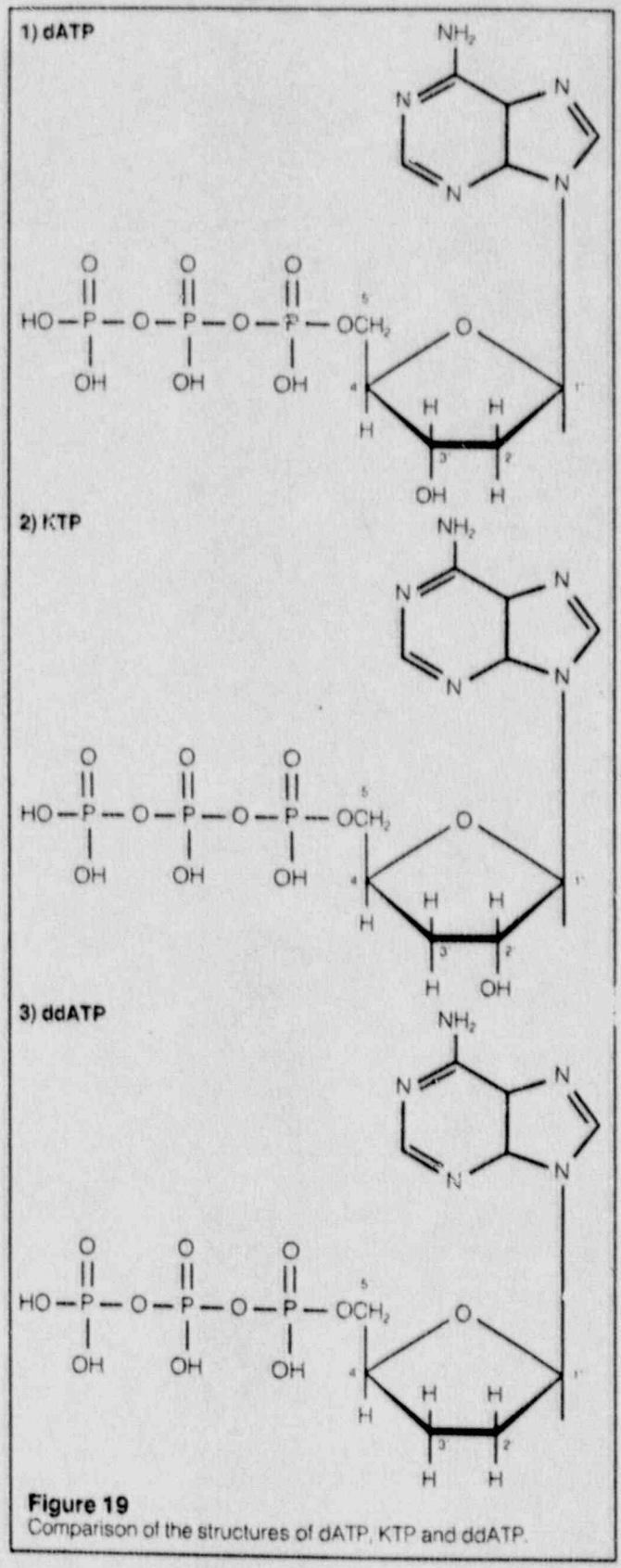

Figure 19
Comparison of the structures of dATP, KTP and ddATP.

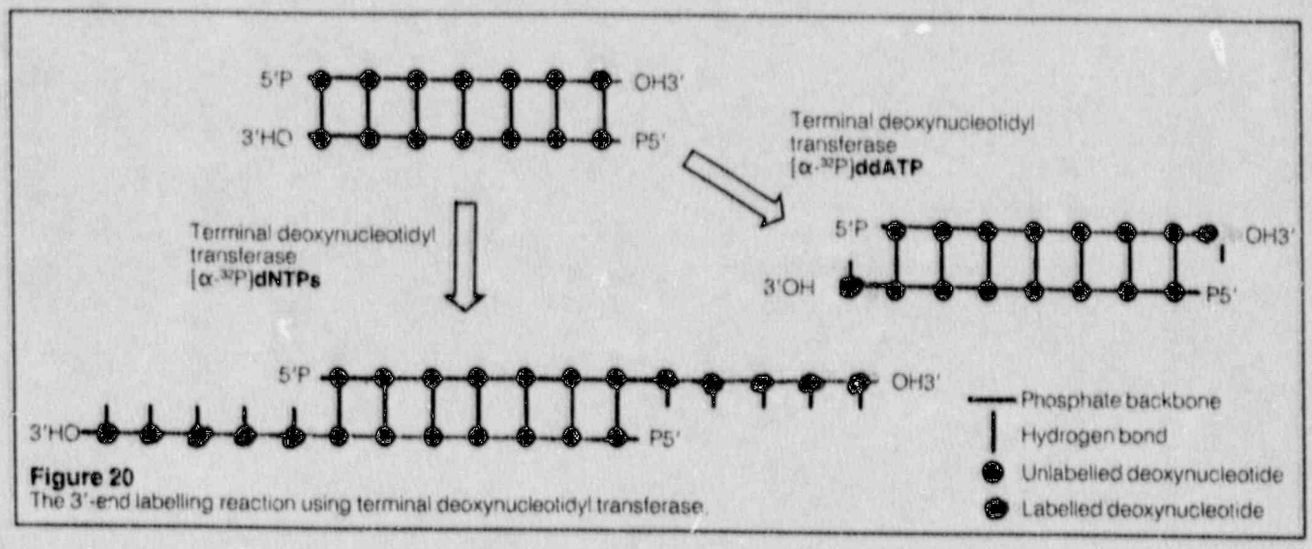

Figure 20
The 3'-end labelling reaction using terminal deoxynucleotidyl transferase.

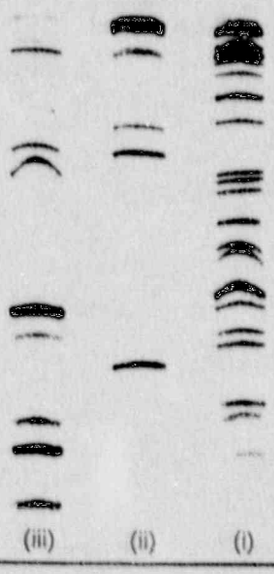

Figure 21
Autoradiograph of restriction fragments labelled using Amersham's 3'-end labelling kit and separated on a 6% polyacrylamide gel.
(i) *Pst*I digest of λ-protruding 3'-ends
(ii) *Hin*dIII digest of λ-recessed 3'-ends
(iii) *Alu*I digest of pBR322-blunt 3'-ends

Table 6. Comparison of 3'-end labelling efficiencies with different labels.

Type of 3'-end	Type of lambda digest	Counts incorporated per 10 picomoles of DNA ends (dpm×10⁻⁶)		
		5'-ends labelled with [γ-32P]ATP	3'-ends labelled with [α-32P]ddATP	[α-32P]KTP
Protruding	*Pst*I	1·9	16·6	6·0
Recessed	*Hin*d III	24·5	12·6	3·5
Blunt	*Alu*I	1·4	61·5	16·5

C. End repair methods

(1) Klenow based method

In the presence of suitable deoxynucleotides (dNTPs), the 5'-3' polymerase activity of the Klenow fragment can be used to fill in from a recessed 3'-end produced by restriction endonuclease cleavage, using the corresponding 5'-extension as template (see figure 22). The product is a blunt-ended molecule. All four supplied dNTPs may be radioactive, so relatively high specific activity probes can be produced. When used for this application, it should be remembered that the enzyme will continue to carry out pyrophosphate exchange (removal of terminal nucleotide by 3'-5' exonuclease activity followed by repolymerization) when all residues have been filled in. At low nucleotide concentrations, this can cause conversion of all free dNTP corresponding to the terminal nucleotide to dNMP, resulting in loss of the terminal nucleotide. For this reason short reaction times (10-15 minutes at room temperature) are recommended. Termination by methods other than heat inactivation is also advisable, as raised temperature increases the rate of the exchange reaction.

Klenow polymerase may also be used to label blunt ends, as the 3'-5' activity of the enzyme is adequate for the removal of terminal 3' nucleotides, allowing subsequent replacement by a labelled equivalent.

The major advantages of the method are that:
(i) it can generally be used after restriction digestion with no intermediate purification.
(ii) relatively high specific activities can be achieved.

The major disadvantage is that it cannot be used efficiently for 3'-overhangs.

(2) DNA polymerase I based method

Although Klenow polymerase is most frequently used, DNA polymerase I holoenzyme is still sometimes used for filling in restriction fragments with 3'-recessed ends. In this case it is necessary to inhibit the 5'-3' exonuclease activity using high salt and low temperature.

The enzyme should not be heat-inactivated as this may lead to transient activation of the 5'-3' exonuclease.

(3) T4 DNA polymerase based method

T4 DNA polymerase has similar properties to the Klenow fragment in that it possesses a 5'-3' polymerase and a 3'-5' exonuclease activity[38, 50]. However, the turnover number for the exonuclease activity of the T4 enzyme is about 250-fold greater than that of the Klenow fragment. This has the practical advantage that it is possible to control experimentally which of the two activities is to predominate simply by omitting or providing deoxynucleoside triphosphates. The enzyme can be used to label DNA in two distinct ways:
(i) 3'-end labelling of DNA.
(ii) labelling by replacement synthesis.

(i) 3'-end labelling of DNA

Using T4 DNA polymerase it is possible to control the precise position of labelling by selective addition of deoxynucleotides. Either [α-^{32}P] or [α-^{35}S] deoxynucleoside triphosphates can be used in this reaction. Three approaches are outlined below.

Recessed 3'-end

In this case the polymerase activity will synthesize a strand complementary to the 5'-overhang. It is thus only

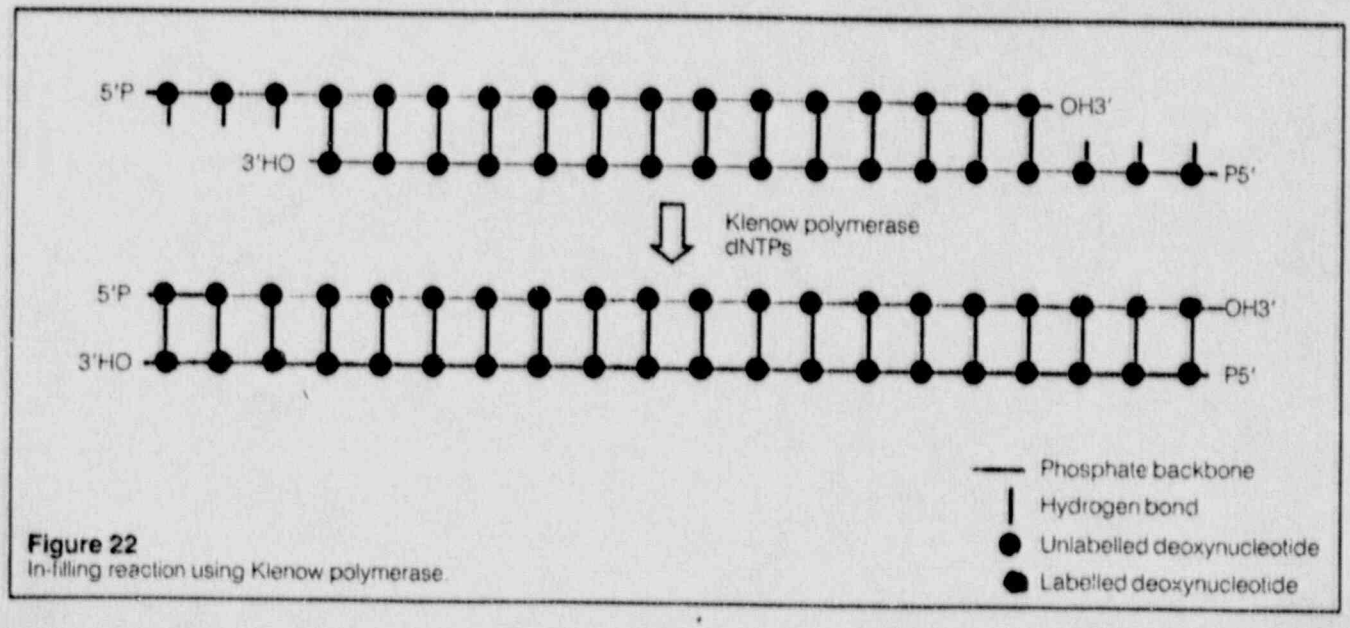

Figure 22
In-filling reaction using Klenow polymerase.

necessary to include a radiolabelled nucleotide complementary to the first residue in the overhang to achieve effective labelling. If all four deoxynucleotides are added the product will be a blunt-ended molecule labelled at or near the terminus. The Klenow fragment of DNA polymerase I will also carry out this reaction.

Blunt end
The enzyme is used in the presence of only one labelled deoxynucleotide. Nucleotides are removed from the 3'-end by the 3'- 5' exonuclease activity until a residue identical to the radiolabelled nucleotide is reached. When this nucleotide is removed, the 5'- 3' polymerase activity of the enzyme inserts a labelled nucleotide in its place. A continuous series of exchange reactions now occurs at this point, the overall effect of which is to produce a molecule labelled at its 3'-end. If the nucleotide used is identical to the initial 3'-residue of the molecule, a labelled blunt-ended molecule will be produced. If any other labelled nucleotide is chosen, then the molecule will be left with a 5'-overhang.

Protruding 3'-end
The 3'-overhang will be removed by the powerful 3'- 5' exonuclease activity until the molecule is blunt-ended, from which point the reaction will proceed as above.

These reactions are most widely used to label restriction enzyme fragments. It is often possible to carry out the initial restriction digestion in T4 polymerase assay buffer to simplify the procedure. However, not all restriction enzymes are active in this buffer and it is usually advisable to carry out a small scale pilot reaction first. It may then be found necessary to include a phenol/chloroform extraction and an ethanol precipitation between the two stages.

(ii) Labelling by replacement synthesis
DNA can be labelled to high specific activity in a two stage reaction. Initially, long recessed 3'-termini are produced by incubation of the DNA with T4 polymerase in the absence of added dNTPs. When these are provided in the second stage, the polymerase activity uses the DNA as a primer-template for the re-synthesis of sequences complementary to the long 5'-overhangs. Such molecules are frequently used as hybridization probes.

The main advantages of the method are:
(i) no hairpin structures occur, in contrast to nick translation.
(ii) either end of the molecule can be isolated following restriction enzyme digestion and gel purification for use as a strand-specific probe.

The main disadvantage is that the first stage must not be allowed to proceed to the centre of the molecule. Reaction conditions are therefore generally chosen to favour incorporation of label near the ends. Hence, uniform labelling is not achieved and, in a mixture of restriction fragments, the extent to which all molecules can be labelled is dictated by the size of the smallest fragment present.

Applications of end-labelled DNA

The applications of end-labelled molecules are somewhat different from those described for uniformly labelled nucleic acids. Although molecules end-labelled with ^{32}P can be used in the detection of mammalian single copy genes, more rapid results will be achieved if a uniform labelling method is employed. Oligonucleotides 5'-end labelled with ^{32}P have been used for both single copy gene detection[13] and the detection of single base mismatches in gel blots or in plaque/colony lifts[95]. 5'-end labelling with T4 polynucleotide kinase is the most convenient means of introducing label into oligonucleotides (see figure 23).

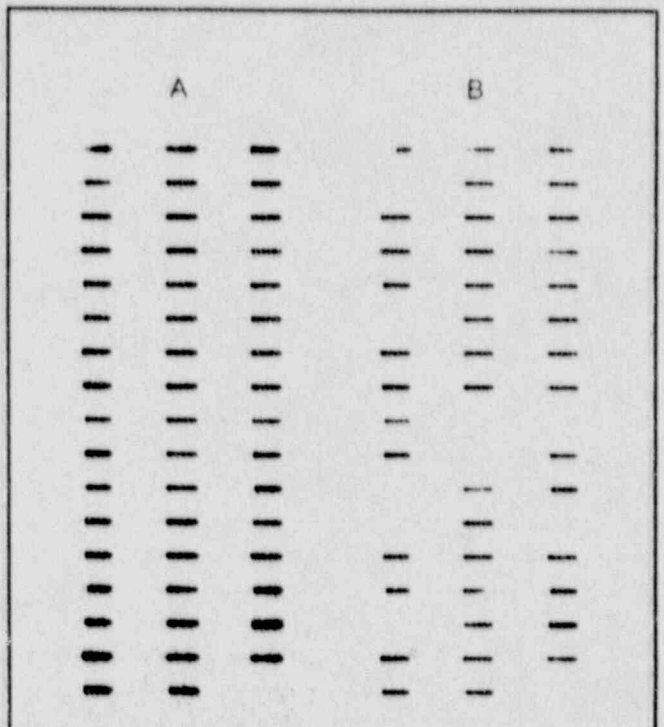

Figure 23
Autoradiograph showing the use of a [γ-^{32}P] ATP 5'-end labelled mutant oligonucleotide probe to detect a single base pair mismatch in the chicken lysozyme gene. The mismatch was introduced using Amersham's *in vitro* mutagenesis system. The nitrocellulose membrane (Hybond C) was autoradiographed using Hyperfilm-MP, after washing at room temperature (A) and at 56°C (B). (At 56°C the mutant oligonucleotide will remain annealed to the mutant template but will dissociate from the wild type template.)

End labelling is used for the most part in situations where direct detection of the labelled molecule (for example by autoradiography) is to take place and where an initial intact molecule is desirable. For example, restriction fragments with recessed 3'-ends can be readily end-labelled with Klenow polymerase and used either as gel markers or for further restriction mapping studies. More detailed mapping by the S1 nuclease technique[93] can also be carried out using an end-labelled restriction fragment.

A major application for end-labelled nucleic acids is sequencing; both enzymatic[21] and chemical[67] methods of RNA sequencing, and the Maxam and Gilbert method for DNA sequencing[56] utilize end-labelled molecules (see page 38). The option of labelling either the 3'- or 5'-ends allows the sequence to be read from either end of the molecule. A disadvantage of this technique with double-stranded DNA is that two 3'- or 5'-ends will be labelled. To avoid overlapping sequences, it is necessary to remove one labelled fragment by either a strand separation gel or restriction digestion followed by gel purification. An alternative is to subclone into a vector that allows only one end of a restriction fragment to be labelled (for example by using an enzyme that cuts at indeterminate residues within its recognition sequence so that a different sequence can be present at either end of an excised insert[89]).

Although not strictly comparable with uniform labelling methods, a table of advantages and disadvantages of end labelling methods in general is given in table 7.

Table 7. Advantages and disadvantages of end labelling approaches.

Advantages

1. Suitable for mapping studies and for sequencing.
2. Fragments remain intact (for example, for gel markers).
3. Most convenient method available for oligonucleotides.
4. Choice of position of label (3' or 5').
5. Variety of enzymes available.

Disadvantages

1. Lower specific activities achievable.
2. Particularly sensitive to contaminating exonuclease activity.

Products for end labelling methods

3'-end labelling kits see page 45
[α-^{32}P]ddATP see page 46
^{32}P-, ^{35}S- and ^{3}H-labelled nucleotides see pages 46, 47
T4 polynucleotide kinase see page 47
Terminal deoxynucleotidyl transferase see page 47
Poly(A) polymerase see page 47
T4 RNA ligase see page 47
E.coli DNA polymerase I 'Klenow' fragment see page 47
T4 DNA polymerase see page 47
E. coli DNA polymerase I see page 47

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5. Direct labelling

The labelling methods described so far have primarily utilized enzymatic reactions to incorporate nucleotides labelled with radioisotopes or non-radioactive reporter molecules, for example biotin. In this section, several examples of direct labelling methods which do not use enzymes to incorporate label are briefly outlined.

The aim of these methods is to attach the label covalently to the nucleic acid probe. However, non-covalent approaches such as intercalation of ethidium bromide are also used. For covalent attachment, both photoactivatable and chemically reactive groups have been used and examples of these methods are shown below.

A. Photoactivatable analogues

For use in non-radioactive labelling, a photoactivatable analogue of biotin has been synthesized and used for rapid preparation of relatively large amounts of DNA and RNA hybridization probes[27]. Upon brief irradiation with visible light, stable linkages are formed with both single-stranded and double-stranded nucleic acids.

Another class of important photochemical reagents for investigation of nucleic acid structure and function are the psoralens. They primarily react with pyrimidine bases and can be detected by enzymatic methods[77].

B. Direct crosslinking of enzymes

Peroxidase and alkaline phosphatase have been directly crosslinked to DNA, thereby obviating the requirement for a reporter group such as biotin[39,71]. For example horseradish peroxidase can be crosslinked to polyethyleneimine with p-benzoquinone and the resulting conjugates covalently linked to DNA using glutaraldehyde[71].

C. Other chemical methods

These include modification of nucleic acids by acetylaminofluorene[86], detection by labelled antibodies, and 5'-end labelling of oligodeoxynucleotides with biotin through an aminoalkylphosphoramide linker arm[12,42]. It is also possible to carry out direct radioiodination of nucleic acids.

Whilst direct labelling may offer more convenience in preparation of probes than enzymatic methods, there is currently little published data on their application and sensitivities achievable.

Summary

The most frequently used methods for uniformly labelling hybridization probes are nick translation, random primer labelling and *in vitro* transcription using phage polymerases. The characteristics of these reactions are summarized in tables 8 and 9. Table 8 details the components of the reactions whilst table 9 lists their major properties. The applications for which these methods are most appropriate are summarized in table 10. Suitable applications for unique primer labelling are also given. A comparable summary of applications for end-labelled nucleic acids is given in table 11.

The remainder of this booklet is largely concerned with these applications, and discusses in particular the major factors that determine the choice of labelling strategy. For convenience these applications will be grouped in four main categories, which encompass the major analytical techniques employing labelled nucleic acids:
(i) Filter hybridization
(ii) *In situ* hybridization
(iii) Nucleic acid sequencing
(iv) Mapping of nucleic acids

The adoption of an appropriate labelling strategy for any of these applications requires the correct choice of both label and labelling method. In chapter 3, those factors affecting the choice of label are discussed, while in chapter 4, factors that are relevant to the choice of labelling methodology are covered. In addition, chapter 4 briefly discusses other features of the above applications that can affect the behaviour of the labelled nucleic acid. Finally, chapter 5 summarizes these considerations and provides guidelines to assist the reader in choosing a labelling strategy.

ITEM 6C

PROCEDURES FOR THE DETECTION OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS IN HUMAN DNA

FBI Laboratory

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I. ISOLATION OF DNA FROM LIQUID BLOOD SAMPLES

1. Liquid blood specimens should be collected in an EDTA vacutainer tube. Mix well before removing aliquots. Blood can be stored at 4C for five days at most before being aliquotted and frozen. Blood is frozen (-80C) in 700 uL aliquots in Sarstedt tubes (screw caps).

2. To the thawed blood add 800 uL 1X SSC and mix. Spin 1 minute in microfuge.

3. Remove and discard 1.0 ml supernatant into disinfectant.

4. Add 1.0 ml 1X SSC, vortex, centrifuge 1 minute, remove all supernatant fluid. Do not remove pellet.

5. To the pellet add: 375 uL 0.2M NaAcetate and vortex briefly.
25 uL 10% SDS
5 uL Proteinase K (20 mg/ml H_2O)
Vortex briefly (1 sec)
Incubate at 56C for 1 hour

NOTE: Processing of the allelic control specimens begins at this step. See Appendix D.

6. Add 120 uL phenol/chloroform/isoamyl alcohol; vortex 30 sec. This step must be carried out in the fume hood!

7. Spin 2 minutes

8. The aqueous layer (on top) is carefully removed and placed in a new 1.5 ml Eppendorf tube. Do not remove the layer of denatured protein that collects at the interface. The new tube now contains the DNA; the old tube containing the phenol and denatured protein can be discarded.

9. To the aqueous layer add 1.0 ml cold, absolute EtOH
Mix by inversion of the tube - At this stage you should see a DNA precipitate. Note: Tubes can be stored at 4C at this stage.
Spin 30 seconds.

10. Remove and discard the supernatant fluid by decantation.

11. To the pellet add 180 uL TE^{-4}.
Vortex.
Incubate at 56C for 10 minutes.
Add 20 uL 2.0M NaAcetate and mix 5 seconds by hand.

12. Add 500 uL cold, absolute EtOH
Mix gently by hand to achieve homogeneous solution.
Spin 10 seconds.
Remove supernatant fluid by decantation.

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13. Wash pellet with 1.0 ml room temperature 70% EtOH.
Spin 30 seconds. Decant supernatant fluid.
Remove the maximum quantity of EtOH with a micropipette (yellow tip).

14. Place tubes in Speed-Vac centrifuge to remove excess EtOH. This process should take about 5 minutes.

15. Add 200 uL TE^{-4}, mix, and incubate at $56^{0}C$ overnight.
Next morning, vortex for 30 seconds.

16. The DNA is ready for quantification by spectrophotometry.

II. ISOLATION OF DNA FROM BODY FLUID STAINS

1. Cut the stain into small pieces and place the pieces into a 1.5 ml tube that has a depression in the cap (Sarstedt).

2. Add 400 uL stain extraction buffer.
   Add 10 uL Proteinase K
   Mix and spin 2 seconds to force cutting into liquid.

3. Incubate at 56^{0}C overnight

4. Punch a hole in the lid and place the cutting pieces in the lid. Spin 5 minutes. Remove the cutting pieces and the cap. Place a new cap on the tube.

5. Add 500 uL phenol/chloroform/isoamyl. This step must be done in the fume hood. Shake the tube vigorously by hand to achieve a milky emulsion in the tube. Spin the tube for 2 minutes.

6. Transfer aqueous phase (top layer) to a new tube. Do not disturb the interface. Discard old tube containing the phenol in the waste box in the hood.

7. To the aqueous layer add 1.0 ml cold absolute EtOH.

8. Mix by hand and place the tube at -20C for 1/2 hour.

9. Warm the tube to room temperature and spin 15 minutes.

10. Remove the alcohol by decantation.

11. Wash pellet with 1.0 ml room temperature 70% EtOH.

12. Spin 5 minutes.

13. Remove the alcohol by decantation. Remove remaining alcohol with a micropipette (yellow tip).

14. Place tube in Speed-Vac to remove remaining EtOH.

15. Resolubilize the DNA in 36 uL TE^{-4} at 56C for from 2 hours to overnight.

III. TEST GEL FOR ASSESSING THE QUALITY AND QUANTITY OF DNA ISOLATED FROM BODY FLUID STAINS

1. The DNA at this stage has been solubilized in 36 uL of TE^{-4} during an incubation at 56°C for from 2 hours to overnight.

2. Remove 4 uL DNA and combine with 2 uL loading solution. Spin 2 sec.

3. Preparation of test gel.

Three sizes of test gels can be run, and each size gel can have multiple origins. The size used and number of origins depends on the number of specimens that one needs to test.

Gel size	Gel vol (ml)	Wells/origin	Origins
Baby (6 X 8.3)	25	14	2
Medium (11 X 14)	100	14/15	2
Large (20 X 25)	325	25	3

All gels use 1% agarose (Sigma type II or Seakem ME) in 1X TAE buffer supplemented with ethidium bromide (EB) at a ratio of 10 uL EB/ 100 ml TAE.

Gel volume (ml)	g agarose/gel	uL EB
25	0.25	2.5
100	1.00	10.0
325	3.25	32.5

Add TAE/EB to the agarose.
Bring to a boil to dissolve agarose.
Equilibrate at 56°C.
Pour agarose into gel form (be sure comb is in place).
Let stand 15 minutes to gel.

4. Pour 1X TAE buffer into electrophoresis tank. The TAE buffer should be supplemented with ethidium bromide (EB) at a ratio of 10 uL EB/ 100 ml buffer.

5. Place the gel into the tank. Enough buffer should be present to cover the gel. Remove comb.

6. The DNA sample mixed with loading solution (6 uL total volume) is pipetted into the well with the gel submerged. Do not

poke the pipette tip through the bottom of the gel!

If the test gel procedure is to be quantitative, DNA standards must be included on the gel. Preparation of the standards is given in the Reagent Section. Load 6 uL of each concentration.

7. Set the voltage at 200 volts. When the bromophenol blue tracking dye has moved 1-2 cm from the origin, the run can be stopped. For 11 X 14 gels, this should be about 20 minutes.

8. Remove the gel from the tank. Examine the gel on the ultraviolet light transilluminator. Intact DNA will move as a band not far from the origin. A smear from the origin to, or past the dye front indicates that the DNA has been fragmented and may not be suitable for restriction. Take a photograph of the gel. Polaroid # 553, f4.5, 1 second, red filter in place. DO NOT EXPOSE YOURSELF TO THE UV LIGHT FOR AN EXCESSIVE AMOUNT OF TIME. ALWAYS WEAR THE FULL FACE SHIELD WHEN WORKING WITH THE TRANSILLUMINATOR.

9. From the photograph, assess the quantity of DNA in test specimens by comparison with the DNA standards. Multiply your estimate by 8 to obtain the total quantity of DNA in the remaining 32 uL of sample.

IV. QUANTIFICATION OF DNA OBTAINED FROM LIQUID BLOOD SAMPLES BY SPECTROPHOTOMETRY

The DNA obtained from a liquid blood sample has been solubilized in 200 uL TE^{-4} (Section I, step 15). The following description of instrument operation is applicable to the Beckman DU 7 only.

1. The concentration of DNA in the sample will be determined on a Beckman DU 7, using a microcuvette that enables the absorbancy of samples as small as 50 uL to be measured. This instrument remains on "idle" when not being used. To activate, push the "ON" button. Follow the menus to set the instrument to read dual wavelengths of 260 and 280 nm and to determine the ratio 260/280.

2. The instrument will self calibrate and then request that you put in the solvent blank. Pipette 50 uL TE^{-4} into the microcuvette. Rap the cuvette gently on the lab bench to remove any air bubbles. Place the cuvette into the sample holder and push "START". The instrument will determine the absorbancy at both 260 and 280 nm and store these readings for the entire working session.

3. Empty the cuvette. There is no need to rinse. Place 50 uL of the DNA sample in the cuvette, put the cuvette in the instrument and push "START". Both absorbancies will be measured and their ratio calculated. At the end of the session you can print out the entire list of values by pushing "COPY."

4. Pipette the 50 uL of sample back into the original sample tube. Rinse the cuvette with TE^{-4} before the next sample is added to the cuvette.

5. Data reduction:

Assume the readings were - $A_{260} = 1.80$
$A_{280} = 1.00$

and the absorbancy ratio - 1.80/1.00 = 1.8

The DNA content of the sample is calculated as follows:

$(A_{260})(50^{*})(0.2)$ = ug DNA/200 uL

(1.80)(50)(0.2) = 18.0 ug DNA

*50 ug DNA/ml yields an A_{260} of 1.0

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V. DIGESTION OF DNA WITH HAE III

Two digestion procedures are given below. One procedure is to be used for the digestion of DNA recovered from body fluid stains; whereas the other should be used if digesting the much larger quantity of DNA recovered from a liquid blood sample.

A. Body fluid stain DNA

1. There should be 32 uL DNA specimen remaining after test gel.

2. Combine the following in the DNA specimen tube:

32 uL	DNA
4 uL	restriction buffer concentrate (REact 2)
4 uL	HAE III (40 international units)
40 uL	

Mix by hand and spin 2 seconds.

NOTE: The volume of restriction enzyme added should never be more than 10% of the final digestion volume. Also, do not permit the HAE III (or any restriction enzyme, for that matter) to warm up. Always keep the enzyme on ice.

3. Incubate at 37C overnight.

B. Liquid blood DNA

1. DNA recovered from liquid blood should be in a volume of 200 uL TE^{-4}.

2. Combine the following in the DNA specimen tube:

200 uL	DNA
25 uL	restriction buffer concentrate (REact 2)
x uL	HAE III
y uL	H_2O
250 uL	

Where: $x = (5)(\text{ug DNA})/(\text{units HAE III/uL})$

$y = 25 - x$

3. Incubate at 37^0C overnight.

VI. REPRECIPITATION OF DIGESTED DNA

A. Body fluid stain DNA

1. To the 40 uL of DNA digest, add 13 uL of 7.0M ammonium acetate. Mix by hand.

2. Add 106 uL cold absolute EtOH and mix by hand.

3. Place tube at -20°C for 15-30 minutes. Don't let the tube freeze.

4. Spin tube 15 minutes. Decant the alcohol.

5. Rinse pellet with 1000 uL room temperature 70% EtOH. Spin for 5 minutes and decant supernatant fluid. Remove remaining EtOH with a micropipette (yellow tip).

6. Put tube in Speed-Vac to remove remaining alcohol. This should take about 5 minutes.

7. Add 16 uL TE^{-4} to the tube and place at 56°C to dissolve the DNA. After restriction the DNA should solubilize quickly.

8. DNA is now ready for another test gel (this one to assess the completeness of restriction) and then an analytical gel.

B. Liquid blood DNA

1. To the 250 uL of restriction digest, add 83 uL 7M NH_4OAc and mix.

2. Add 666 uL 100% EtOH and mix by hand.

3. Place tube at -80°C for 15-30 minutes. Don't let the tube freeze.

4. Spin tube 15 minutes. Decant the alcohol.

5. Rinse pellet with 1000 uL room temperature 70% EtOH. Spin and decant supernatant fluid. Remove remaining EtOH with a micropipette (yellow tip).

6. Put tube in Speed-Vac to remove remaining alcohol. This should take about 5 minutes.

7. Add 16 uL TE^{-4} to the tube and place at 56°C to dissolve the DNA. After restriction the DNA should solubilize within 30-60 minutes.

9. DNA is now ready for another test gel (this one to assess the completeness of restriction) and then an analytical gel.

CONTROL NO. 87285

VII. TEST GEL TO MEASURE COMPLETENESS OF RESTRICTION DIGESTION

1. Remove 2 uL of DNA and combine with 1 uL loading solution in a separate tube. Spin 2 seconds. Pipette the entire 3 uL into test gel well. Run gel under same conditions as described in Section III.

2. Completely digested DNA will be present on this test gel as a smooth streak from the dye front back toward the origin. If a fluorescent large band remains near the origin, the digestion is incomplete and must be repeated. To redigest, add 18 uL TE^{-4} to the 14 uL of DNA that remain to restore the volume to 32 uL. Redigest as per the original digestion procedure.

VIII. RESOLUTION OF DNA FRAGMENTS ON AN ANALYTICAL GEL

The analytical gels are composed of 1% agarose in 1X TAE buffer. The gel dimensions are 11 X 14 X 0.65 cm (100 ml).

1. Preparation of the analytical gel:

Prepare 100 ml of 1X TAE buffer. Add 10 uL ethidium bromide (EB) to the buffer.

Weigh out 1.0 g agarose (Sigma type II or Seakem ME) into a flask or bottle.

Add 100 ml TAE-EB

Bring to a boil to dissolve agarose

Place at 56^{0}C to equilibrate

Place the gel tray on a leveling platform.

Place a 14-well comb into the gel tray.

Pour agarose into gel form.

Let stand at least 15 minutes to cool.

2. Pour 1X TAE buffer into the BRL gel tank. Supplement the tank buffer with EB at ratio of 10 uL EB/100 ml TAE.

3. Place the gel into the tank with the well comb nearest you. The buffer should cover the gel to a depth of at least 0.5 cm. Remove the comb.

WELL NUMBER 1 IS DEFINED AS THE WELL AT THE FAR LEFT SIDE OF THE GEL.

WELL 1 IS RESERVED FOR THE COMBINATION MARKER (VISUAL + SIZE)

WELL 2 IS RESERVED FOR THE HAE III DIGESTED ALLELIC CONTROL

WELLS 6, 10, AND 14 ARE RESERVED FOR SIZE MARKERS

4. To the 14 uL digested DNA add 4 uL loading solution, mix, spin 2 seconds and carefully pipette the entire specimen into the well.

5. Add other samples to their wells.

6. Set the voltage at 30 volts (maximum amperage) for a run time of 17 hours.

CONTROL NO. 87285

7. After the electrophoresis is complete, the gel can be examined on the UV transilluminator to evaluate the fragment separation. Photograph gels with Polaroid #553 (ASA 400) for 1 second at f4.5 with a red filter.

IX. SOUTHERN BLOTTING OF GELS ONTO NYLON MEMBRANES

1. Slide the gel from the tray into a plastic box that contains 0.4M NaOH. Gently shake for 30 minutes.

Soak BRL blot pad in a separate container of 0.4M NaOH for 15 minutes. Discard the dirty NaOH and refill with fresh NaOH for an additional 15 minutes. During the same time period, fill a sponge with 0.4M NaOH and place it into a plastic dissecting tray. In a separate container, immerse Zeta-Probe (BioRad) membrane (exactly 11 X 12.5 cm) in 0.4M NaOH for 15 minutes. Membrane should be handled only by gloved hands.

2. Place the soaked blot pad onto the sponge.

3. Carefully remove the gel from the NaOH. When removed, cover with a glass plate. The top of the gel is defined as the gel surface that contains the formed wells. Invert the gel, remove the top glass plate and slide the gel onto the blot pad. The original gel top should now be face down on the blot pad with the gel origin nearest to you. With gloved fingers, press down carefully on the gel to remove any air bubbles.

4. Without delay, place the presoaked Zeta-Probe membrane onto the gel. Be sure the edges of the membrane are square with the gel edges. Roll a glass pipette up and down the membrane several times to remove any air bubbles.

Cover the membrane with a piece of Whatman #3 that has been cut to 11 X 12.5 cm and wetted with 0.4M NaOH. Roll the surface.

Place 9 blot pads on top of the Whatman #3.
Place 2 - 15 X 20 X 0.4 cm glass plates on top of the sandwich.

5. Allow the transfer to proceed for 6 hours at room temperature.

6. Remove blot pads and 3 mm paper. Grasp the membrane at the right corner (origin end), remove and turn it over. Label the membrane with a pen where your thumb touched the membrane.

7. Wash the membrane once with 0.2 M Tris, pH 7.5 + 2X SSC for 15 minutes with gentle shaking. Blot the membrane on a sheet of Whatman #1.

8. At this stage you can label the membrane with special ink that exposes the x-ray film.

9. Sandwich each membrane between 3 mm Whatman (tape edges) and place in an 80^{0}C vacuum oven for 30 minutes.

X. HYBRIDIZATION

Normally 60 ml of hybridization solution is needed in the hybridization containers when hybridizing 11 X 12.5 cm membranes. Add the membranes to the hybridization solution one at a time, making certain that each is covered with solution before the next is added. As many as 6 membranes can be hybridized in the same container. After membranes are in the solution, tilt the container to pool the hyb solution at one corner. Add the labelled probes (VNTR probe and size marker probes) and agitate container to mix. Incubate at 65C overnight with constant shaking.

HYBRIDIZATION SOLUTION

20.4 ml STERILE H_2O
12.0 ml 50% PEG
4.5 ml 20% SSPE
21.0 ml 20% SDS
57.9 ml

+ probe (Volume = 2.1 ml + x uL probe)
See Appendix C, step 12.

XI. POST-HYBRIDIZATION WASHES

1. Pour off the hybridization solution slowly. The membranes will stick to the bottom of the container. Capture the last drops that collect at the corner of the container with a Kimwipe. Discard the Kimwipe into a radioisotope waste container.

2. Carry out the following washes, using enough wash solution to fill the container one-half full.

A. 15 minutes in 2X SSC + 0.1% SDS at room temperature

B. 15 minutes in 2X SSC + 0.1% SDS at room temperature

C. **10 minutes**[A] in 0.1X SSC + 0.1% SDS **at 65C**[B]

[A]Length of this wash depends on VNTR probe. Ten minutes is used for probes YNH-24, 3'HVR, and V1. Probe MS-1 requires at least a 45 minute wash.

[B]The solution for this wash must be at 65^0 before use.

3. Lightly blot the membrane on #1 Whatman - DO NOT LET THE MEMBRANE DRY OUT!

XII. AUTORADIOGRAPHY

1. Wrap the damp membranes in Glad or Saran wrap. **Do not use the Reynolds food wrap for this step!** In the darkroom under red light illumination, place the membranes DNA side down onto XAR film. Tape the membranes to this film. Place another sheet of XAR onto the back of the membranes and close the cassette. Place the cassette at -80^{0}C.

2. The XAR film on the back side of the membranes can be removed after a short exposure period which can be as short as a few hours or as long as several days. The back film is to be used as a guide for determining the length of time the front film needs to be left in place.

NOTE: Application of 2 ug DNA per analytical gel lane and hybridization under the conditions described in this procedure will yield satisfactory autorad results within 1-2 days.

XIII. BLOT STRIPPING PROCEDURE

1. Remove plastic wrap from membranes.

2. Place membranes in the following solution:

   110 ml formamide
   20 ml 20X SSPE
   10 ml 20% SDS
   Q.S. with H_2O to 200 ml.

3. Shake membranes for 45-90 minutes at 65°C

4. Rinse the stripped blot in 200 ml of 0.1X SSC + 0.1% SDS for 1 minute at room temperature.

6. Place the blot on filter paper to remove excess fluid. Relabel the membrane with ballpen if required.

7. Place the blot in the hybridization solution for the next probing.

NOTE: If membranes are to be stored for an indefinite period of time, carry out the stripping and rinsing steps as described. Then, rewrap the membranes with plastic wrap and freeze at -80.

CONTROL NO. 87289

XIV. RESOLUTION OF SPERM CELL DNA AND VAGINAL CELL DNA

1. Remove the swab from the applicator stick and place into a 1.5 ml tube.

2. Add 450 uL HEPES-buffered saline, pH 7.5, or phosphate buffered saline to the swab. Add 50 uL 20% Sarkosyl to the tube and mix. AFTER 2 HRS CAN RUN P_{30} ON A SMALL SAMPLE, BEFORE ADD SARKOSYL

3. Rock tube overnight at 4°C.

4. Punch a hole in the lid of the tube. Place the swab into the lid and centrifuge for 3 minutes.

5. Remove and discard supernatant (SPNT) fluid. ~~BULK OF ♀~~ SOME ♀ DNA PRESENT

6. Place a new cap on the tube.

7. Put the swab back into the tube and add:

400 uL	Tris-EDTA-NaCl (TNE)
50 uL	10% SDS
50 uL	H2O
5 uL	PaseK (PROTEINASE K)
505 uL	

Mix and place the tube at 37C for 2 hours.

8. Punch a hole in the lid of the tube. Place the swab into the lid and spin for 5 minutes.

9. Remove the supernatant fluid and save (female fraction). Discard the swab.

BULK

10. Place a new cap on the tube.

PHENOL / CHLORO EXT

11. To the pellet add:

ETOH, etc.

150 uL	TNE
100 uL	10% sarkosyl
40 uL	0.39M dithiothreitol (DTT) - SPERM HEAD MEMB. RICH IN DISULFIDE BONDS
100 uL	H2O
10 uL	PaseK
400 uL	

Mix and incubate at 37C for 2 hours.

12. Extract the incubation mixture with an equal volume of phenol:chloroform:isoamyl alcohol. Continue processing as if a regular stain extract were being handled.

APPENDIX A

REAGENTS FOR DNA TYPING PROCEDURES

* Solutions that must be autoclaved

1. ***AMMONIUM ACETATE 7M** - 53.96 g anhydrous NH_4OAc bring to 100 ml with H_2O

2. **DNA QUANTIFICATION STANDARDS**- For use on test gels

Lambda phage DNA at 250 ug/ml = stock

Carry out serial doubling dilutions of the stock with TE^{-4} to obtain the solutions shown below. Combine 1.0 ml aliquots of diluted standards with loading solution as shown.

1 ml at 125	ug/ml +	0.5 ml	loading	solution	= 500 ng/6 uL
1 ml at 62.5	ug/ml +	"	"	"	= 250 ng/6 uL
1 ml at 31.3	ug/ml +	"	"	"	= 125 ng/6 uL
1 ml at 15.6	ug/ml +	"	"	"	= 63 ng/6 uL
1 ml at 7.8	ug/ml +	"	"	"	= 31 ng/6 uL
1 ml at 3.9	ug/ml +	"	"	"	= 15 ng/6 uL

NOTE: Similar quantitative standards of DNA can be obtained commercially.

3. ***EDTA 0.5M**- EDTA-$2H_2O$ 186.1 g
dissolve in 800 ml of water - add about 15-20 g of NaOH pellets to drop the pH toward 8.0. When fully dissolved, add more NaOH to bring the pH to 8.0.
Adjust volume to 1.0 L.

4. **ETHIDIUM BROMIDE**- 5 mg ethidium bromide/ml H_2O. Keep bottle wrapped in foil to protect from light.
<u>MUTAGENIC SUBSTANCE!!</u>

5. **HERRING SPERM DNA**- herring sperm DNA - 500 mg
Q.S. H_2O 50 ml

Denature by placing in boiling water for 5 minutes.

6. **LOADING SOLUTION**-

Glycerol	50%
Bromophenol blue	0.1%
EDTA	0.1M

solvent is TE^{-4}

7. ***NaCl 5M-** NaCl 292.2 g
dissolve and adjust volume to 1.0 L

8. **NaOH 4M -** dissolve 800 g NaOH pellets in 4200ml H_2O
adjust volume to 5.0 L with H_2O

9. **PEG 50% -** polyethylene glycol (MW 8000) 50g
bring to 100 ml with H_2O

10. **PHENOL/CHLOROFORM/ISOAMYL ALCOHOL**

CAUTION - PHENOL IS VERY CAUSTIC - BE CAREFUL !

Melt phenol at 65°C and pour 100g into a Bellco bottle. Add 200 mg 8-hydroxyquinoline and mix the solution thoroughly.

Add an equal volume of 1.M Tris, pH 7.5, transfer to a separatory funnel and mix. Let phases separate.

Drain lower phase (phenol) into the bottle. Drain upper phase into a beaker.

Add equal volume of 0.01M Tris, pH 7.5 to the phenol, transfer to the separatory funnel and mix.

Capture lower phase. Capture the upper phase and measure its pH. If the pH of the upper phase is 7.5, then cease equilibration procedure. If pH is <7.5 repeat the equilibrations against 0.01M Tris until the pH of the upper phase equals 7.5.

Combine the phenol with a solution composed of 100 ml chloroform + 4 ml isoamyl alcohol.

Cover the solution with 0.01M Tris and store at 4°C.

11. **PROTEINASE K-** 500 mg proteinase K
dissolve in 25 ml H_2O
aliquot into convenient size volumes
and freeze.

12. **SDS 10%-** SDS 100 g
700 ml H_2O - heat to about 65C to dissolve
adjust volume to 1.0 L

13. **SDS 20%-** SDS 200 g
700 ml H_2O - heat to about 65C to dissolve
adjust volume to 1.0 L

14. ***SSC 20X-** NaCl 175.3 g
Na_3citrate 88.2 g
dissolve in 800 ml H_2O

adjust to pH 7.0 with HCl
bring to 1.0 L with H_2O

15. **0.1X SSC: 0.5% SDS -** 5 ml 20X SSC
25 ml 20% SDS
adjust volume to 1.0 L with H_2O

16. **2X SSC: 0.1% SDS-** 100 ml 20X SSC
5 ml 20% SDS
adjust volume to 1.0 L with H_2O

17. **0.1X SSC: 0.1% SDS-** 5 ml 20X SSC
5 ml 20% SDS
adjust volume to 1.0 L with H_2O

18. **20X SSPE-** 3.6 M NaCl 210.4 g
0.2 M NaH_2PO_4 (anhy) 24.0 g
0.02M $Na_2EDTA-2H_2O$ 7.4 g

dissolve and titrate to pH 7.0 with NaOH
Q.S. 1.0 L with H_2O

19. **STAIN EXTRACTION BUFFER -** 0.010M TRIS 1.21g
0.01M $Na_2EDTA-2H_2O$ 3.70g
0.100M NaCl 5.84g
0.039M DTT 6.02g
dissolve in 500 ml H_2O
adjust pH to 8.0 with HCl
add 100 ml 20% SDS
adjust volume to 1.0 L with H_2O

20. ***TAE 20X -** TRIS base 96.6 g
Glacial HAc 22.8 ml
0.5 M EDTA, pH 8.0 40.0 ml
adjust volume to 1.0 L with H_2O

21. ***TE^{-4}-** TRIS base (10 mM) 1.21 g
Na_2EDTA (0.1 mM) 0.037 g
dissolve Tris in 800 ml H_2O and adjust pH to 7.5
add EDTA, after solution, recheck the pH
bring volume to 1.0 L

22. ***TRIS 2M- pH 7.5** TRIS base 242.2 g
dissolve in 800 ml H_2O
adjust pH to 7.5 with concentrated HCl
adjust volume to 1.0 L with H_2O

23. ***TRIS 2M- pH 8.0** TRIS base 242.2 g
dissolve in 800 ml H_2O
adjust pH to 8.0 with concentrated HCl
adjust volume to 1.0 L with H_2O

24. **0.2M TRIS: 2X SSC, pH 7.5-** 100 ml 2M Tris
100 ml 20X SSC
adjust volume to 1.

25. **TNE-** TRIS base 0.121 g
NaCl 0.584 g
EDTA 0.037 g
dissolve in 80 ml H_2O
titrate to pH 8.0 with NaOH
Q.S. 100 ml with water

26. **SARKOSYL -20%** - N-lauroylsarcosine 20g
Q.S. with H_2O to 100 ml
filter sterilize through 0.22 u filter

APPENDIX B

MOLECULAR WEIGHT AND VISUAL MARKERS

There are three types of markers used in this RFLP procedure. (1) **Visual** marker; (2) **Combination** marker; and (3) **Molecular Weight** marker (AKA ladder). Each marker is available commercially in ready to use form.

The molecular weight markers are purchased also in radiolabeled form for use during hybridization.

A. Handling of markers

1. Visual marker: For use on yield gels and post-restriction digestion gels.

   A. Supplied already mixed with loading solution.

   B. Use 10 uL in lane #1 of each gel.

2. Combination marker: For use in well #1 of each analytical gel.

   A. Calculate the volume of combination marker needed for gels. The marker is already mixed with loading solution. Each gel requires 14 uL marker.

   B. Place the tube containing the correct volume of combination marker into a 65°C water bath for 5 minutes immediately prior to use.

3. Molecular weight marker: For use in wells 6, 10, and 14 of analytical gels.

   A. Calculate the volume of molecular weight marker needed for gels. The marker has already been mixed with loading solution. Each well requires 14 uL. Thus, 3 X 14 uL are needed for each analytical gel.

   B. Place the tube containing the correct volume of molecular weight marker into a 65°C water bath for 5 minutes immediately prior to use.

CONTROL NO 87285

B. Use of radiolabeled molecular weight marker

The radiolabeled molecular weight marker can be obtained commercially. It is furnished ready for use after addition of the probe denaturation solution and boiling. One unit of labeled molecular weight marker contains 1 X 10^6 DPM of each DNA species and is designed for use in 60 ml hybridization solution.

A. Transfer one unit of radiolabeled marker into a 20 ml tube that contains:

0.6 ml 0.2M NaOH
1.5 ml denatured herring sperm DNA

B. Place tube into boiling water for 5 minutes.

C. Add entire solution to hybridization solution in the hybridization container.

CONTROL NO. 87285

APPENDIX C

Several locus-specific DNA probes can be obtained commercially in prelabeled form. These probes are furnished as 100 ng probe labeled with 30 X 10^6 dpm in a microfuge tube and are ready to use for hybridization after addition to the probe denaturation solution (see final step in this appendix).

LABELING OF LOCUS-SPECIFIC DNA PROBES

Probe labeling is carried out using the BRL **Random Primers DNA Labeling System**. The protocol recommended by BRL has been modified as follows:

1. Place 1 uL (100 ng) probe in a 1.5 ml screw-cap tube. Add 22 uL H_2O and mix.

2. Place the tube in boiling water for 8 minutes. Immediately after heating, place the tube into a slurry of crushed ice and water for 5 minutes.

3. While on ice, add to the tube:

   2 uL dATP
   2 uL dGTP
   2 uL dTTP
   15 uL random primers buffer mixture
   5 uL ^{32}P-dCTP (50 uCi at 3000 Ci/mmole)*

   mix

   1 uL Klenow fragment

   mix and then spin for 2 seconds

   50 uL = final labeling volume

4. Incubate at room temperature (25^{0}C) for 3 hours to overnight.

* It is very easy to contaminate the tube threads with radioactivity. Once the threads are contaminated, the radioactivity will be transferred to the worker's fingers and thence transferred to anything else that is touched. To avoid such a senario, spin the tube each time its contents are mixed. Check fingers for radioactivity frequently.

5. After incubation, precipitate the DNA with spermine.

50 uL reaction mixture
140 uL TE^{-4}
4 uL herring sperm DNA at 10 mg/ml

mix

4 uL 0.1 M spermine-4HCl

on ice for 15 minutes (can go less time)

6. Spin 10 min microfuge at 4C
Remove supernantant and place in radioactive storage bottle
Rinse pellet with 396 uL TE-4 + 4 uL spermine
vortex briefly
Spin 2 min at 4C
Remove and discard supernatant (place in radioactive storage bottle)

7. Resuspend pellet in 520 uL TE-4
+ 40 uL 5 M NACl
mix and place at 56°C for 15-30 minutes

8. Remove 2 uL labeled probe and place in the exact bottom of a 1.5 ml screw-cap tube. If 2 uL is deposited onto the side of the tube it will not be counted properly. Spin 2 seconds if necessary to place the 2 uL at the bottom.

9. Place the tube containing 2 uL probe into radioactivity counter. Counter is a Dupont 'Bench-Count' model BC2000. Start the counter and count to 2% precision.

10. Calculate dpm isotope present in probe preparation

Example: counts/minute (cpm) = 10,000
counting efficiency = 6.8 % (instrument specific)
volume probe counted= 2 uL

Calculations: (10000 cpm)(1/0.068)(1/2) = 73529 dpm/uL

(73529 dpm/uL)(560 uL) = 4.1×10^7 dpm total

4.1×10^7 dpm/0.1 ug DNA = 4.1×10^8 dpm/ug

11. Calculate uL probe that must be added to hybridization solution to achieve 5×10^5 dpm/ml solution.

Example: 60 ml hybridization solution
5×10^5 dpm/ml hybridization solution
probe label = 73529 dpm/uL

(60 ml)(5×10^5 dpm/ml)/(73529 dpm/uL) = 408 uL

Immediately before labeled probe is to be added to the hybridization chamber, it must be boiled. Combine the following in a 15 ml screw-cap plastic tube: *p244X

0.6 ml 0.2M NaOH
1.5 ml denatured herring sperm DNA
x uL labeled probe (e.g. 408 uL)

boiling water 5 minutes
Add entire volume to hybridization solution

APPENDIX D

ALLELIC CONTROL

Allelic control is obtained commercially. Use cell line K562.

1. The allelic control is furnished as 2.5 X 10^6 cells/tube.

2. Process cell pellet beginning with I. Isolation of DNA from liquid blood samples, step 5.

3. Exactly 0.1 ug allelic control DNA must be mixed with loading solution and placed in well #2 of each analytical gel.

ITEM 7

RADIOACTIVE MATERIAL EXPERIENCE

Charles Barna:

1.) Thyroid Uptake Testing (T-3, T-4) using ^{125}I & ^{131}I. Akron Medical Laboratories, Akron, Ohio. (1969-1973)

2.) DNA sequencing seminar, Nov. 1988 at Michigan State University. ^{32}P labeling was used. Safety Lecture was given by Office of Radiation, Chemical and Biological Safety, Michigan State University.

3.) DNA typing, 4 week coarse, Jan. 1989, at the FBI Training Academy in conjunction with the University of Virginia. ^{32}P labels were used. Four hours of class room lecture were provided on Radiation Safety.

Julie A. Howenstine:

1.) 40 hours of coursework through the Michigan State University College of Veterinary Medicine in Radiology. This course included lectures on measurement of radiation exposure, radiation safety and the biophysical effects of radiation.

2.) 15 months of clinical veterinary medical experience in the use of diagnostic X-rays.

3.) 16 hours of lecture and lab experience at a DNA sequencing seminar, Nov. 1988 at Michigan State University. ^{32}P labeling was used. A safety lecture was given by Office of Radiation, Chemical and Biological Safety, Michigan State University specifically dedicated to the use of ^{32}P and included provision of the USNRC Regulatory Guide 8.13.

4.) DNA Typing, 4 week course, Jan. 1989, at the FBI Training Academy in conjunction with the University of Virginia. ^{32}P labels were used. Four hours of classroom lecture were provided on radiation safety including provision of the USNRC Rules and Regulations parts 19 and 20 and Regulation 8.13.

Item #8

Michigan State Police Laboratories
Radiation Safety Manual

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1

PREFACE

This manual has been compiled in order to present quidelines and procedures for obtaining radioactive materials by the Michigan Department of State Police employees and to provide general information concerning radiation safety requirements for the Michigan Department of State Police laboratories. Information is also presented on the responsibilities of the radiation worker along with a description of the rules and regulations governing occupational exposure to ionizing radiation. Questions can be directed to the Radiation Safety Officer Charles Barna, telephone 332-2521, extension 371.

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GENERAL

Scope of the Manual

This manual describes the rules and procedures to be followed in obtaining and using radioactive materials on the Department of State Police laboratories. Standards for this manual are based upon the U.S. Nuclear Regulatory Commission's Code of Federal Regulations, Title 10, Parts 19 & 20, and the Michigan Department of Public Health's Division of Radiological Health Document titled "Ionizing Radiation Rules Governing Radioisotope Material and Electronic Product Radiation". These and other radiation safety related documents are kept on file at the Department of State Police Laboratories and upon request, can be borrowed or copied.

Training of Radiation Workers

All Department of State Police radioisotope workers are required to attend a radiation safety class sponsored by the Office of Radiation, Chemical & Biological Safety (ORCBS) at Michigan State University and pass an exam before the employee will be allowed to work with radioisotopes at the laboratories of Department of State Police. Also, each radioisotope worker must sign a statement that he/she has read this manual and understands its contents (see Appendix A). Additionally, the enclosed "Michigan State University Radiation Safety Study Guide" and "Radiation Safety Training Guide" are part of the training materials. At the discretion of the Department of State Police Radiation Safety Officer, additional training may be required.

Responsibilities of the Radioisotope Laboratory Workers

Radioisotope workers are directly responsible for compliance with all regulations governing radiation safety in the laboratory and for safe practices by other investigators or technicians who work under their supervision. They have an obligation to:

(1) Insure that individuals working under their control are properly supervised and have the training required to enable safe working habits and prevention of exposure to others or contamination of the surroundings. (Inadequate supervision and lack of training have been cited in radiation lawsuits as indicative of negligence).
(2) Be aware of the unknown radiation hazards inherent in a proposed activity. If these hazards are not covered by the general program of laboratory safety, they are responsible for instructing personnel in safe practices or in directing personnel to sources of information concerning safe practices.
(3) Be aware of the various forms of radiation which are present.
(4) Avoid any unnecessary exposure, either to themselves or to other workers under them.
(5) Understand the risks associated with the possession, use and shipment of all radioactive materials. Because federal and state regulations control the use and shipping of radioactive materials and other certain chemicals, they must be aware of these laws and comply with them. If you are not sure of the proper procedures, call the Radiation Safety Officer
(6) Keep current working records of the receipt and the disposition of radioactive material in their possession including use in research, waste disposal, transfer, storage, etc.
(7) Post warnings and restrict entry to areas that contain potentially hazardous radioactivity or chemicals

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(8) Once notified in writing by a female radiation worker that she is pregnant the Radiation Safety Officer will be immediately notified and aid in complying with NRC reg. guide 8.13 (see Appendix D).

Failure to comply with the rules and regulations set forth above and throughout this manual may lead to disciplinary actions and/or the cessation of all radioisotope shipments and experiments. If it becomes necessary to halt the purchase of radioisotopes due to a violation of the Michigan Department of State Police safety regulations, the Radiation Safety Officer (RSO) and address the safety concerns and violations before reinstatement of the use of radioactive materials can occur. The RSO reserves the right to permanently terminate any radioisotope use when appropriate.

Ordering Radioactive Materials

Any purchase of radioactive materials must be approved by the Radiation Safety Officer. All requisitions should be sent to the purchasing department directly which then will contact the Radiation Safety Officer for approval. The information that purchasing needs from the approved user is their name, department, account number, requisition number, element and mass number, chemical form, activity and company from which the radioisotope will be purchased. All shipments of radioactive materials received on Department of State Police property must be inventoried. The inventory will be maintained by the Radiation Safety Officer.

Shipment of Radioactive Materials off The Michigan State Police Laboratory

All shipments of radioactive materials off the Michigan Department of State Police Laboratory must have prior consent of the RSO. Federal and State law requires that the shipper must obtain the receiver's approval and their respective Nuclear Regulatory License number or the State License number prior to the shipment of the material. All shipments must be in accordance with the packaging and labeling requirements set forth by the Department of Transportation (DOT) and an appropriate record be made of the dose rates on contact with the package and dose rates at three feet from the package (an ion chamber must be used). Also, the record must contain information on the shipper, receiver, nuclide(s) and activity, phone numbers of shipper and receiver, and performance of a survey for removable radioactivity. If assistance is needed, call the Radiation Safety Officer

Receiving and Monitoring Radioisotope Shipments

(1) Monitor the radiation level of the package (wear protective gloves when doing this).
(2) Wipe the outside of the package for removable contamination.
(3) Note radiation units stated on the package, verify and record shipment monitoring record.
(4) Place package in a vented hood.
(5) Open outer and inner package and verify that the contents agree in name and quantity with the packing slip.
(6) Measure the radiation field of the unshielded container. If necessary, place container behind shielding.
(7) Check for possible breakage of container seals, loss of liquid, or change of color of the packing material. Report all leakage and broken containers to the Radiation Safety Officer immediately.
(9) Wipe test the inner contents of package and record results on shipment monitoring record.

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Table I

Removable Radioactive Contamination Limits

	Alpha Emitters dpm/100cm^2	High Risk Beta or Gamma Emitters dpm/100cm^2	Low Risk Beta or Gamma Emitters dpm/100cm^2
Unrestricted Areas	22	220	2,200
Restricted Areas	220	2,200	2,2000
Personal Clothing Outside Restricted Areas	22	220	2,200
Personal Clothing Inside Restricted Areas	220	2,200	2,2000
Skin	22	220	2,200

Radioisotope Inventory

A routine inventory of radioactive materials is conducted periodically. The Radiation Safety Officer will maintain a list of isotopes possessed under the NRC license. The Radiation Safety Officer is required to review this list and make necessary corrections to the inventory.

Terminating Employment at Department of State Police

All radiation workers that terminate their education and/or employment at Department of State Police shall notify the RSO and their respective film badges must be returned.
The RSO maintains film badge records for all radiation workers. This information can be forwarded to any future employer at the request of the employee. When requesting this data, please include employment dates, full legal name while employed at Department of State Police and social security number.

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REGULATIONS REGARDING RADIATION SAFETY

Ionizing Radiation

Ionizing radiation, simply put, is the ability to remove electrons from atoms. The result of ionization is the production of free electrons with their negative charges and ionized atoms with their positive charges. The types of radiation involved that can remove atomic electrons can be classified into two groups: 1) photons such as X and gamma rays, and 2) particles such as beta rays, alpha particles, neutrons and protons. Photons are pure energy and have no mass whereas the particles have typically both mass and charge. Both remove electrons but by their natures, interact with matter differently.

Ionized atoms, regardless of how they were formed, are much more active chemically than neutral atoms. These chemically active ions can form compounds that interfere with the process of cell division and metabolism. The degree of damage suffered by an individual exposed to ionizing radiation is a function of several factors: type of radiation involved, intensity of the radiation, its energy, and length of exposure.

External and Internal Radiation Hazards

Injury, as a result of being irradiated, is caused mainly by ionizations within the tissues of the body. There are two main potential hazards connected with work involving radioactivity: external exposure to radiation and internal deposition of radioactive materials.

External hazards arise when the radiation source is external to the body and the body is penetrated by ionizing radiation. These radiations can be X or gamma rays, neutrons, alpha particles or beta particles. Beta particles do not normally penetrate beyond the skin, but when sufficiently intense, can cause skin and/or eye damage. Very energetic beta rays such as those emitted from P-32 can penetrate several centimeters into the skin and shielding may be needed in order minimize the external radiation exposure. Typically, a half-inch thickness of lucite is an effective shield for most beta rays. Alpha particles, because of their higher mass, slower velocity, and greater electrical charge compared to beta rays, are capable of travelling a few inches in air and rarely penetrate the outer dead skin layer of the body. Typically, alpha particles are not an external radiation hazard. X and gamma rays along with neutron radiation, on the other hand are very penetrating, and are of primary importance when evaluating the external radiation exposure. The onset of first observable effects of acute radiation exposure (diminished red-blood cell count) may occur at a dose of approximately 100 REM of radiation. The lethal dose for humans wherein 50% of the exposed population may die from a one time exposure to the whole body is about 500 REM assuming no medical intervention.

Exposure to external radiation may be controlled by limiting the working time in the radiation field, by working at a distance from the source of radiation, and/or by inserting shielding between the worker and the source.

Internal hazards arise when radiation is emanated from internal deposits of radioactive materials within the body. Although external hazards are primarily caused by X-rays, gamma rays and neutrons, all forms of radiation, including alpha and beta particles, can cause internal radiation hazards. Alpha particles create a high concentration of ions along its path and can cause severe damage to internal organs and tissues when they are ingested, injected or inhaled. Once these particles get into the body, serious damage can occur since there is no protective dead skin layer to shield the organs and the tissues. The internal hazard is not limited to the intake of large amounts at one time. Often a chronic hazard can arise from small accumulations of radioactive materials over a long period of time.

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It is known that many substances taken into the body will concentrate in certain body organs. For example, iodine will concentrate in the thyroid. When iodine is inhaled or ingested, the body cannot distinguish stable iodine from radioactive iodine, and on the average about 35% of the inhaled iodine will become affixed to the thyroid gland within 24 hours. Other elements such as calcium, strontium, radium and plutonium concentrate in the bones. Here, high dose rates can occur to the bone over very long periods of time since the body eliminates these materials very slowly once they are incorporated into the bone structure. The blood forming organs are very radiosensitive as these cells are in the S-phase of mitotic activity more often than other cells. Hence, if there is a significant long-term exposure to radioisotopes, chronic diseases such as leukemia and/or osteosarcoma can occur. The induction time for the onset of these types of diseases is typically in excess of 20 years.

Personnel Monitoring

Film badges must be worn routinely by all personnel where significant exposure to penetrating radiation (i.e.P-32) is possible. The badges are changed monthly and each individual is responsible for seeing that his/her badge has the current month's dosimeter inserted within the holder. These badges are your legal documentation of external radiation exposure received while working at Department of State Police. These badges are not to leave your immediate work area. They are not to be taken home or used off laboratory where a non-occupational exposure may occur. If you are travelling to another facility, do not bring your Department of State Police radiation badge. It is the responsibility of that particular facility to see that a new dosimeter is issued. These badges are heat sensitive and if left in a car where the temperature may be high, a dose will be recorded and it will become very difficult to distinguish a true radiation dose from a dose caused by exposure to excessive heat. Also, for those individuals who use X-ray equipment and/or high energy beta emitters, finger ring badges should be used in conjunction with the whole-body film badge. This whole-body film badge should be worn on the torso with the name tag facing the source of suspected radiation. With finger ring badges, the same holds true as the name tag must directly face the radiation source. Care should be taken to make sure that your badges do not become contaminated with radioactive materials. Lost or misplaced badges should be reported immediately to the RSO. At any time, any individual can call the RSO for the film badge dose data. The RSO will not contact every badge holder every month. Only those with significant doses (i.e. greater than 100 mrem per month to the whole body) will be contacted. If you suspect that you might have received a high dose, call the RSO and your badge will be sent out immediately and a spare badge will be issued for the interim period. On an emergency basis, film badges can be sent off and results obtained within a week.

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Radiation Dose Units

The roentgen, abbreviated as "R", is the unit for measuring the quantity of X or gamma ray radiation in terms of the amount of ionization produced in air. One roentgen is the quantity of X or gamma ray radiation that will produce ions (of one sign) carrying a charge of 2.58E-04 coulombs per kilogram of air. An exposure to one roentgen of radiation will yield 89.6 ergs of energy deposition per gram of air. If human tissue is exposed to one roentgen of radiation, 96 ergs of energy will be deposited per gram of tissue. The roentgen is easy to measure as an ion chamber is a device that will measure the ions produced in air directly. The ion chamber has a read-out in milli-roentgen per hour and is a very close approximation of tissue exposure.

The RAD and the REM are the two main radiation units used when assessing radiation dose. The RAD (radiation absorbed dose) refers to the energy deposition by any type of radiation in any type of material. Specifically, one rad equals 100 ergs of energy deposition per gram of absorber. The REM (radiation equivalent man) takes into account the biological effectiveness of different radiations. Some types of radiation cause more damage to biological tissue than other types. For example, one rad of alpha particles is twenty times more damaging than one rad of gamma rays. To account for these differences, a number called a quality factor (QF) is used in conjunction with the radiation absorbed dose in order to determine the dose equivalent in rem:

REM Dose = RAD Dose X QF

For X-rays, gamma rays, and beta particles the quality factor is one. Therefore, the roentgen, rad and rem are approximately equal when considering the above types of radiations.

The dose rate is proportional to the radiation flux (number of particles or photons per square centimeter per second) and is expressed in rem/hour or millirem/hour. Your radiation film badge readings are reported in millirem. The dose rate can be estimated by using as ion chamber as previously mentioned when the radiation source is a gamma or X-ray emitter. The ion chamber can be useful in estimating dose for most beta radiations, but in the case of alpha or neutron radiations, special detectors are required. If you find that you have a need to monitor neutron radiation dose rates, call the RSO for additional information.

Maximum Permissible Exposure

Exposure standards have been established and set at a level where apparent injury during a normal lifetime to ionizing radiation is not expected. This limit is called the "maximum permissible exposure"

Maximum permissible exposure to external radiation is given in Table II. However, personnel should not completely disregard exposures at or below these limits. It is the responsibility of each individual to keep his exposure to all radiation as low as practical and to avoid all exposures to radiation when such exposures are unnecessary.

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TABLE II

MAXIMUN PERMISSIBLE EXPOSURES TO EXTERNAL RADIATION IN MILLIREM FOR RADIATION WORKERS*

Part of Body	Quarterly
Whole Body, Head and Trunk, Active Blood Forming Organs, Lens of Eye, Gonads	1,250
Hands and Forearms, Feet and Ankles	18,750
Skin of Whole Body	7,500

* These limits are for those workers who are 18 years of age or older. For those less than 18 years of age, the dose limitations are 10% of the limits listed above. Therefore, minors must receive special permission in order to work in a radiation area and the RSO must be notified.

An exception to the above stated limits can be allowed when there is documented evidence that a worker's previous exposure is low enough, the Department of State Police Radiation Safety Officer may permit a dose of up to three rems in one calender quarter. The State of Michigan regulations require the annual dose to be less than five rems in all circumstances. In addition, a worker's accumulated dose may not exceed 5(N-18) rems, where N is the person's age in years, i.e. the lifetime occupational dose may not exceed an average of five rems for each year above the age of 18.

Female Staff of Child Bearing Age

A special situation arises when a radiation worker becomes pregnant. Under these conditions, radiation exposure could also involve exposure to the embryo or fetus. A number of studies have indicated that the embryo or fetus is more sensitive than the adult, particularly during the first three months of pregnancy . This can be a problem since many women are unaware of their pregnancy during the first month or two of gestation. Hence, the NRC and the State of Michigan requires that all occupationally exposed women be instructed concerning the health protection problems associated with prenatal radiation exposure and sign a statement the they are familiar with the risks involved as stated in the NRC Regulatory Guide 8.13.

There are relatively few places in the Michigan State Police Laboratories where radiation levels are high enough that a fetus would receive 500 millirem before birth. (Maximum permissible exposure during gestation period is 500 millirem.) If a radiation worker is pregnant, she should notify her supervisor in writing to this effect. By doing so, a complete assessment of her radiation exposure potential can be made. If appropriate, a change in work assignment or location may be required in order to assure a low radiation exposure potential to the fetus. Failure to notify a pregnant worker's supervisor of her condition will maintain the dose limit to the whole body at 1,250 mR per calendar quarter under the law.

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Exposure of Non-Radiation Workers

Visitors to a radiation laboratory who are not classified as radiation workers by their employers and must not receive a radiation dose in excess of:

A. Two millirem in any one hour.
B. 125 millirem in any one calendar quarter.

Posting of Radioisotope Areas and Warning Signs

All rooms or areas in which regulated quantities of radioactive materials are used or stored must be posted with a "Caution Radioactive Material" sign, a NRC Licensing and Regulation Information Bulletin" sign, and a NRC-3 form "Notice To Workers" sign. Door signs must include emergency personnel names and phone numbers, and where he\she can be reached in the event of an emergency. These items may be obtained from the RSO.

Warning signs are used to mark areas where hazardous radiation levels could exist. In addition, entrance to any room containing radioactive material should be labeled with the radiation caution symbol and the phrase "caution radioactive materials". The terms "Radiation area" and "high radiation area" are defined here and should be used whenever appropriate on warning signs. A "radiation area" means any area accessible to personnel in which radiation exists at such a level that a major portion of the body could receive a dose of five mR or more in one hour or 100 mR in any five consecutive days. Properly worded signs should be posted to designate the area as restricted for access.

A "high radiation area" means any area accessible to personnel in which there exists radiation at such levels that a major portion of the body could receive a dose of 100 mR in one hours time. Ropes or fences should be used in addition to warning signs to clearly mark the restricted area.

Warning signs are available at the RSO. Indiscriminate use of warning signs is not permitted. If you have questions, call RSO.

Table III

DEFINITION OF RADIATION WARNING SIGNS

Amount of Radition	Designation
Any Radioactive Material or Radiation	Caution or Danger "Radioactive Material" or Radiation
5 to 100 mrem/hour	Caution or Danger "Radiation Area"
100 mrem/hour or greater	Caution or Danger "High Radiation"

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Labeling Requirements

Ali trays, racks, stock solutions, tools , etc. which contain radioactive material or are contaminated must be labeled with the radioactive materials tape which contains information on the type of radiation present, date, and the total activity in disintegrations per minute or in microcuries. It is not reasonable to expect that each tube or vial be labeled, but the tray or rack that holds them must be labeled (e.g., scintillation vials do not need to be individually labeled but the tray or box that they are stored in must have the above described label.)

General Rules for Radioisotope Laboratories

(1) Do not eat, drink or smoke in radiation areas. "Eating" includes consuming gum, candy beverages and chewing tobacco.
(2) Refrigerators must not be used jointly for foods and radioactive materials.
(3) Gloves should be worn during operations in which contamination of the hands is possible.
(4) Do not pipette radioactive liquids by mouth.
(5) Store and transport radioactive materials in such a manner as to prevent breakage and spillage.
(6) Use ventilation hoods or glove boxes if the radioactivity can become airborne.
(7) The individual(s) responsible for any contamination will be required to decontaminate the area of concern under the supervision of the RSO.
(8) Regularly check your hands, clothing and shoes for contamination prior to leaving the work area after working with radioactive material.
(9) Always dispose of radioactive waste in a radioactive waste container.
(10) Always wear your assigned film badge(s)
(11) Women must receive a copy of the NRC Guide 8.13 (prenatal radiation exposure) and present a signed consent form to the principle investigator.

RSO Services Offered to the Department of State Police Laboratory

The RSO offers a wide range of services that include environmental monitoring, waste reduction and separation consultations, advice on shielding concerns, internal dosimetric evaluation, and a wide selection of safety related materials and catalogues that an investigator may find useful when designing or redesigning a radioisotope laboratory. The RSO also keeps all pertinent historical records regarding radiation surveys and audits, personnel monitoring and radioisotope purchases.

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RADIATION LABORATORIES

Laboratory Design and Equipment

Working with radioactive materials requires the use of specially designed laboratories and equipment. Smooth, continuous, non-absorbant surfaces such as stainless steel or linoleum are essential as base materials in a radiation work area. In areas where contamination is likely those base materials should be covered with absorbent and disposable material like polyback absorbant lab paper. Equipment such as glassware, tools, syringes, etc. used in the handling of radioactive materials should not be used for other work or allowed to leave the lab unless it can be shown that the equipment is free from removable contamination. It is strongly recommended that a marked storage cabinet be used to store this type of equipment. Also, fume hoods with flow rates not less than 100 linear feet per minute should be used whenever working with radioactive materials where the potential for vaporization/volatilization exists.

Monitoring Instruments

Every laboratory using radioactive materials must possess or have available for immediate use appropriate radiation monitoring equipment. This equipment must be in good working order, calibrated and looked at by the RSO once a year. Results of this calibration and examination will be forwarded on to the laboratory supervisor. Equipment that has not passed this annual examination must be removed from service until it is repaired or replaced to the satisfaction of the Radiation Safety Officer.

Radiation Surveys

The Radiation Safety Officer or his designate will make surveys of the radioisotope labs during and after each experimental procedure when radioisotopes are used. Written records will be maintained for inspection by the U,S. Nuclear Regulatory Commission. Such things as inventory assessment, contamination control, personnel monitoring, training and waste disposal methodology will be addresses during these surveys. Copies of the results of these surveys will be maintained in a permanent file and a subsequent inspection will be conducted in the event problems have been detected that need corrective action. By doing this, the potential for internal contamination cab be evaluated and addressed. When removable radioactivity is found to occur, the area must be decontaminated and then re-surveyed and documented. Table I lists the limits in which removable radioactivity should not exceed. However, all detectable levels of removable contamination should be removed whenever possible. It is understood that certain areas may be routinely contaminated, such as internal parts of equipment and the inside areas of glassware, and that it may not be practical to clean these surfaces. If this occurs, signs must be posted and protective clothing and gloves should be used when in contact with these areas.

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Security of Radioactive Materials

Precautions must be taken to prevent the tampering of radioactive materials by unauthorized personnel. This means that radioactive materials should be kept in locked containers or rooms that are not readily accessible to unauthorized visitors. Any loss of radioactive materials must be reported to the Radiation Safety Officer immediately.

Disposal of Radioactive Waste

All radioactive waste shall be separated from non-radioactive waste. Under no circumstances is it permissible to dispose of any radioactive material into the non-radioactive trash or into any drains. The issues of radioactive waste disposal is becoming very complex due not only to the radioactive nature of the waste and its inherent disposal problems, but also of recent concern is the chemical hazards associated with the same waste. Hence, it is now possible to have not only radioactive waste, but hazardous waste which is regulated differently. Essentially, all waste needs to be properly manifested regarding not only the the isotope and activity, but also its contents and chemistry and/or presence of biohazards.

RADIOLOGICAL HEALTH EMERGENCIES

The term "radiological health emergency" applies to any incident where the use of ionizing radiation might produce a significant radiation exposure or contamination to personnel or work areas. In the event of a radiological health emergency, notify the Radiation Safety Officer during normal work hours. If the accident occurs in the evening or on weekends, call OPERATIONS Ext 100 immediately. Your call will be directed to a radiation health specialist who will need the following information in order to evaluate the seriousness of the situation: What radionuclides are involved and what amount of activity has been released? How many persons have been exposed? What chemical form is the radionuclide in? Is it a gas or is it a liquid, a solvent, acid, base, carcinogen, etc.? Has the spill or condition stabilized (example: is the building afire or is the source still leaking or is the spill spreading)? In the event of serious injury due to exposure and/or contamination, an ambulance may be dispatched at the discretion of the radiation safety specialist whereby victims will be transported to Sparrow Hospital in Lansing, Michigan for treatment. Upon arrival at Sparrow Hospital, the victims(s) will be met by appropriate radiation safety personnel who will oversee the treatment and decontamination procedure.

Handling of Radioactive Spills

In the event of a MINOR spill of radioactive material, the following procedure should be followed:

(1) Notify all persons in the area that a spill has occurred.
(2) Prevent the spread by covering the spill, if necessary, with absorbant paper.
(3) Using disposable gloves, carefully fold up the the absorbant paper and pad and deposit in an appropriated radioactive waste container.

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(4) Document spill in radiation survey log book and perform a follow-up radiation survey using the appropriate monitoring equipment in order to evaluate the presence of contamination on an individual's skin and clothing and on lab equipment.
(5) Report the incident to the Radiation Safety Officer Ext. 371

In the event of a MAJOR spill, the following procedure should be instituted:

(1) Notify all persons in the area that a major spill has occurred and vacate the area.
(2) If possible, prevent the spreading of the radioactive material by using absorbant paper. Do not attempt to clean it up. Confine all potentially contaminated individuals in order to prevent the further spread of contamination.
(3) If possible, shield the source, but only if it can be done without significantly increasing your radiation exposure.
(4) Leave the affected room and lock the doors in order to prevent entry.
(5) Call the Radiation Safety Officer ext371 if incidents occur during normal work hours. Call the Operations after normal hours Ext. 100
(6) Remove all contaminated clothing and await instructions concerning clean-up from the Radiation Safety Officer or Office of Radiation, Chemical & Bio. Safety
Michigan State Univ.
C-124 Engineering Research Bldg.
E. Lansing, MI 48824
(517) 355-0153

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APPENDIX A

RADIATION WORKER CONSENT FORM

Work in a laboratory where potentially hazardous materials and equipment could pose a risk of illness or injury requires knowledge, skill and common sense in order to achieve safe laboratory practice. Towards this end, it is critical that all workers in radioisotope laboratories avail themselves of a maximum of safety training and safety related information.

I verify that I have had access to and/or have received the following safety information:

(1) RSO Radiation and Chemical Safety Training Class.
(2) Radiation Safety Certification Examination
(3) Radiation Safety Manual (_____1988 Edition)
(4) Radiation Safety Study Guide
(5) NRC Reg. Guide 8.13 (Female Radiation Workers Only)
(6) Hazardous Waste Handling & Disposal Procedures

I have read and understand the written information listed above. I agree to comply with the policies, procedures and practices delineated in the above documents to the best of my ability.

I understand that this statement does not negate any rights and responsibilities as set forth in the Employee Rights and Responsibilities Handbook. I further understand that this statement of consent is required strictly as an acknowledgement of my responsibilities and that I have completed the requisite training for my particular job description at Department of State Police.

_____ _____
(Principal Investigator Signature) (Signature)

_____
(Position/Title)

_____
(Department)

_____
(Date)

Appendix A - Radiation Worker Consent Form.....
Appendix B - Instruction Concerning Prenatal Radiation Exposure

Appendix B

U.S. NUCLEAR REGULATORY COMMISSION

Revision 2
December 1987

REGULATORY GUIDE

OFFICE OF NUCLEAR REGULATORY RESEARCH

REGULATORY GUIDE 8.13
(Task OP 031-4)

INSTRUCTION CONCERNING PRENATAL RADIATION EXPOSURE

A. INTRODUCTION

Section 19.12, "Instructions to Workers," of 10 CFR Part 19, "Notices, Instructions, and Reports to Workers; Inspections," requires that all individuals working in or frequenting any portion of a restricted area[1] be instructed in the health protection problems associated with exposure to radioactive materials or radiation, in precautions or procedures to minimize exposure, and in the regulations that they are expected to observe. The present 10 CFR Part 20, "Standards for Protection Against Radiation," has no special limit for exposure of the embryo/fetus.[2] This guide describes the instructions an employer should provide to workers and supervisors concerning biological risks to the embryo/fetus exposed to radiation, a dose limit for the embryo/fetus that is under consideration, and suggestions for reducing radiation exposure.

This regulatory guide takes into consideration a proposed revision to 10 CFR Part 20, which incorporates the radiation protection guidance for the embryo/fetus approved by the President in January 1987 (Ref. 1). This revision to Part 20 was issued in January 1986 for comment as a proposed rule. Comments on the guide as it pertains to the proposed Part 20 are encouraged. If the new Part 20 is codified, this regulatory guide will be revised to conform to the new regulation and will incorporate appropriate public comments.

Any information collection activities mentioned in this regulatory guide are contained as requirements in 10 CFR Parts 19 or 20, which provide the regulatory basis for this guide. The information collection requirements in 10 CFR Parts 19 and 20 have been cleared under OMB Clearance Nos. 3150-0044 and 3150-0014, respectively.

B. DISCUSSION

It has been known since 1906 that cells that are dividing very rapidly and are undifferentiated in their structure and function are generally more sensitive to radiation. In the embryo stage, cells meet both these criteria and thus would be expected to be highly sensitive to radiation. Furthermore, there is direct evidence that the embryo/fetus is radiosensitive. There is also evidence that it is especially sensitive to certain radiation effects during certain periods after conception, particularly during the first 2 to 3 months after conception when a woman may not be aware that she is pregnant.

Section 20.104 of 10 CFR Part 20 places different radiation dose limits on workers who are minors than on adult workers. Workers under the age of 18 are limited to one-tenth of the adult radiation dose limits. However, the present NRC regulations do not establish dose limits specifically for the embryo/fetus.

The NRC's present limit on the radiation dose that can be received on the job is 1,250 millirems per quarter (3 months).[3] Working minors (those under 18) are limited to a dose equal to one-tenth that of adults, 125 millirems per quarter. (See § 20.101 of 10 CFR Part 20.)

Because of the sensitivity of the unborn child, the National Council on Radiation Protection and Measurements (NCRP) has recommended that the dose equivalent

[1] Restricted area means any area that has controlled access to protect individuals from being exposed to radiation and radioactive materials.

[2] In conformity with the proposed revision to 10 CFR Part 20, the term "embryo/fetus" is used throughout this document to represent all stages of pregnancy.

[3] The limit is 3,000 millirems per quarter if the worker's occupational dose history is known and the average dose does not exceed 5,000 millirems per year.

USNRC REGULATORY GUIDES

Regulatory Guides are issued to describe and make available to the public methods acceptable to the NRC staff of implementing specific parts of the Commission's regulations, to delineate techniques used by the staff in evaluating specific problems or postulated accidents, or to provide guidance to applicants. Regulatory Guides are not substitutes for regulations, and compliance with them is not required. Methods and solutions different from those set out in the guides will be acceptable if they provide a basis for the findings requisite to the issuance or continuance of a permit or license by the Commission.

This guide was issued after consideration of comments received from the public. Comments and suggestions for improvements in these guides are encouraged at all times, and guides will be revised, as appropriate, to accommodate comments and to reflect new information or experience.

Written comments may be submitted to the Rules and Procedures Branch, DRR, ADM, U.S. Nuclear Regulatory Commission, Washington, DC 20555.

The guides are issued in the following ten broad divisions:

1. Power Reactors
2. Research and Test Reactors
3. Fuels and Materials Facilities
4. Environmental and Siting
5. Materials and Plant Protection
6. Products
7. Transportation
8. Occupational Health
9. Antitrust and Financial Review
10. General

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to the unborn child from occupational exposure of the expectant mother be limited to 500 millirems for the entire pregnancy (Ref. 2). The 1987 Presidential guidance (Ref. 1) specifies an effective dose equivalent limit of 500 millirems to the unborn child if the pregnancy has been declared by the mother; the guidance also recommends that substantial variations in the rate of exposure be avoided. The NRC (in § 20.208 of its proposed revision to Part 20) has proposed adoption of the above limits on dose and rate of exposure.

In 1971, the NCRP commented on the occupational exposure of fertile women (Ref. 2) and suggested that fertile women should be employed only where the annual dose would be unlikely to exceed 2 or 3 rems and would be accumulated at a more or less steady rate. In 1977, the ICRP recommended that, when pregnancy has been diagnosed, the woman work only where it is unlikely that the annual dose would exceed 0.30 of the dose-equivalent limit of 5 rems (Ref. 3). In other words, the ICRP has recommended that pregnant women not work where the annual dose might exceed 1.5 rem.

C. REGULATORY POSITION

Instructions on radiation risks should be provided to workers, including supervisors, in accordance with § 19.12 of 10 CFR Part 19 before they are allowed to work in a restricted area. In providing instructions on radiation risks, employers should include specific instructions about the risks of radiation exposure to the embryo/fetus.

The instructions should be presented both orally and in printed form, and the instructions should include, as a minimum, the information provided in Appendix A (Instructor's Guide) to this guide. Individuals should be given the opportunity to ask questions and in turn should be questioned to determine whether they understand the instructions. An acceptable method of ensuring that the information is understood is to give a simple written test covering the material included in Appendix B (Pregnant Worker's Guide). This approach should highlight for instructors those parts of the instructions that cause difficulties and thereby lead to appropriate modifications in the instructional curriculum.

D. IMPLEMENTATION

The purpose of this section is to provide information to applicants and licensees regarding the NRC staff's plans for using this regulatory guide.

Except in those cases in which an applicant or licensee proposes an acceptable alternative method for complying with specified portions of the Commission's regulations, the NRC will use the material described in this guide to evaluate the instructional program presented to individuals, including supervisors, working in or frequenting any portion of a restricted area.

APPENDIX A

INSTRUCTOR'S GUIDE

EFFECTS ON THE EMBRYO/FETUS OF EXPOSURE TO RADIATION AND OTHER ENVIRONMENTAL HAZARDS

In order to decide whether to continue working while exposed to ionizing radiation during her pregnancy, a woman should understand the potential effects on an embryo/fetus, including those that may be produced by various environmental risks such as smoking and drinking. This will allow her to compare these risks with those produced by exposure to ionizing radiation.

Table 1 provides information on the potential effects resulting from exposure of an embryo/fetus to radiation and nonradiation risks. The second column gives the rate at which the effect is produced by natural causes in terms of the number per thousand cases. The fourth column gives the number of additional effects per thousand cases believed to be produced by exposure to the specified amount of the risk factor.

The following section discusses the studies from which the information in Table 1 was derived. The results of exposure of the embryo/fetus to the risk factors and the dependence on the amount of the exposure are explained.

1. RADIATION RISKS

1.1 Childhood Cancer

Numerous studies of radiation-induced childhood cancer have been performed, but a number of them are controversial. The National Academy of Science (NAS) BEIR report reevaluated the data from these studies and even reanalyzed the results. Some of the strongest support for a causal relationship is provided by twin data from the Oxford survey (Ref. 4). For maternal radiation doses of 1,000 millirems, the excess number of deaths (above those occurring from natural causes) was found to be 0.6 death per thousand children (Ref. 4).

1.2 Mental Retardation and Abnormal Smallness of the Head (Microcephaly)

Studies of Japanese children who were exposed while in the womb to the atomic bomb radiation at Hiroshima and Nagasaki have shown evidence of both small head size and mental retardation. Most of the children were exposed to radiation doses in the range of 1 to 50 rads. The importance of the most recent study lies in the fact that investigators were able to show that the gestational age (age of the embryo/fetus after conception) at the time the children were exposed was a critical factor (Ref. 7). The approximate risk of small head size as a function of gestational age is shown in Table 1. For a radiation dose of 1,000 millirems at 4 to 7 weeks after conception, the excess cases of small head size was 5 per thousand; at 8 to 11 weeks, it was 9 per thousand (Ref. 7).

In another study, the highest risk of mental retardation occurred during the 8 to 15 week period after conception (Ref. 8). A recent EPA study (Ref. 16) has calculated that excess cases of mental retardation per live birth lie between 0.5 and 4 per thousand per rad.

1.3 Genetic Effects

Radiation-induced genetic effects have not been observed to date in humans. The largest source of material for genetic studies involves the survivors of Hiroshima and Nagasaki, but the 77,000 births that occurred among the survivors showed no evidence of genetic effects. For doses received by the pregnant worker in the course of employment considered in this guide, the dose received by the embryo/fetus apparently would have a negligible effect on descendents (Refs. 17 and 18).

2. NONRADIATION RISKS

2.1 Occupation

A recent study (Ref. 9) involving the birth records of 130,000 children in the State of Washington indicates that the risk of death to the unborn child is related to the occupation of the mother. Workers in the metal industry, the chemical industry, medical technology, the wood industry, the textile industry, and farms exhibited stillbirths or spontaneous abortions at a rate of 90 per thousand above that of workers in the control group, which consisted of workers in several other industries.

2.2 Alcohol

It has been recognized since ancient times that alcohol consumption had an effect on the unborn child. Carthaginian law forbade the consumption of wine on the wedding night so that a defective child might not be conceived. Recent studies have indicated that small amounts of alcohol consumption have only the minor effect of reducing the birth weight slightly, but when consumption increases to 2 to 4 drinks per day, a pattern of abnormalities called the fetal alcohol syndrome (FAS) begins to appear (Ref. 11). The syndrome consists of reduced growth in the unborn child, faulty brain function, and abnormal facial features. There is a syndrome that has the same symptoms as full-blown FAS that occurs in children born to mothers who have not consumed alcohol. This naturally occurring syndrome occurs in about 1 to 2 cases per thousand (Ref. 10).

TABLE 1

EFFECTS OF RISK FACTORS ON PREGNANCY OUTCOME

Effect	Number Occurring from Natural Causes	Risk Factor	Excess Occurrences from Risk Factor
		RADIATION RISKS	
		Childhood Cancer	
Cancer death in children	1.4 per thousand (Ref. 5)	Radiation dose of 1000 millirems received before birth	0.6 per thousand (Ref. 4)
		Abnormalities	
		Radiation dose of 1000 millirads received during specific periods after conception:	
Small head size	40 per thousand (Ref. 6)	4-7 weeks after conception	5 per thousand (Ref. 7)
Small head size	40 per thousand (Ref. 6)	8-11 weeks after conception	9 per thousand (Ref. 7)
Mental retardation	4 per thousand (Ref. 8)	Radiation dose of 1000 millirads received 8 to 15 weeks after conception	4 per thousand (Ref. 8)
		NONRADIATION RISKS	
		Occupation	
Stillbirth or spontaneous abortion	200 per thousand (Ref. 9)	Work in high-risk occupations (see text)	90 per thousand (Ref. 9)
		Alcohol Consumption (see text)	
Fetal alcohol syndrome	1 to 2 per thousand (Ref. 10)	2-4 drinks per day	100 per thousand (Ref. 11)
Fetal alcohol syndrome	1 to 2 per thousand (Ref. 10)	More than 4 drinks per day	200 per thousand (Ref. 11)
Fetal alcohol syndrome	1 to 2 per thousand (Ref. 10)	Chronic alcoholic (more than 10 drinks per day)	350 per thousand (Ref. 12)
Perinatal infant death (around the time of birth)	23 per thousand (Refs. 13, 14)	Chronic alcoholic (more than 10 drinks per day)	170 per thousand (Ref. 15)
		Smoking	
Perinatal infant death	23 per thousand (Refs. 13, 14)	Less than 1 pack per day	5 per thousand (Ref. 13)
Perinatal infant death	23 per thousand (Refs. 13, 14)	One pack or more per day	10 per thousand (Ref. 13)

For mothers who consume 2 to 4 drinks per day, the excess occurrences number about 100 per thousand; and for those who consume more than 4 drinks per day, excess occurrences number 200 per thousand. The most sensitive period for this effect of alcohol appears to be the first few weeks after conception, before the mother-to-be realizes she is pregnant (Refs. 10 and 11). Also, 17% or 170 per thousand of the embryo/fetuses of chronic alcoholics develop FAS and die before birth (Ref. 15). FAS was first identified in 1973 in the United States where less than full-blown effects of the syndrome are now referred to as fetal alcohol effects (FAE) (Ref. 12).

2.3 Smoking

Smoking during pregnancy causes reduced birth weights in babies amounting to 5 to 9 ounces on the average. In addition, there is an increased risk of 5 infant deaths per thousand for mothers who smoke less than one pack per day and 10 infant deaths per thousand for mothers who smoke one or more packs per day (Ref. 13).

2.4 Miscellaneous

Numerous other risks affect the embryo/fetus, only a few of which are touched upon here. Most people are familiar with the drug thalidomide (a sedative given to some pregnant women), which causes children to be born with missing limbs, and the more recent use of the drug diethylstilbestrol (DES), a synthetic estrogen given to some women to treat menstrual disorders, which produced vaginal cancers in the daughters born to women who took the drug. Living at high altitudes also gives rise to an increase in the number of low-birth-weight children born, while an increase in Down's Syndrome (mongolism) occurs in children born to mothers who are over 35 years of age. The rapid growth in the use of ultrasound in recent years has sparked an ongoing investigation into the risks of using ultrasound for diagnostic procedures (Ref. 19).

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APPENDIX B

PREGNANT WORKER'S GUIDE

POSSIBLE HEALTH RISKS TO CHILDREN OF WOMEN WHO ARE EXPOSED TO RADIATION DURING PREGNANCY

During pregnancy, you should be aware of things in your surroundings or in your style of life that could affect your unborn child. For those of you who work in or visit areas designated as Restricted Areas (where access is controlled to protect individuals from being exposed to radiation and radioactive materials), it is desirable that you understand the biological risks of radiation to your unborn child.

Everyone is exposed daily to various kinds of radiation: heat, light, ultraviolet, microwave, ionizing, and so on. For the purposes of this guide, only ionizing radiation (such as x-rays, gamma rays, neutrons, and other high-speed atomic particles) is considered. Actually, everything is radioactive and all human activities involve exposure to radiation. People are exposed to different amounts of natural "background" ionizing radiation depending on where they live. Radon gas in homes is a problem of growing concern. Background radiation comes from three sources:

	Average Annual Dose
Terrestrial - radiation from soil and rocks	50 millirem
Cosmic - radiation from outer space	[illegible] millirem
Radioactivity normally found within the human body	25 millirem
	125 millirem*
Dosage range (geographic and other factors)	75 to 5,000 millirem

The first two of these sources expose the body from the outside, and the last one exposes it from the inside. The average person is thus exposed to a total dose of about 125 millirems per year from natural background radiation.

In addition to exposure from normal background radiation, medical procedures may contribute to the dose people receive. The following table lists the average doses received by the bone marrow (the blood-forming cells) from different medical applications.

X-Ray Procedure	Average Dose*
Normal chest examination	10 millirem
Normal dental examination	10 millirem
Rib cage examination	140 millirem
Gall bladder examination	170 millirem
Barium enema examination	500 millirem
Pelvic examination	600 millirem

*Variations by a factor of 2 (above and below) are not unusual.

*Radiation doses in this document are described in two different units. The rad is a measure of the amount of energy absorbed in a certain amount of material (100 ergs per gram). Equal amounts of energy absorbed from different types of radiation may lead to different biological effects. The rem is a unit that reflects the biological damage done to the body. The millirad and millirem refer to 1/1000 of a rad and a rem, respectively.

NRC POSITION

NRC regulations and guidance are based on the conservative assumption that any amount of radiation, no matter how small, can have a harmful effect on an adult, child, or unborn child. This assumption is said to be conservative because there are no data showing ill effects from small doses, the National Academy of Sciences recently expressed "uncertainty as to whether a dose of, say, 5 rad would have any effect at all." Although it is known that the unborn child is more sensitive to radiation than adults, particularly during certain stages of development, the NRC has not established a special dose limit for protection of the unborn child. Such a limit could result in job discrimination for women of child-bearing age and perhaps in the invasion of privacy (if pregnancy tests were required) if a separate regulatory dose limit were specified for the unborn child. Therefore, the NRC has taken the position that special protection of the unborn child should be *voluntary* and should be based on decisions made by workers and employers who are well informed about the risks involved.

For the NRC position to be effective, it is important that both the employee and the employer understand the risk to the unborn child from radiation received as a result of the occupational exposure of the mother. This document tries to explain the risk as clearly as possible and to compare it with other risks to the unborn child during pregnancy. It is hoped this will help pregnant employees balance the risk to the unborn child against the benefits of employment to decide if the risk is worth taking. This document also discusses methods of keeping the dose, and therefore the risk, to the unborn child as low as is reasonably achievable.

RADIATION DOSE LIMITS

The NRC's present limit on the radiation dose that can be received on the job is 1,250 millirems per quarter (3 months).* Working minors (those under 18) are limited to a dose equal to one-tenth that of adults, 125 millirems per quarter. (See § 20.101 of 10 CFR Part 20.)

Because of the sensitivity of the unborn child, the National Council on Radiation Protection and Measurements (NCRP) has recommended that the dose equivalent to the unborn child from occupational exposure of the expectant mother be limited to 500 millirems for the entire pregnancy (Ref. 2). The 1987 Presidential guidance (Ref. 1) specifies an effective dose equivalent limit of 500 millirems to the unborn child if the pregnancy has been declared by the mother; the guidance also recommends that substantial variations in the rate of exposure be avoided. The NRC (in § 20.208 of its proposed revision to Part 20) has proposed adoption of the above limits on dose and rate of exposure.

ADVICE FOR EMPLOYEE AND EMPLOYER

Although the risks to the unborn child are small under normal working conditions, it is still advisable to limit the radiation dose from occupational exposure to no more than 500 millirems for the total pregnancy. Employee and employer should work together to decide the best method for accomplishing this goal. Some methods that might be used include reducing the time spent in radiation areas, wearing some shielding over the abdominal area, and keeping an extra distance from radiation sources when possible. The employer or health physicist will be able to estimate the probable dose to the unborn child during the normal nine-month pregnancy period and to inform the employee of the amount. If the predicted dose exceeds 500 millirems, the employee and employer should work out schedules or procedures to limit the dose to the 500-millirem recommended limit.

It is important that the employee inform the employer of her condition as soon as she realizes she is pregnant if the dose to the unborn child is to be minimized.

INTERNAL HAZARDS

This document has been directed primarily toward a discussion of radiation doses received from sources outside the body. Workers should also be aware that there is a risk of radioactive material entering the body in workplaces where unsealed radioactive material is used. Nuclear medicine clinics, laboratories, and certain manufacturers use radioactive material in bulk form, often as a liquid or a gas. A list of the commonly used materials and safety precautions for each is beyond the scope of this document, but certain general precautions might include the following:

1. Do not smoke, eat, drink, or apply cosmetics around radioactive material.
2. Do not pipette solutions by mouth.
3. Use disposable gloves while handling radioactive material when feasible.
4. Wash hands after working around radioactive material.
5. Wear lab coats or other protective clothing whenever there is a possibility of spills.

Remember that the employer is required to have demonstrated that it will have safe procedures and practices before the NRC issues it a license to use radioactive material. Workers are urged to follow established procedures and consult the employer's radiation safety officer or health physicist whenever problems or questions arise.

*The limit is 3,000 millirems per quarter if the worker's occupational dose history is known and the average dose does not exceed 5,000 millirems per year.

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1. TYPES OF IONIZING RADIATION

Beta Radiation

A beta particle is an electron emitted by an unstable nucleus in which a neutron is transformed spontaneously into a proton. Beta rays are typically emitted with energies ranging up to about 2 MeV which implies that its range in aluminum is less than 1/8 inch. Therefore, an aluminum shield can be used to discriminate between beta and gamma rays emitted into a G-M detector.

Beta radiation loses energy readily to atomic electrons as it passes through matter, and can eventually stop if the absorber is thick enough. X-rays can be produced when high speed electrons are slowed down rapidly by striking a target. When beta radiation from a radioactive source impinges on a shielding material, this kind of X-ray, called bremsstrahlung, is produced. The efficiency of this process is greater for shielding material of high atomic number. This is the reason for using low atomic number materials such as Lucite for shielding beta sources.

"Beta plus" or positron radiation results from transformation of a proton into a neutron by the same nuclear process. Positrons are detected as 511 keV gamma rays because they combine readily with electrons and annihilate each other.

Gamma and X- Radiation

Gamma rays are photons with well-defined energies which are emitted by nuclei undergoing radioactive decay. X-rays are photons emitted by atomic electrons which have been perturbed from their normal orbits around the nucleus by some disturbance (for example the change in the charge of the nucleus caused by radioactive decay). X- and gamma rays having energy below 5 MeV are absorbed or scattered by atomic electrons through the processes called the photoelectic effect and Compton scattering. At higher energies, the pair production process becomes the dominant way photons are absorbed. Considering all three processes, the most effective shielding materials are those with a high density of electrons, which are elements of high atomic number and high density, such as lead. Remember, anything works if it is thick enough. Space and cost are typically major concerns when selecting shielding material.

Alpha Radiation

Alpha particles are helium nuclei emitted from heavy nuclei (greater than a mass of 210) which has undergone decay and have an energy ranging from 4 MeV to 9 MeV. The range of an alpha particle is very small and therefore, a sheet of paper or even the dead skin layer of the body can readily shield these particles.

Since alpha particles deposit their energy in a small region around the source, they therefore can be more dangerous than other radionuclides if the radiation gets within the body as the internal

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organs of the body have no protective dead skin layer, and small portions of the body can receive high radiation doses once these materials are incorporated into specific organ structures such as bone.

Neutron Radiation

Neutrons are produced by nuclear reactions. Since neutrons are not charged they penetrate shielding more easily than alpha and beta rays. Neutrons are not emitted by most radioactive sources except for the nuclides which decay by spontaneous fission, such as ^{252}Cf (californium), and alpha emitting sources like radium or plutonium in combination with beryllium.

Neutrons lose energy mainly by collisions with nuclei, not by collisions with atomic electrons, and this collision probability decreases when the neutron energy increases. When a neutron scatters off a nucleus it gives up some of its energy to the target nucleus. The maximum energy transfer occurs when the target nucleus has the same mass as the neutron. This is why paraffin or other substances that contain hydrogen are sometimes used in shielding neutron sources. Certain elements, such as boron and cadmium, also have especially large nuclear cross sections for capture of very low energy neutrons. Neutron shields containing hydrogen (which lowers the energy) and boron (which captures the low-energy neutrons) can be useful in shielding small sources.

Questions for Review

1) Name 4 types of ionizing radiation.

2) What process produces neutron radiation?

3) How would you shield a low energy neutron beam?

4) What material is used to shield sources of gamma rays? What precaution should be taken when shielding a gamma source that also emits betas?

5) Under what circumstances would exposure to alpha radiation NOT be harmful to the body? Radionuclides that emit alpha particles are considered especially hazardous. Explain this.

2. SHIELDING PRINCIPLES AND METHODS

Distance

The inverse square law applies for point sources of radiation (in a vacuum, i.e. neglecting attenuation by any material traversed by the radiation). The source of radiation can be regarded as the center of a sphere from which many radii (rays) emerge. Radiation is characterized by rays, that is, particles emitted from the source move on straight lines.

If the radiation intensity (or dose rate) is measured at a point whose distance from the source is known, the intensity (or dose rate) at any other distance from the source can be calculated from the inverse square law, expressed by the following equation:

$$D_1 R_1^2 = D_2 R_2^2, \text{ where}$$

D_1 is the dose rate at distance R_1, and

D_2 is the dose rate at distance R_2.

In practice, the inverse square law is a good approximation only when the distance R from source to detector is much larger than the dimensions of both the source and the detector.

Example: The dose rate of gamma rays from a ^{60}Co source is measured to be 70 mR/hr at a distance of 30 cm. What is the dose rate at 90 cm?

$$70 \times (30)^2 = D_2 \times (90)^2$$

$$D_2 = 7.8 \text{ mR/hr}$$

Absorber

Any material object that interacts with and reduces the amount of radiation is called an absorber. It absorbs energy from the radiation.

Certain radiations (neutrons and gamma rays) are attenuated exponentially with respect to absorber thickness. For these the "half-value layer" is a useful concept. The half value layer is the thickness of shielding required to reduce the radiation levels to one half of the original intensity. The half value layer depends on the particle energy, type of radiation and on the material properties of the absorber.

Example: The half value layer for 0.5 MeV gamma rays absorbed in lead is 0.40 cm. How thick must the walls of a lead container be to reduce the gamma radiation from a source of 0.5 MeV gamma rays to 1/16 of the unshielded intensity?

Answer: 1/16 = 1/2 x 1/2 x 1/2 x 1/2, so 4 half value layers are needed. Wall thickness required is 4 x 0.4 cm, or 1.6 cm.

Half Value Layers (in cm) for Gammas and X-Radiation in Selected Materials

Energy [MeV]	Lead	Iron	Alumi-num	Water	Air*	Stone concrete
0.3	.16	.85	2.46	5.82	5.13	2.76
0.5	.40	1.06	2.94	7.53	6.24	3.39
1.0	.82	1.47	4.22	9.76	8.45	4.65
1.5	1.17	1.83	5.06	12.16	10.34	5.72
2.0	1.36	2.07	6.19	13.86	12.38	6.66
2.5	1.44	2.29	6.79	15.75	13.86	
3.0	1.47	2.48	7.37	17.77	15.07	8.15
Density [g/cc]	11.35	7.86	2.82	1.0	.00129	2.35

* multiply by 1000

Ref.-- "Health Physics and Radiological Health Handbook", B. Shleien and M.S. Terpilak, eds., Nucleon Lectern Associates (1984) 133

Absorbed Dose

The dose of radiation absorbed by the body depends on radiation intensity and the time of exposure:

Dose = Dose rate x time.

Example: If the daily dose limit is 100 mrem, the time that a worker may remain in a 1.5 rem/hr radiation field is:

0.10 rem / 1.5 rem/hr = 0.067 hr, i.e. 4.0 min.

Range

This term applies to charged particles in some absorber. The range is the distance traveled by the particle in the absorber as it slows down from its initial energy to zero energy. The range depends on the particle charge, mass and energy, and on absorber properties (mainly electron density). Note that the range of 5 MeV alpha particles (typical for alpha-emitting radionuclides) is 3.7 cm in air.

Radioactive Decay

Each radionuclide decays with a charateristic physical half-life, defined as the time required for one half of the nuclei in a sample to decay. The probability per unit time that any particular nucleus in the sample will undergo radioactive disintegration is

constant. The activity (disintegrations per unit time) is proportional to the number of radioactive atoms in the sample. This implies that the dose rate will decrease with time.

If the activity of a substance having half life T is measured to be A_o at some initial time, the activity after a time t has elapsed is an exponential function which may be written as follows:

$$A = A_o e^{-(0.693t/T)}$$

Summary

1) Increase distance from source whenever possible (e.g. erect fences and warning signs).

2.) Minimize working time near the source.

3.) For charged particles (alpha and beta) -- use absorber thicker than the range of the particle to completely absorb the particles.

4.) For X- or gamma rays -- determine the required thickness of lead or other suitable shielding either by experiment or by calculating with a table of half-value layers (or equivalent data, such as mass attenuation coefficient).

5.) For neutrons -- similar to 4), except concrete and iron are better absorbers of fast neutrons than lead.

Questions for Review

1) A beam of neutrons is attenuated by placing a steel plate in the beam. Without the plate present the flux of neutrons is 2000 neutrons/sq cm/s. With a 2-inch thick plate in the beam the flux is reduced to 500 neutrons/sq cm/s.

a) What is the "half value layer" in steel for neutrons of the energy found in this beam?

b) Using the answer from part (a) calculate the flux that will be measured when a 4-inch thick plate is placed in the beam.

2) What materials are especially effective at absorbing very low energy (thermal) neutrons?

3) What factors can be used to control the dose of radiation absorbed by the body when working with radioactive material?

4) What is the definition of "range"?

5) For which of the following radiations can a range in aluminum be determined?

	Energy	Radiation
1.	5 MeV	alpha particles
2.	1.2 MeV	beta particles
3.	1.2 MeV	gamma rays
4.	3.5 MeV	neutrons

6) The activity of a pure radioactive source of ^{64}Cu (beta and gamma emitter, half life 12.71 h) is measured at time b to be 6×10^6 disintegrations per second. What will the activity be 38 hours later? At time b the radiation dose rate is measured to be 16 mrem/h at a certain distance from the source. What dose rate will be measured 38 h later at the same distance?

3. UNITS AND DEFINITIONS

Contamination

(1) radioactive material which is inadvertently released.
(2) radioactive material in a form that makes it easily transferrable onto or into a person, creating an internal hazard.

Energy units

'eV equals electron volt which is the amount of potential an electron has.

Multiply MeV by 1.602×10^{-13} to obtain joules.

Multiply joules by 1×10^{7} to obtain ergs.

Quantity of radiation:

Roentgen -- Amount of X- or gamma radiation that will produce in air ions (of one sign) carrying a charge of 2.58×10^{-4} coulombs per kilogram of air. In terms of energy deposition in air this corresponds to depositing 86.9 ergs/g in air. One roentgen (abbreviated 1 R) also will deposit 96 erg/g in biological tissue.

particle flux --particles/sq. cm/second

fluence -- number of particles per unit area of irradiated surface, regardless of rate (example: neutrons/sq. cm.

Absorbed dose: Energy deposited by radiation in an absorber exposed to the radiation.
1 gray = 1 joule/kg = 100 rad
1 rad = 100 erg/g

Quality factor -- The quality factor Q is a multiplier used to take into account the different ability of radiation types to cause damage to biological systems. It is a pure number (dimensionless). Examples of Q values are the following:

Radiation type	Q
X, gamma, beta	1
slow neutron	3
fast neutron	10
alpha	20

Dose equivalent -- Energy deposited in biological tissue corrected for the ability of the particular type of radiation to cause biological damage. Dose equivalent is the absorbed dose of gamma rays that would produce the same biological effect as the actual radiation used. There are two units for dose equivalent in common use:

sievert -- dose equivalent [Sv] = Q x absorbed dose [Gy]
rem -- dose equivalent [rem] = Q x absorbed dose [rad]

Dose rate

1 Sv/hr is the same as 100 rem/hr
1 rem/hr is the same as 10000 micro-Sv/hr
1 mrem/hr is the same as 10 micro-Sv/hr

Activity -- Rate of nuclear disintegrations in a sample of radioactive material. The following units are used to express activity:

disintegration /min.
1 bequerel = 1 disintegration/s
1 Curie = 3.70×10^{10} disintegration/s

Summary of SI units (international standard)

Measured quantity	Unit	Abbreviation	Definition
1. Absorbed dose	gray	Gy	1 joule/kg
2. Dose equivalent	sievert	Sv	Q x absorbed dose
3. Activity	bequerel	Bq	1 disintegration / s

Questions for Review

1) Define absorbed dose, quality factor and dose equivalent.

2) Suppose the radiation field of a source of gamma rays produces 1 roentgen of gammas in 10 minutes in the air at a certain point in space.

a) What is the absorbed dose in the air at that point in a 10 minute exposure?

b) If the air is replaced by biological tissue, what is the absorbed dose in the tissue in 10 minutes?

c) What is the dose equivalent for the tissue in part b)?

3) A tumor is irradiated with fast neutrons to an absorbed dose of 100 rads. What is the dose equivalent obtained by the tumor?

4) A Pu-Be neutron source has an emission rate of 727,000 neutrons/s. What is the flux (in neutrons/cm^2/s) at 2 meters distance?

5) Indium exposed to neutrons forms the radioactive ^{116}In nucleus with half life 54.2 min. If the activity of ^{116}In in a sample is initially 0.32 MBq, what will the activity be after the following times have elapsed: 1 h? 1 day? 1 week?

4. RADIATION DETECTORS

Ion Chambers

An ion chamber consists of a negative and a positive electrode in a chamber filled with gas (usually air). Sufficient voltage is applied to collect ion pairs from the interaction of radiation with the gas. The collected charge and resulting current depends on the average number of radiations, thus the exposure rate of the chamber to ionizing radiation (compare this description to the definition of the roentgen).

Ion chambers measure exposure rates, for example, milliroentgen/hour. They should not be used for routine surveys to detect radiation as other kinds of detectors are more sensitive. Also, they must have thin windows to be resposive to betas, but the cap which covers the window should be present to get the proper detector response to high energy gamma rays. Keep in mind that the roentgen is defined in terms of the ionizing effect of X- and gamma radiation on air.

Geiger-Mueller Counter

The GM counter is constructed much like the ion chamber except that the voltage on the electrodes is high enough that all radiations produce essentially the same number of ion pairs, thus a large current pulse. It is sensitive to small amounts of alpha, beta, and gamma radiation, and is used to detect the presence of radioactivity. Only simple electronics are needed to read out the pulses. A thin window GM counter is less than 0.5% efficient for detecting gammas and about 10% efficient for detecting betas. Depending on energy and window thickness, alpha particles may be detected within 1 or 2 cm of the alpha source.

A sheet of paper will absorb alphas and will transmit betas; 1/8 inch aluminum will stop many betas. These materials are convenient for distinguishing between the various possible types of radiation detected by the G-M counter.

Sodium-Iodide Detector

The NaI detector consists of a NaI crystal coupled to a photomultiplier tube. The crystal produces scintillations of light when irradiated with gammas or X-rays. The PM tube collects the light and produces current pulses proportional to the radiation energy deposited in the crystal. A 2"x 2" NaI detector is about 25% efficient for detecting gamma rays greater than 400MeV. For photons less than 100KeV, a low energy gamma (LEG) probe is best and has an efficiency of up to 50% for ^{125}I. For intermediate energies from 100 KeV to 400 KeV, a 1"x 1" NaI detector preforms well with an efficiency of about 25%.

Alpha Particle Scintillation Probe

This detector works like the NaI detector except that a zinc sulfide screen takes the place of the NaI crystal as the detector. Alphas interacting with ZnS produce light, which is transmitted via a plastic light guide to a PM tube. This detector is about 30-40% efficient for detecting 5 MeV alpha particles.

Film Badges

The Landauer film badge has four thicknesses of plastic, a hole, and a metal foil, all in front of film. From the response of the film to the filtered radiation it is possible to distinguish between betas, gammas, and x-rays with some rough energy information. The B1 series also contains a plastic chip in which neutrons will cause protons to recoil and thus ionize. The tracks of ionization damage can be observed after etching the chip. Film badges are "permanent" records of radiation as they can be stored and reviewed at some later date. They are the most useful for legal records.

Efficiency

Efficiency = (counts/min.) / (disintegrations/min.)

Efficiency is defined for pulse-type detectors only, that is, detectors whose output is a pulse for each particle detected. It may be regarded as the fraction of particles counted compared with the total number emitted if the radionuclide being measured emits one particle per disintegration. The definition given above is used even when estimating the activity of sources of unknown composition.

As defined here, efficiency takes both detector sensitivity and source-detector geometry (solid angle) into account. This implies that the position and size of the source will be preserved or reproduced when the detector is used to measure activity. Often the efficiency is measured with the standard source in contact with the detector face.

Survey Procedures

When using monitoring instruments you should keep the following points in mind:

1) Take the response time of the instrument into account, to avoid moving it too fast (about 0.5 inch/s suggested).

2) Hold the detector close to the surface, on account of the small range of alpha and beta particles.

3) Distinguish alpha, beta and gamma radiations using appropriate detectors and absorbers.

Questions for Review

1) List the radiation detectors that would best detect the presence of each type of radiation:

Neutrons
Alphas
Betas
Gammas

2) What is the most efficient type of portable detector for gammas?

3) What is the most convenient detector for measuring dose rate of gammas? Of neutrons?

4) Which detectors are used to survey for low level radioactive contamination?

5) Define efficiency and list the detectors in the chapter to which the concept of efficiency applies.

6) Why are some neutron detectors placed in a cadmium container? What is the purpose of the paraffin surrounding the detector?

5. OTHER MONITORING INSTRUMENTS

Ratemeters

A ratemeter displays the number of pulses ("counts") per minute corresponding to ionization events in the detector. A ratemeter is appropriate for use with GM counters, sodium iodide detectors, alpha particle scintillation probes and neutron detectors. It is not used with ion chambers.

A ratemeter actually displays a time average counting rate. The averaging time is typically selectable with a switch, e. g. 5 s or 11 s to reach 90 percent of final reading. Ratemeter readings are subject to statistical fluctuations because of random effects in the detection process and in the radiation source.

Scalers

A scaler registers the total number of detector pulses ("counts") regardless of the counting rate. Some scalers are equipped with a clock that can automatically end the counting after a preset time. A scaler can measure radiation intensity (counting rate) more precisely than a ratemeter because (1) the averaging time can be made as long as desired, and (2) averaging over a longer time increases the statistical accuracy of the average counting rate measured by the scaler.

Detector power supplies

Many ratemeters and scalers designed for use with portable detectors have a detector power supply built in. The power supply voltage is regulated, and the voltage is adjustable (e.g. 0.5 to 1.5 kV) to match the requirements of the detector. If an external voltmeter is connected, it must have a very high input resistance to avoid overloading the power supply and thereby getting an inaccurate voltage reading. For example, the output impedance of the detector power supply in a Ludlum Model 3 portable ratemeter is 1 megohm. It will overload on resistive loads below 60 megohms. The manufacturer recommends using a voltage divider with 1000 megohms input resistance, or an electrostatic voltmeter to measure the detector voltage.

CONTROL NO. 87285

DARKROOM

HOT LAB

HOOD

DRAWING A

4'-0"

5'-0"

4'-0"

8'-6"

8'-1"

8'-0"

6'-0"

12'-8"

24"

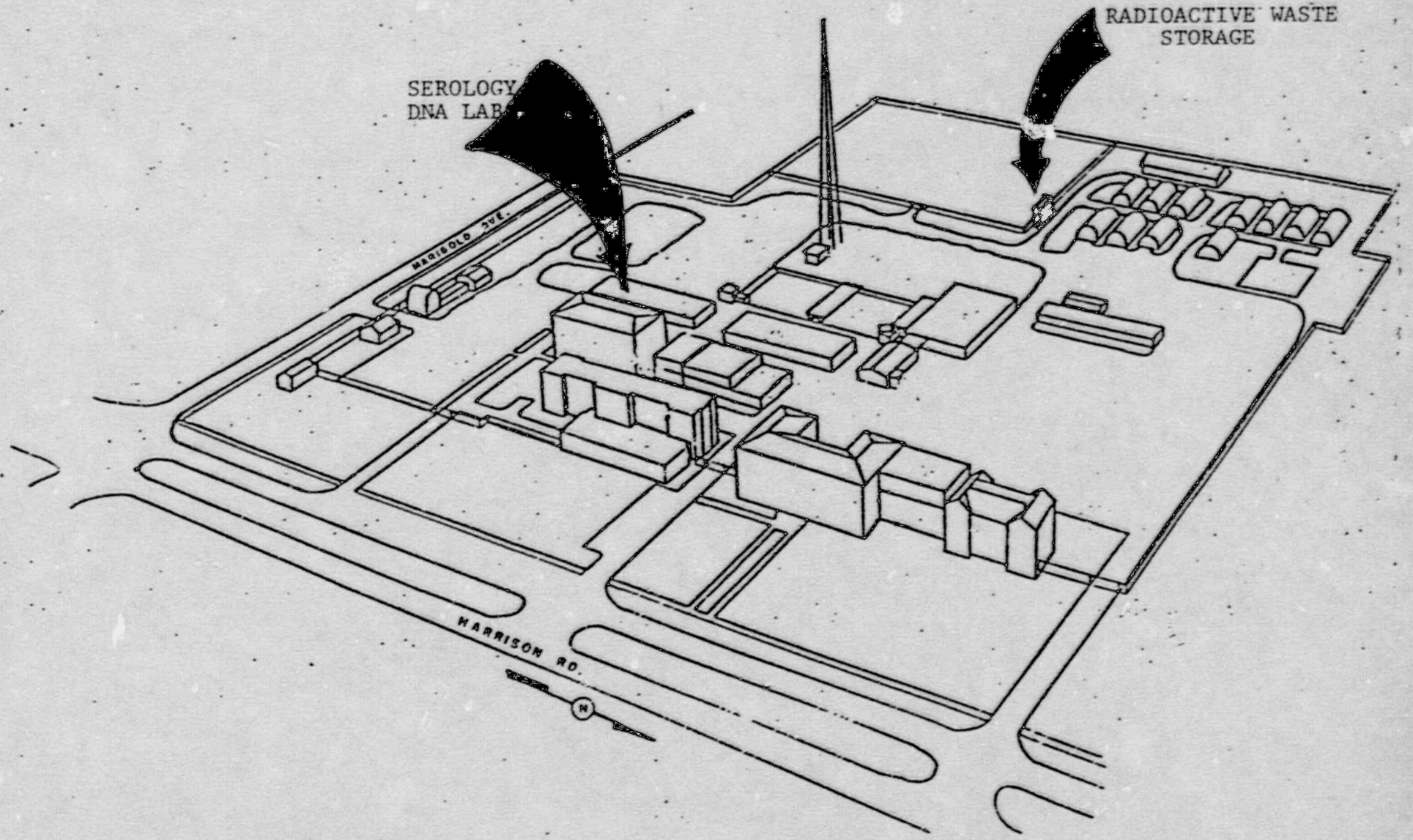
29'-1"

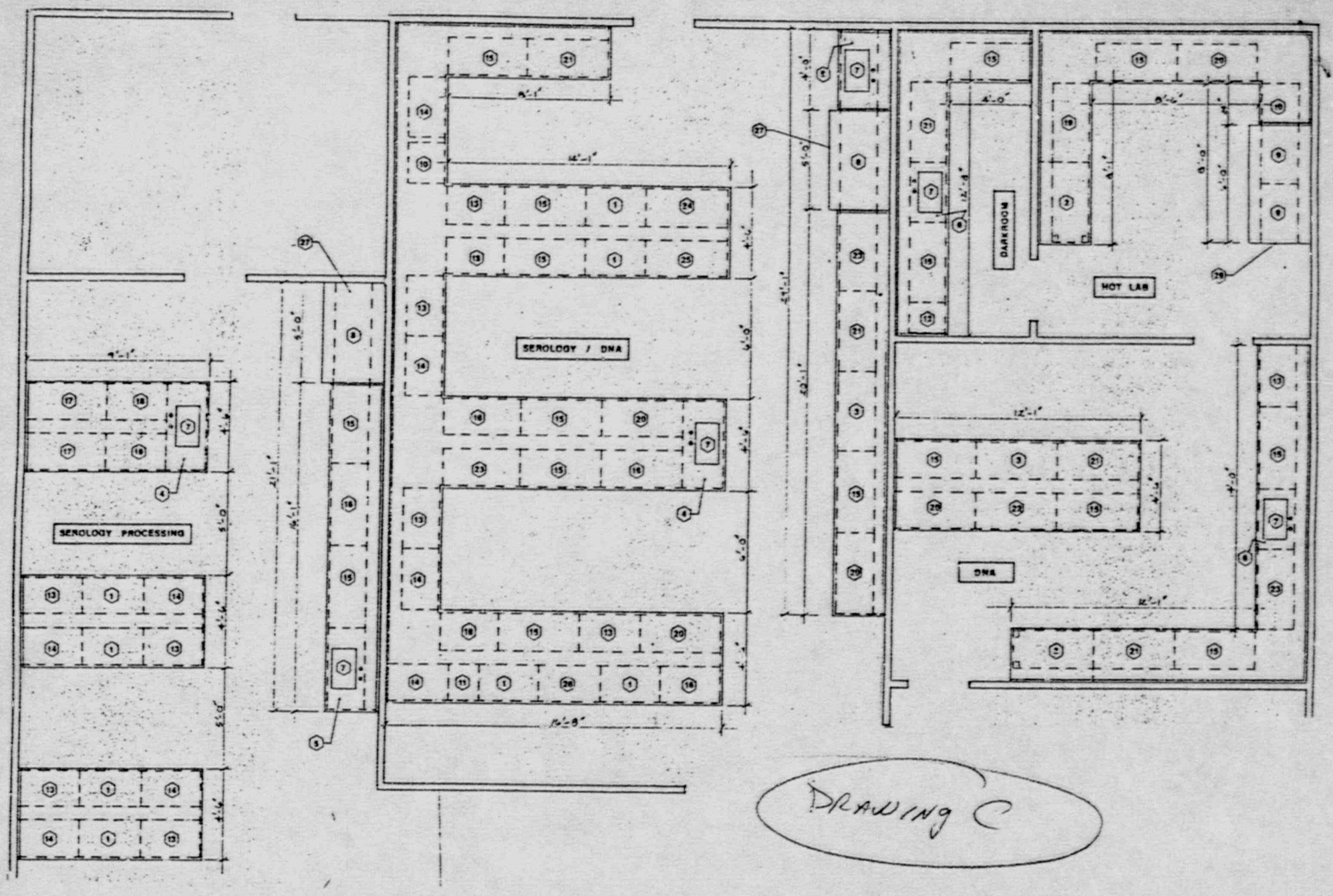
20'-1"

Scale: 3/8" = 1'

MICHIGAN STATE POLICE COMPLEX
714 S. Harrison
East Lansing, MI 48823

DRAWING B

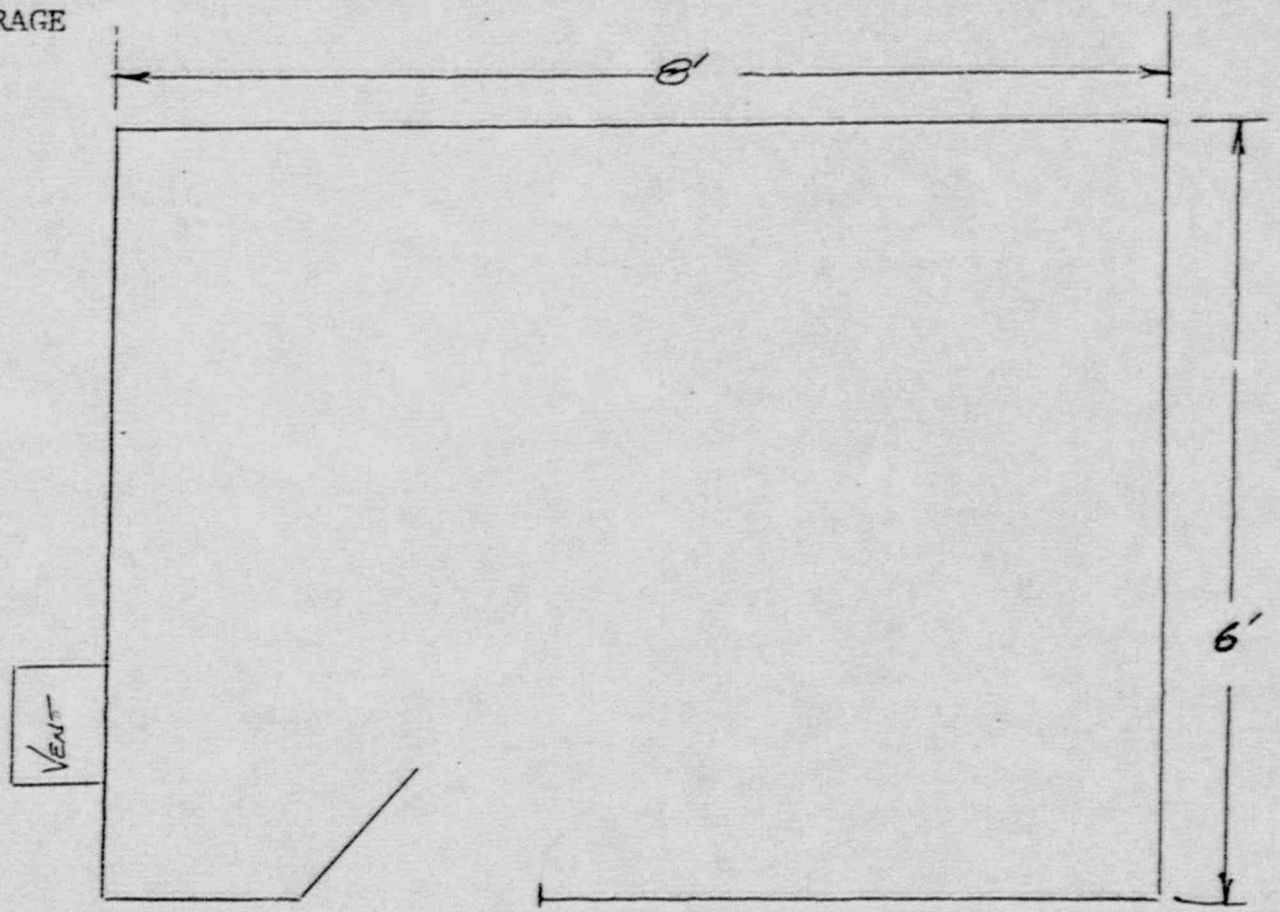




DRAWING C

DRAWING D

HAZARDOUS CHEMICAL STORAGE
OUT BUILDING



N

NOT TO SCALE

ITEM # 11

Received 4-1988

MICHIGAN STATE UNIVERSITY

PHYSICAL PLANT DIVISION
POWER AND WATER DEPARTMENT
T.B. SIMON POWER PLANT

EAST LANSING • MICHIGAN • 48824-1217

April 8, 1988

Mrs. E. Eaton
City of East Lansing
Water Department
East Lansing, MI 48824

Dear Mrs. Eaton:

Subject: WATER UTILIZATION REPORT
1st. Quarter 1988

The following tabulation summarizes total gallons of water pumped by the University Water System and also the amount supplied to various agencies by the University.

	JANUARY	FEBRUARY	MARCH
TOTAL GALLONS SERVICE WATER			
UNIVERSITY WATER SYSTEM Total gallons pumped:	90,339,000	90,286,000	82,159,000
TOTAL GALLONS SUPPLIED			
STATE CONTROL LABORATORY Meter # 2708391 3-31-88 1150.24	485,400	427,100	544,000
M.S.U. CREDIT UNION Bldg. Meter # 10981281 3-31-88 139.05	34,400	31,800	38,900 1,145M
Irrigation Meter # 5247415	0	0	0 10.1M
STATE POLICE Bldg. Meter # 5992318 3-31-88 12,974.52	3,155,000	3,297,000	3,560,000 10,91M

Sincerely yours,

J. F. Kavanagh
Director

365.3314

QTREPORT
c: N. E. Craig
file

CONTROL NO. 8728.5

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MICHIGAN STATE UNIVERSITY

PHYSICAL PLANT DIVISION
POWER AND WATER DEPARTMENT
T.B. SIMON POWER PLANT

EAST LANSING • MICHIGAN • 48824-1217

July 13, 1988

Mrs. E. Eaton
City of East Lansing
Water Department
East Lansing, MI 48824

Dear Mrs. Eaton:

Subject: WATER UTILIZATION REPORT
2nd. Quarter 1988

The following tabulation summarizes total gallons of water pumped by the University Water System and also the amount supplied to various agencies by the University.

	APRIL	MAY	JUNE
TOTAL GALLONS SERVICE WATER			
UNIVERSITY WATER SYSTEM Total gallons pumped:	94,405,000	105,547,000	94,811,000
TOTAL GALLONS SUPPLIED			
STATE CONTROL LABORATORY Meter # 2708391	473,300	444,700	488,200
M.S.U. CREDIT UNION Bldg. Meter # 10981281	64,600	53,200	92,800
Irrigation Meter # 5247415	0	174,000	310,100
STATE POLICE Bldg. Meter # 5992318	3,762,000	4,448,000	5,133,000

Sincerely yours,

R. L. Ellerhorst

QTREPORT
c: N. E. Craig
file

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MICHIGAN STATE UNIVERSITY

PHYSICAL PLANT DIVISION
POWER AND WATER DEPARTMENT
T.B. SIMON POWER PLANT

EAST LANSING • MICHIGAN • 48824-1217

10-24-88

October 20, 1988

Mrs. E. Eaton
City of East Lansing
Water Department
East Lansing, MI 48824

Dear Mrs. Eaton:

Subject: WATER UTILIZATION REPORT
3rd. Quarter 1988

The following tabulation summarizes total gallons of water pumped by the University Water System and also the amount supplied to various agencies by the University.

	JULY	AUGUST	SEPTEMBER
TOTAL GALLONS SERVICE WATER			
UNIVERSITY WATER SYSTEM Total gallons pumped:	93,955,000	91,378,000	88,226,000
TOTAL GALLONS SUPPLIED			
STATE CONTROL LABORATORY Meter # 2708391	672,100	877,400	653,300
M.S.U. CREDIT UNION Bldg. Meter # 10981281	84,700	64,300	48,700
Irrigation Meter # 5247415	347,100	328,200	284,600
STATE POLICE Bldg. Meter # 5992318	5,607,000	5,307,000	4,398,000

Sincerely yours,

R. L. Ellerhorst

QTREPORT
c: N. E. Craig
ORCBS, K. Bronson
file

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MICHIGAN STATE UNIVERSITY

PHYSICAL PLANT DIVISION
POWER AND WATER DEPARTMENT
T.B. SIMON POWER PLANT

EAST LANSING • MICHIGAN • 48824-1217

January 23, 1989

Received
1-25-89

Mrs. E. Eaton
City of East Lansing
Water Department
East Lansing, MI 48824

Dear Mrs. Eaton:

Subject: WATER UTILIZATION REPORT
4th. Quarter 1988

The following tabulation summarizes total gallons of water pumped by the University Water System and also the amount supplied to various agencies by the University.

	OCTOBER	NOVEMBER	DECEMBER
TOTAL GALLONS SERVICE WATER			
UNIVERSITY WATER SYSTEM Total gallons pumped:	101,003,000	87,469,000	62,094,000
TOTAL GALLONS SUPPLIED			
STATE CONTROL LABORATORY Meter # 2708391	703,300	620,100	669,700
M.S.U. CREDIT UNION Bldg. Meter # 10981281	36,700	33,900	33,000
Irrigation Meter # 5247415	168,400	-	-
STATE POLICE Bldg. Meter # 5992318	3,729,000	3,321,000	3,601,000

1993 M

104 M

Sincerely yours,

R. L. Ellerhorst

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c: N. E. Craig
ORCBS, K. Bronson
D. Coon
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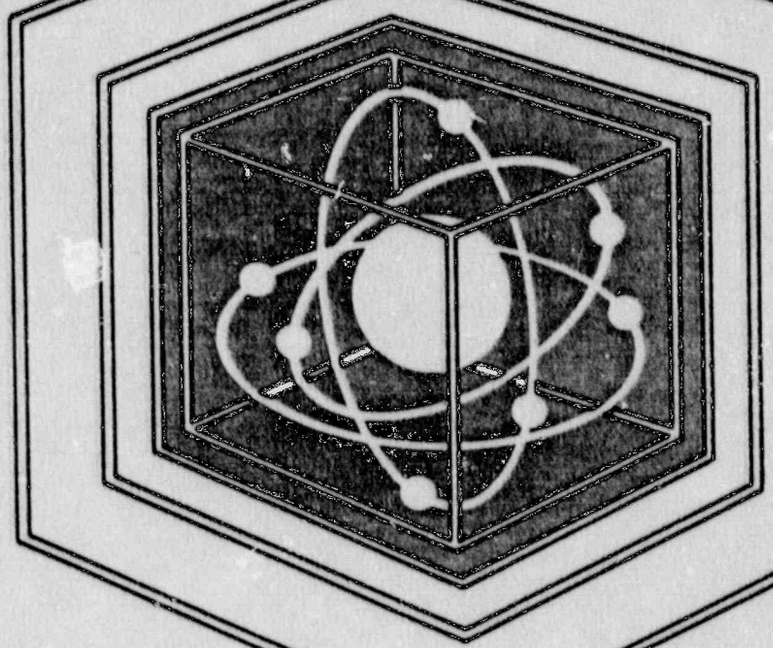
Radiation Safety Study Guide

Office of Radiation, Chemical and Biological Safety



Michigan State University

CONTROL NO. 87285

MICHIGAN STATE UNIVERSITY

Office of Radiation, Chemical and Biological Safety
C124 Engineering Research Complex
Phone 355-0153

BASIC RADIATION SAFETY STUDY GUIDE

September 10, 1987

OFFICE OF RADIATION, CHEMICAL AND BIOLOGICAL SAFETY

BASIC RADIATION SAFETY STUDY GUIDE

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INTRODUCTION

This Study Guide serves as a text for the basic radiation protection information required for radiation workers. This guide is offered to satisfy State and Federal regulations requiring that individuals working with radioactive materials and radiation producing machines be adequately trained. The practical problem sets at the end of each chapter and their detailed solutions also make the Guide useful for self-study in the event that participation in the formal course is not possible.

The concepts and ideas presented in the text require a working knowledge of biology, physics and advanced mathmatics. While the material is presented in its most basic form, undoubtedly some ambiguities will remain. A biblography and an extensive reference section is provided for the interested student to further his or her education in this area.

The Office of Radiation, Chemical and Biological Safety is available to answer any question or to aid any user in the Radiation Science field. We welcome all inquiries and would appreciate comments and suggestions on how to improve this Study Guide.

Note: The Study Guide is an adjunct to the MSU Radiation Safety Manual. Call the Office of Radiation, Chemical and Biological Safety (5-0153 or 3-6675) to request copies.

CHAPTER I FUNDAMENTAL RADIATION CONCEPTS

1. The Radioactive Atom

All matter is composed of elements and all elements are composed of atoms. The atom contains a nucleus consisting of protons and neutrons with electrons revolving in circular and eliptical orbits about the nucleus. Electrons carry a negative charge, protons carry a positive charge, and the neutrons have no electrical charge. An atom normally has one electron in orbit for each proton in the nucleus, leaving the atom electrically neutral.

The atomic structure of an element is denoted as:

$$_{Z}^{A}X$$

where: X is the chemical symbol of the element,

Z is the atomic number, defined as the number of protons in the nucleus. This determines the chemical identity of the element.

A is the mass number. It is the sum of the numbers of protons and neutrons in the nucleus.

A - Z gives the number of neutrons. An element may have different numbers of neutrons and still be chemically the same.

Each individual arrangement of protons and neutrons is referred to as a nuclide. Nuclides which differ only in the number of neutrons are called isotopes. Thus, isotopes can be defined as a group of nuclides having the same atomic number, but different atomic masses. Examples of isotopes of Hydrogen:

$_{1}^{1}H$ Hydrogen

$_{1}^{2}H$ Deuterium

$_{1}^{3}H$ Tritium

Most nuclides are unstable or "radioactive". Radioactivity is defined as the spontaneous disintegration of unstable nuclei with the resulting emission of nuclear energy that results in the formation of new elements. Radioactive nuclides attempt to reach stability by one or more of five (5) modes as outlined in the next section.

2. Radioactive Decay Modes

A) Alpha Decay

i) Generalized Atomic Equation:

$$^{A}_{Z}X \rightarrow {}^{A-4}_{Z-2}Y + \alpha$$

$\alpha = {}^{4}_{2}He$ (Helium Nucleus)

occurs with $Z > 82$

ii) Reaction in Nucleus:

Alpha particles are emitted when the neutron to proton ratio is too low. Sometimes the nucleus, after emitting an alpha particle, is still left in a semi-excited state. To rid itself of excess energy, the nucleus emits gamma rays of energies corresponding to the differences between the excited state and the ground or stable state. Alpha particles are emitted with discrete energies.

iii) Specific Examples of Alpha Decay:

$$^{241}_{95}Am \rightarrow {}^{237}_{93}Np + 5.49\ \text{MeV}\ \alpha\ (85\%) + 5.44\ \text{MeV}\ \alpha\ (13\%) + \gamma\text{'s}$$

$$^{226}_{88}Ra \rightarrow {}^{222}_{86}Rn + 4.78\ \text{MeV}\ \alpha\ (94.3\%) + 4.59\ \text{MeV}\ \alpha\ (5.7\%) + \gamma\text{'s}$$

B) Beta (β^-) Decay

i) Generalized Atomic Equation:

$$^{A}_{Z}X \rightarrow {}^{A}_{Z+1}Y + \beta^- + {}^{0}_{0}\nu$$

β^- = electron emitted from nucleus = $^{0}_{-1}e$

$^{0}_{0}\nu$ = neutrino

ii) Reaction in Nucleus:

When the neutron to proton ratio is too high, a neutron decays into a proton and electron with the electron being ejected from the nucleus. Beta particles are emitted with a continuous energy spectrum up to some maximum value. Beta decay is accompanied by a neutrino, a massless, chargeless particle in order to conserve energy. Beta decay is also accompanied by the emission of gamma rays when the nucleus is left in an semi-excited state following the emission of a beta particle.

iii) Specific Examples of Beta Decay:

$$^{3}_{1}H \rightarrow {}^{3}_{2}He + 0.0186\ \text{MeV}\ \beta^-\ (100\%) + {}^{0}_{0}\nu$$

$$^{14}_{6}C \rightarrow {}^{14}_{7}N + 0.156\ \text{MeV}\ \beta^-\ (100\%) + {}^{0}_{0}\nu$$

$$^{131}_{53}I \rightarrow {}^{131}_{54}Xe + 0.6\ \text{MeV}\ \beta^-\ (85\%) + 0.315\ \text{MeV}\ \beta^-\ (15\%) + \gamma\text{'s} + {}^{0}_{0}\nu$$

C) Positron (β^+) Decay

i) Generalized Atomic Equation:

$$^{A}_{Z}X \rightarrow {}^{A}_{Z-1}Y + \beta^+ + {}^{0}_{0}\nu$$

β^+ = beta particle with a positive charge = positron = $^{0}_{+1}e$

ii) Reaction in Nucleus:

When the neutron to proton ratio is too low and alpha decay is not energetically possible, the nucleus emits a positron resulting from the transformation of a proton into a neutron. When the positron comes in contact with a free electron, the two particles combine and are annihilated, giving rise to two gamma rays whose energies correspond to the rest mass equivalent of the particles (0.511 MeV/γ). Positron decay is also accompanied by gamma and/or X-Ray emission.

iii) Specific Examples of Positron Decay:

$$^{19}_{10}Ne \rightarrow {}^{19}_{9}F + 2.22 \text{ MeV } \beta^+ + \gamma\text{'s}$$

$$^{22}_{11}Na \rightarrow {}^{22}_{10}Ne + 0.546 \text{ MeV } \beta^+ + \gamma\text{'s}$$

D) Electron Capture

i) Generalized Atomic Equation:

$$^{A}_{Z}X + {}^{0}_{-1}e \rightarrow {}^{A}_{Z-1}Y + \text{X-Rays} + {}^{0}_{0}\nu$$

ii) Reaction in Nucleus:

One of the orbital electrons is captured by the nucleus and combines with a proton to form a neutron. Electron capture competes with positron decay when there is a low neutron to proton ratio. If the atom is unable to meet the energy requirements of positron decay, then decay occurs by electron capture. Whenever an atom decays by electron capture, X-Rays are emitted that are characteristic of the daughter nuclide; γ-rays may also be emitted.

iii) Specific Examples of Electron Capture:

$$^{54}_{25}Mn + {}^{0}_{-1}e \rightarrow {}^{54}_{24}Cr + 0.835 \text{ MeV X-Rays} + {}^{0}_{0}\nu$$

$$^{125}_{53}I + {}^{0}_{-1}e \rightarrow {}^{125}_{52}Te + 0.035 \text{ MeV } \gamma\text{-ray } (6.67\%) + \text{X-Rays} + {}^{0}_{0}\nu$$

E) Nuclear Transition-Gamma Ray Emission

i) Generalized Atomic Equation:

excited nucleus → excited metastable nucleus → ground state nucleus

ii) Reaction in Nucleus:

Gamma rays carry away the excess energy of a partially excited nucleus following particle emission from an excited parent nucleus.

iii) Specific examples of nuclear transition can be seen from previous examples of alpha, beta$^-$, and positron decays.

3. The Radioactive Decay Equation

A radioactive nuclide disintegrates or decays spontaneously at a rate depending on the number of original atoms present and upon its decay constant, lambda (λ). This constant, λ, is defined as the instantaneous fraction of atoms decaying per unit time. Each radioactive nuclide has its own characteristic decay constant.

The instantaneous time rate of change of the number of atoms, N, for a radionuclide is given by:

$$\frac{dN}{dt} = -\lambda N$$

If we started with N_o radioactive atoms at some time t = 0, the number of atoms present at some other time N_t, can be obtained by integrating:

$$\frac{dN}{N} = -\lambda dt$$

$$\int_{N_o}^{N_t} \frac{dN}{N} = -\int_{o}^{t} \lambda dt$$

$$\ln\left[\frac{N_t}{N_o}\right] = -\lambda t$$

$$\frac{N_t}{N_o} = e^{-\lambda t}$$

$$N_t = N_o\, e^{-\lambda t}$$

The $e^{-\lambda t}$ term indicates that the radioactive atoms decay exponentially. This equation, $N_t = N_o\, e^{-\lambda t}$, is called the decay equation.

If we were to substitute into the decay equation the time, T, it takes for the reducation of a quantity of radioactive atoms to half of the original, we get:

$$N_T = \frac{1}{2} N_o$$

$$\frac{1}{2} N_o = N_o e^{-\lambda T_{1/2}}$$

$$\frac{1}{2} = e^{-\lambda T_{1/2}}$$

$$\ln \frac{1}{2} = -\lambda T_{1/2} \qquad \ln \frac{1}{2} = \ln 1 - \ln 2; \quad \ln 1 = 0$$

$$-\ln 2 = -\lambda T_{1/2}$$

$$T_{1/2} = \frac{\ln 2}{\lambda} \qquad \ln 2 = 0.693$$

$$\therefore \lambda = \frac{0.693}{T_{1/2}}$$

Thus, the decay constant, λ, can be calculated for any radioactive nuclide from its half-life.

4. Radioactivity Units

The instantaneous number of atoms, N, remaining at a particular instant in time is given by:

$$A = \lambda N$$

Where A is the activity, defined as the instantaneous number of atoms decaying per unit time. The activity determines the quantity of radioactive material in a sample. The special unit for activity is called the Curie(Ci), and is based on the decay rate of 1 gram of radium:

$$1 \text{ Curie} = 3.7 \times 10^{10} \text{ disintegrations per second (dps)}$$

OR

$$1 \text{ Curie} = 2.22 \times 10^{12} \text{ disintegrations per minute (dpm)}$$

Because the Curie is a very large quantity, fractions of the Curie are often used:

1 millicurie = (mCi) = 2.22×10^{9} dpm = 10^{-3} Curies

1 microcurie = (uCi) = 2.22×10^{6} dpm = 10^{-6} Curies

1 nanocurie = (nCi) = 2.22×10^{3} dpm = 10^{-9} Curies

1 picocurie = (pCi) = 2.22 dpm = 10^{-12} Curies

Since radioactive material is measured in units of activity, the decay equation now takes the form:

$$A = A_o e^{-\lambda t}$$

Where: A = Activity after some time t

A_o = Original activity of the sample

λ = The radioactive decay constant equal to $\frac{0.693}{T_{1/2}}$

t = Decay time

Note - the decay time and half-life must be in the same units of time.

It should be mentioned that the International System (SI) of units has defined the Becquerel (Bq) as the unit of activity, equal to 1 disintegration per second. The Becquerel is already in use in some parts of the world and will eventually replace the Curie.

5. Interactions of Radiations with Matter

Radiation interacting with matter can be either scattered or absorbed. The mechanisms of the absorption of radiation is of interest because: a) absorption in the body tissues may result in biological injury; b) absorption is the principle upon which detection of radiation is based; c) the degree of absorption is the primary factor in determining proper shielding requirements.

The transfer of energy from emitted radiations to matter occurs in two major ways: ionization and excitation.

Ionization: The process resulting in the removal of an electron from an atom, leaving the atom with a net positive charge.

Excitation: Addition of energy to an atomic system, transferring it from the ground state to an excited state.

Radiation can be classified into two groups:

1) Particulate radiation (charged particles) such as alpha and beta particles; or

2) Electromagnetic radiation such as X or gamma rays.

A) Interaction of Charged Particles

All atoms are normally electrically neutral. When a charged particle strikes an orbital electron it ejects it from the atom, resulting in the formation of an ion pair. Since the removal of the electron from the atom decreases the total number of negative charges by one, it leaves the atom with a net positive charge. The ion pair consists of:

1) The positively charged atom

2) The negatively charged electron

Such particles capable of creating ion pairs in this manner are called ionizing radiation.

The term used to compare and relate the ionizing powers of different types of charged particles is called the "specific ionization". Specific ionization is defined as the number of ion pairs per unit path length formed by ionizing radiation in a medium:

$$\text{Specific ionization} = \frac{\text{\# of ion pairs formed}}{\text{cm of path}}$$

The specific ionization is dependent on the velocity of the charged particle (and therefore its energy), and the density of the absorbing material (the number of atoms available for ionization).

i) Alpha Particles

An alpha particle is a helium nucleus stripped of its orbital electrons. It is emitted from a radioactive atom with a velocity of about 1/20 that of the speed of light and with energies ranging from 4 to 9 MeV. Alphas cause ionizations in matter when they are deflected by the positive charge of a nucleus and pull the orbital electrons (attracted by the alpha's positive charge) along with them. Alpha particles also cause excitation along their path by pulling inner orbital electrons to outer orbits. No ion pair is formed, but energy is lost from the alpha particle and added to the atom. The added energy is then given off by the atom as fluorescent radiation or low energy X-Rays when the electrons drop back down to the inner orbital vacancies.

Because of its relatively large mass (2 neutrons and 2 protons), high electrical charge (2+) and low velocity, the specific ionization of an alpha particle is very high. That is, it creates many ion pairs in a very short path length. Because of this, it loses all of its energy in a very short distance. The range in air is only several centimeters even for the most energetic alpha particles.

Since the alpha particle has a very limited range in matter, it presents no external radiation hazard to man. Many alpha particles cannot penetrate the protective layer of skin. However, once inside the body, surrounded by living tissue, damage will be to the local area in which the alpha emitter is deposited. Thus, alpha emitters are an internal hazard and intake to the body must be prevented. (See Chapter IV, "Radiation Protection Techniques").

ii) Beta Particles

Beta particles are emitted from the nucleus of a radioactive atom with a wide range of energies up to some maximum value. When a beta is emitted that is below the maximum value, the neutrino carries away the rest of the energy.

Beta particles, like alpha particles, lose their energy by ionization and excitation, but because of their small mass (1/7300 of an alpha) and lower charge (1/2 of that of an alpha) the interactions take place at less frequent intervals. Therefore, the beta particles do not produce as many ion pairs per centimeter of path as alpha particles, and thus, have a greater range in matter. The beta particle's range in matter depends on the energy and the composition of the material. (See Appendix II, "Penetration Ability of Beta Radiation").

Beta particles can interact with a nucleus of an element and give rise to X-rays by a method called Bremsstrahlung. Bremsstrahlung(German for "Breaking Radiation") occurs when a high speed beta particle approaches the nucleus of an atom. The electrical interaction between the negative beta particle and the positively charged nucleus causes the beta particle to be deflected from its original path or stopped all together. This stoppage or deflection results in a change in velocity of the beta particle with the emission of X-rays of various energies. The likelihood of Bremsstrahlung production increases with increasing atomic number of the absorber. For this reason, beta shields are made from low atomic numbered materials, like aluminum or plastics.

Beta particles require an energy of greater than 70 keV to penetrate the protective layer of the skin, and thus, are somewhat of an external hazard. The beta can also constitute an internal hazard. A beta particle has a greater range in tissue compared to an alpha particle due to its low specific ionization- it gives up less energy per unit volume of tissue and, therefore, is not as effective in causing damage as an alpha particle.

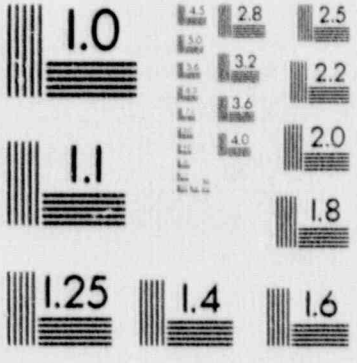
B) Interaction of X-Rays and Gamma-Rays

From a practical radiation protection point of view, X-rays and gamma rays are identical, differing only in their place of origin. Gamma rays are emitted from excited nuclei with a discrete energy. X-rays are emitted when the extra-nuclear atomic structure undergoes a transition; i.e., an outer shell electron replaces a missing lower shell electron and an X-ray is produced. The energy of the X-ray is approximately equal to the difference in the electron energy levels.

Since X and γ rays are chargeless, they do not interact by electrostatic forces as in the case of charged particles, which cause ionization of matter directly along their path of travel. However, X and gamma rays do have sufficient energy to release high energy secondary charged particles (electrons) from matter through one of three basic interactions: The Photoelectric Effect, the Compton Effect, and Pair Production. The high speed electrons resulting from these interactions then cause ionization of the medium.

2

IMAGE EVALUATION TEST TARGET (MT-3)



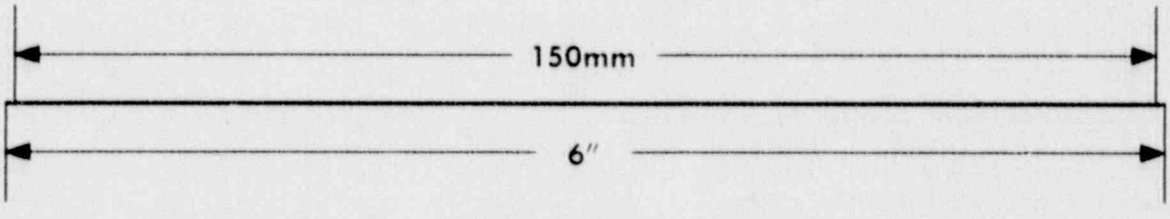


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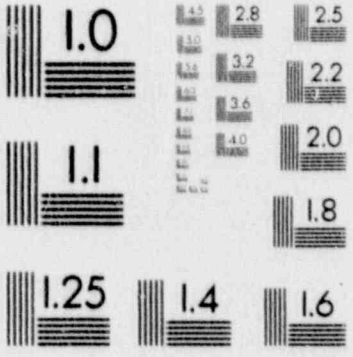
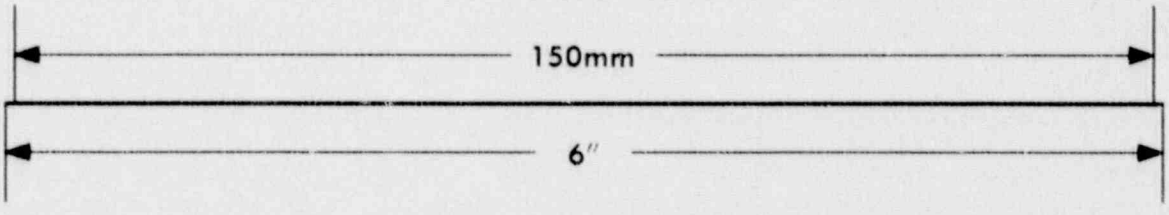
IMAGE EVALUATION TEST TARGET (MT-3)





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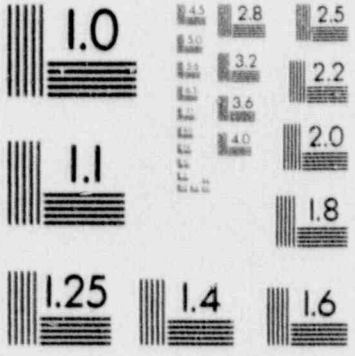
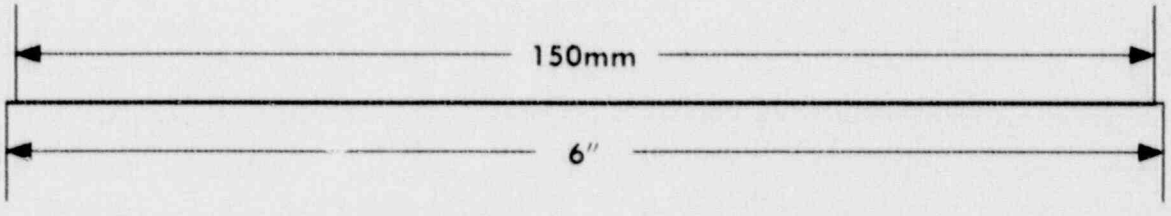
2

IMAGE EVALUATION TEST TARGET (MT-3)





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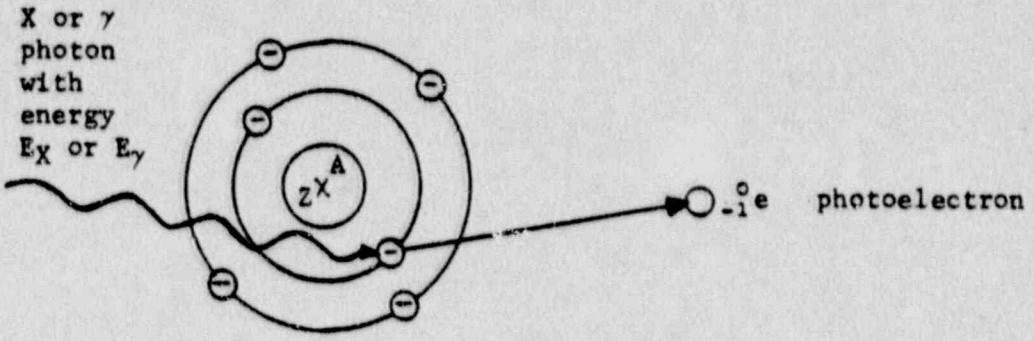
i) The Photoelectric Effect

The Photoelectric Effect is the interaction of X- or γ-ray photons* as well as other photons (such as light), whereby all of the energy of the photon is transferred to an inner shell electron (usually the K shell), ejecting it from the atom and leaving the atom with an inner shell vacancy. This shell vacancy creates an excitation energy which corresponds to the Binding Energy (BE) of the ejected photoelectron.



$$KE_{photoelectron} = E_X \text{ or } E_\gamma - \text{BE of inner shell electron ejected}$$

The Kinetic Energy (KE) of the photoelectron is equal to the energy of the X- or γ-ray photon minus the BE of the electron ejected.

If the X or γ photon does not have sufficient energy to knock the inner shell electron loose, the reaction will not occur.

The resultant atom is now in an excited state and will decay to the ground state by emission of X-rays and fluorescent radiation with the total energy equal to the BE of the photoelectron. The energies of the secondary radiations are usually much lower than the primary X- or γ-ray energies.

Application of the Photoelectric Effect

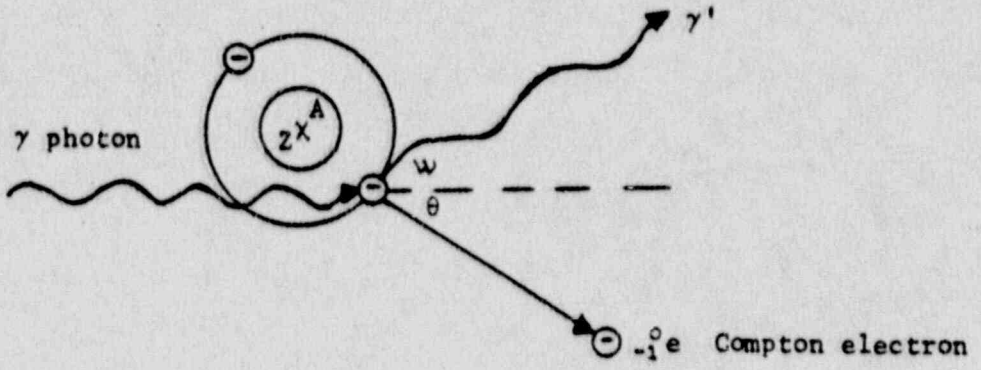
Gamma rays emitted from excited nuclei, and X-rays emitted from excited atoms, have discrete energy characteristics of the specific nuclides and elements, respectively. Thus, the energy of these γ of X photons can be used as "finger prints" to identify unknown nuclides and elements.

---

*A photon, as described by the Quantum Theory, is a "particle" or "quantum" that contains a discrete quantity of electromagnetic energy which travels at the speed of light, or 3×10^8 meters per second.

ii) The Compton Effect (named after A.H. Compton)

Photons with energies much greater than the BE of the electrons in an atom may interact through essentially elastic scattering interactions in which the total KE of the system is conserved. In this interaction, the electron appears to the photon as a free electron.



The primary γ loses part of its energy to the Compton electron which gets scattered at an angle θ from the original direction of the incident γ, while the Compton scattered γ (γ') is scattered at an angle ω (see diagram). In this process the scattered photon and Compton electron share the energy of the incident photon (γ).

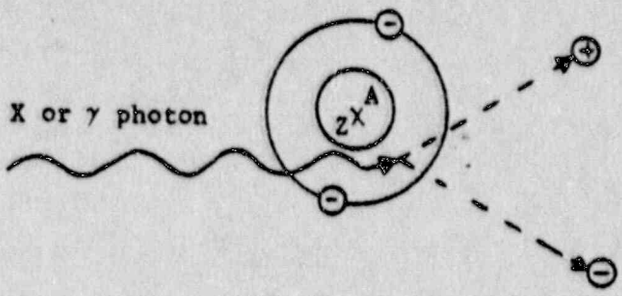
The KE carried off by the Compton electron may be deposited locally (i.e., absorbed immediately by the surroundings). However, the energy carried off by the Compton scattered photon is not deposited locally. Therefore, this scattered photon can significantly contribute to the dose outside a shielding apparatus.

Application of the Compton Effect

Due to its characteristic peaks, the Compton Effect aids in the identification of unknown nuclides. However, in a detecting system, the compton scattered electron can mask lower energy photons interacting by the photoelectric effect making interpretation of results difficult.

iii) Pair Production

High energy gamma photons transfer their energy primarily by pair production. A high energy X- or γ-ray passing close to a nucleus suddenly disappears and an electron and a positron appear in its place. This interaction must take place in the neighborhood of a nucleus to conserve momentum. (See diagram on the following page.)



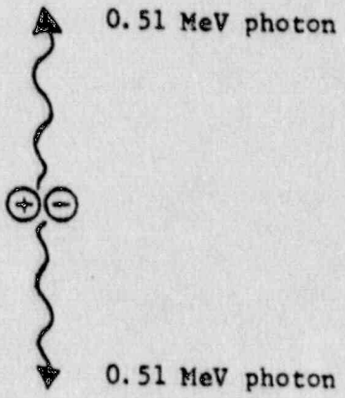
$E_\gamma = KE_+ + KE_- + 2\ m_o c^2$

$m_o c^2$ represents the energy required to create an electron at rest (0.51 MeV)

Since both particles are created from energy supplied by the incident photon, the process is energetically possible only if E_γ or E_X is greater than 1.02 MeV.

When the positron slows down (i.e., loses its KE), it will annihilate itself by combining with an electron. This produces two photons with an energy of 0.51 MeV each. This "annihilation radiation" represents the energy equivalent of the rest mass of two electrons which is converted to pure energy according to the principles of Einstein's theories, in particular, $E = mc^2$;

where E = energy of two 0.51 MeV photons
m = the rest mass of two electrons (1/1840 amu)
c = the velocity of light (3×10^8 m/sec)



Applications of Pair Production

Again, due to characteristic peaks observed for various known nuclides, Pair Production is an aid in the identification of unknowns.

6. Radiation Units

Radiations are measured in three basic units - the Roentgen, the rad, and the Rem:

The Roentgen (R) is a measure of radiation exposure and is defined as that amount of X or gamma radiation which produces in 1 cc of air at standard temperature and pressure, secondary energetically charged particles that when completely stopped, produce ions of either sign equal to one electrostatic unit of charge.

The rad (radiation absorbed dose) is a measure of energy deposition in any medium by all types of radiation. The rad is equal to 100 ergs/gram. The SI unit for absorbed dose is the Gray(Gy), equal to 1 Joule/kg. 1 Gy = 100 rads.

Note- In radiation protection, the Roentgen and rad are often used interchangably since in tissue, 1 Roentgen = 93 ergs/gm and 1 rad = 1.08 Roentgens. Strictly speaking, though, the Roentgen is a unit of exposure and applies only to X or gamma radiations. Roentgens can be directly measured - portable survey instruments are calibrated in units of R/Hr (exposure rate-see Chapter II). The rad on the other hand, refers to the quantity of radiation absorbed per unit mass and thus, is a unit of dose.

The Rem (Roentgen equivalent man) is a unit of dose equivalent used for radiation safety purposes. The Rem is defined as the dose (in rads) multiplied by an appropriate Quality Factor (QF). The Quality Factor is a term used to take into account the different abilities of radiation types to cause damage in a biological system. Below is a table listing Quality Factors for various types of radiations:

Radiation	Quality Factor
X, γ or β	1
Slow neutrons (low KE)	3
Fast neutrons (high KE); Protons	10
Alpha particles	20

Thus, the Rem allows us to add doses of different radiation types to obtain a total biologically effective dose.

Example: What is an individuals dose equivalent from 10 mR of gamma rays, 5 mrads of β^- particles and 10 mrads of fast neutrons? (m = milli = 1/1000)

Dose Equivalent = mrads x QF = mRems

Gamma dose equivalent	=	10	x 1	=	10
Beta dose equivalent	=	5	x 1	=	5
Fast neutron dose equivalent	=	10	x 10	=	100
			TOTAL		115 mRems

The SI unit for dose equivalent is the Sievert (Sv) and is equal to 1 Joule/kg. 1 Sievert = 100 Rem.

Problem Set 1

Multiple choice questions may have more than one correct response. Refer to Appendix IV for reference data.

1. The structural difference between various nuclides of an element are due to different numbers of:

   a) electrons

   b) protons

   c) neutrinos

   d) neutrons

2. Beta decay results in:

   a) decrease in atomic number and mass number of nucleus

   b) decrease in atomic number

   c) increase in atomic number

   d) increase in atomic number and mass number

   e) increase in atomic number and decrease in mass number

3. One millicurie equals:

   a) 3.7×10^{7} dps

   b) 3.7×10^{10} dps

   c) 2.22×10^{9} dpm

   d) 2.22×10^{6} dpm

   e) none of the above

4. The decay constant, λ, is equal to:

   a) A/N

   b) $0.693/T_{1/2}$

   c) 0.693/t

   d) e^{-NT}

5. Gamma rays interact directly with matter by:

   a) ionization and excitation

   b) compton scattering

   c) pair production

   d) photoelectric effect

6. A charged particle interacts with matter by:

   a) a compton scattering

   b) photoelectric effect

   c) excitation and ionization

   d) pair production

7. The activity of a radioactive sample is measured in which of the following units?

   a) Roentgens

   b) Curies

   c) Rems

   d) Rads

8. The Rem is equal to:

   a) Roentgens x Quality Factor

   b) Roentgens x Rads

   c) Roentgens/Quality Factor

   d) Rads x Quality Factor

9. An exposure to 1 mR of gamma, 10 mRad of β^- particles, and 5 mRad of fast neutron radiations would give an individual a dose equivalent of:

   a) 16 mRem

   b) 16 uCi

   c) 61 mRem

   d) 61 mRads

10. List the names and give specific examples for the types of radioactive decay processes in which particles are emitted:

	Name of Process	Example
a)	_____	_____
b)	_____	_____
c)	_____	_____

11. Now, do the same for two types of decay which do not emit particles:

a)	_____	_____
b)	_____	_____

12. A particular radioisotope sample with a half-life of 30 minutes is determined to have an activity of 10,000 dpm at noon.

a) What is the value of its decay constant (λ)? (show units too) _____

b) How many radioactive atoms must have been present in the sample at noon? _____

c) How many dpm will it exhibit at 1:30 PM? _____

13. At 9:00 AM, Tuesday, you assay an unknown radioactive sample and get 15,000 dpm. The next day at 9:00 AM you assay the sample again and find it has decayed to 3,885 dpm. What is the half-life of the isotope? What is the isotope?

14. Assume that you have converted an ancient piece of wood to benzene for Carbon-14 dating. You obtained 3 grams of benzene. The disintegration rate of this sample you found to be 18 dpm. Your modern carbon sample has a disintegration rate of 9 dpm per gram of benzene. Calculate the age of the wood sample.

15. A user requires 2 mCi of Cu-64 for his experiments. If the delivery time is three days, what activity must the Vendor ship in order for the user to receive the correct activity?

CONTROL NO 87285

CHAPTER II RADIATION INSTRUMENTATION

1. Portable Survey Instruments

The major principle for sensing and measuring radiations in survey instruments is based on the ionizations radiation produces when interacting in a gas filled detector. As described in the previous chapter, radiations passing through matter create ion pairs. In a detector, these ion pairs are collected to form a electrical signal through the use of an electric field. The signal, either a current or a pulse, is then used to register the presence or amount of radiation. There are a number of different types of radiation detectors, each operating on this basic principle, but designed for specific purposes. The two major types of portable radiation survey instruments, the Ion Chamber and Geiger Counter are discussed below.

A) Ionization Chambers

Ionization chamber type instruments are designed to measure exposure rates of ionizing radiations in units of mR/Hr or R/Hr. The detector is usually cylindrical, filled with air and fixed to the instrument. When radiation interacts with the air in the detector, ion pairs are created and collected generating a small current. Since we have defined the Roentgen in terms of the amount of ionization charge deposited in air, measurement of this ionization current will indicate the exposure rate.

B) Geiger Counter

The most common type of portable radiation survey instrument is the Geiger Counter, also known as a Geiger-Muller (GM) Counter. The GM counter's detector consists of a tube filled with a mixture of "Q-gas", containing 98% helium and 1.3 % butane; and usually can be removed from the instrument to survey an area. Instead of measuring the average current produced over many interactions, as in Ion Chambers, the output is recorded for each individual interaction in the detector. Thus, a single ionizing event causes the GM tube to produce a "pulse" or "count". Because all pulses from the tube are the same size regardless of the number of original ion pairs that initiated the process, the GM counter cannot distinguish between radiation types or energies. This is why most GM counters are calibrated in "counts per minute" (cpm). However, GM counters can be use to measure exposure rates in mR/Hr or R/Hr as long as the energy of the X or gamma radiation is known and the instrument is calibrated for this particular fixed energy. At best, for a given X or gamma ray energy, the count rate will respond linearly with the intensity of the radiation field. However, in most applications, the radioactive source will have X or gamma rays of various energies which can result in erroneous and unreliable readings. Therefore, GM counters are primarily used to detect the presence of radioactive material.

2. Use of Radiation Survey Instruments

Radiation Instruments are designed with specific purpose in mind. Choose the instrument depending on your particular needs. Generally, Geiger Counters are more sensitive than Ion Chambers and can monitor low levels of contamination in the laboratory. If you wish to measure radiation levels in the laboratory, then an Ion Chamber is the proper instrument to use. Each instrument comes with

an operating manual that describes its function and limitations such as warm up time, battery life, operating temperature range, minimum sensitivities, etc. Outlined below are simple instructions on the proper use of portable radiation survey instruments.

a) Read the instrument's operating manual. Gain familiarity with the controls and operating characteristics.

b) Check the batteries. Most instruments have a battery check indicator. Replace weak batteries. Turn off the instrument when not in use. When storing the instrument for extended periods, remove the batteries to prevent damage from leakage.

c) Check the operability of the detector. Pass the detector over a radioactive check source (sometimes attached to the side or end of the instrument) to verify that the detector responds to radiation.

d) Determine the instrument's response time. By passing the detector at varying speeds over a check source, you can determine how long it takes for the detector to respond to the radiation. It is possible to miss contamination or radiation fields if the detector is moved too rapidly over the area being surveyed.

e) Determine the operating background. Note the instrument's response in an area free of contamination or radiation levels. This is normally due to natural sources of radiation called "background" (see Chapter III, Part 3). Subtract this value from the "gross" reading to obtain the "net" result due to the sample (S) itself: $S_{net} = S_{gross} - S_{background}$.

When using portable instruments, caution should be used when extending detector cords as this may generate electrical noise and register as "counts". Also, thin window GM tubes used to detect alpha and low energy beta particles are fragile and can easily break if dropped or punctured. In a mixed beta-gamma field, the reading due to beta radiation only will be the reading with the beta shield off the detector minus the reading with the beta shield on the detector.

3. Calibrations and Efficiency

In order for the results of a survey instrument to be meaningful, the instrument must be calibrated. Calibrations should be performed at least every six months or when battery or test functions indicate a problem. Ion Chambers are usually calibrated against Cs-137, Co-60, or an X-ray radiation field. The true exposure rate is determined by multiplying correction factors (if any) by the reading on the instrument. GM counters are usually calibrated against a specified reference standard at a fixed distance from the detector (usually 1 centimeter) and a variable pulse generator.

Efficiencies for instruments expressing results in terms of count rates can be calculated from the following formula:

$$\text{Efficiency} = \frac{\text{Observed Standard Count Rate (cpm)}}{\text{Known Standard Disintegration Rate (dpm)}}$$

Divide the observed sample count rate by the detector efficiency to obtain the actual disintegration rate.

Example: A Carbon-14 standard has a disintegration rate of 85,000 dpm. Your GM counter measures a count rate of 4500 cpm. If the background is 250 cpm, what is the efficiency of the counter?

$$\text{Efficiency} = \frac{4500 \text{ cpm} - 250 \text{ cpm}}{85{,}000 \text{ dpm}} = 0.05 \text{ c/d} \times 100 = 5\%$$

4. Counting Statistics

Since radioactive decay is a random process, the number of disintegrations in a given time will fluctuate around an average value. The best estimation of this function is given by the standard deviation (σ). For a single measurement, N, the standard deviation represents 68% probability that the actual value lies within the range $N \pm \sigma$. The standard deviation for a large number of measured events (i.e. counts) is given by the square root of N:

$$\sigma = \sqrt{N}$$

However, it is usually the counting rate which is of interest and the standard deviation becomes:

$$\sigma_r = \frac{\sqrt{N}}{t} \qquad t = \text{counting time}$$

Example: What is the standard deviation of the count rate for a sample that yielded 1,000 counts in two minutes and for a sample that yielded 10,000 counts in twenty minutes?

$$\text{Count Rate} = \frac{1000 \text{ counts}}{2 \text{ minutes}} \pm \frac{\sqrt{1000}}{2} = 500 \pm 15.8 \text{ cpm}$$

$$\text{Count Rate} = \frac{10000 \text{ counts}}{20 \text{ minutes}} \pm \frac{\sqrt{10000}}{20} = 500 \pm 5 \text{ cpm}$$

One can see that in counting, greater statistical accuracy can be achieved by increasing the total counts which is usually accomplished by increasing the counting time of the sample. Generally, between 1,000 and 10,000 counts are needed for a sample to have statistical validity.

5. Liquid Scintillation Counting Techniques

The following is a laboratory experiment on liquid scintillation counting techniques.

Experiment # 9: LIQUID SCINTILLATION COUNTING TECHNIQUES

A widely used technique for the measurment of radioactivity of weak beta emitters as H^3, C^{14} and S^{35} is liquid scintillation counting. Here, the sample is dissolved in a solvent which also contains a soluble phosphor. This mixture is then placed in a position where a photomultiplier can detect the light emitted from the counting vial. The method offers advantages in that the problem of self-absorption is eliminated, there are no problems of uniformity of plating, films of sample cannot peel off and either be lost or contaminate the counter. The main problems encountered are those of solubility of both the sample and the phosphor in a suitable solvent.

The simplest liquid scintillation systems consist of a single photomultiplier tube attached to a scaler. Such a system is quite adequate for either carbon-14 or sulphur-35 counting if there is sufficient activity in the sample. The background counting rate is quite high, however, due to noise characteristics of the photomultiplier tube which produces pulses of approximately the same size range as the samples. The use of this type of system for counting tritium is quite limited due to the small pulses originating from the sample with the high background counting rate in the same range of pulse heights. The use of a single channel analyzer with such a system helps for the counting of isotopes with a beta energy equal or greater than carbon-14, but background counting rates are quite high for tritium.

More refined systems for liquid scintillation counting incorporates a second photomultiplier tube which looks at the sample. By feeding the two tubes into a circuit which passes only coincident pulses, such as would result from a scintillation in the sample vial, the background due to random pulses originating in the photomultiplier tubes is greatly reduced. The output of such units are usually fed into a single channel analyzer or into two such units for simultaneous

counting of two beta emitting isotopes. Such systems may exhibit efficiencies as high as 90% for carbon-14 and 60% for tritium with backgrounds of 20 cpm when adjusted for optimum conditions. The background rates are primarily due to cosmic and environmental activity, and the activity in the counting vials and solvent.

Solvents.

The choice of solvent for use in the liquid scintillation system is based primarily on two factors: solubility of the phosphor in it, and the efficiency of transfer of the beta energy dissipated within it to the phosphor. The following solvents have proven useful in this system:

Primary Solvent	Efficiency
toluene	100
xylene	100
methoxybenzene	100
1,4-dioxane	70
acetone	10
ethyl ether	3
ethyl alcohol	0

Of these, toluene is the most widely used.

Phosphors.

Compounds which have proven useful as phosphors in liquid scintillation counting are a class which contain conjugated aromatic rings. The most popular of these are listed below.

Phosphor	Emission nm max.	Rel Pulse Height
"PPO" 2,5-diphenyloxazole	380	1.03
p-terphenyl	346	1.00
"PBD" 2-(4-biphenyl)-5-phenyl-1,3,4-oxadiazole	370	1.24

Secondary Solute.

The wavelength of the light emitted from the phosphor is often less than that to which the photomultiplier is most efficient. A wavelength shifter is thus used as a secondary solute to increase the efficiency of detection.

Wavelength Shifter	λ,nm
"POPOP" 2-p-phenylenebis (5-phenyloxazole)	420
"dimethyl-POPOP"	430
"α-NPO" 2-(α-napthyl), 5-phenyloxazole	400-420

Counting Methods.

Many solubility problems exist, and numerous solutions to this problem are possible and have been made. Obviously samples soluble in toluene are the simplest to count. A few procedures for solublizing samples are listed below:

Aqueous samples - make a tertiary mixture with toluene-PPO-ethanol

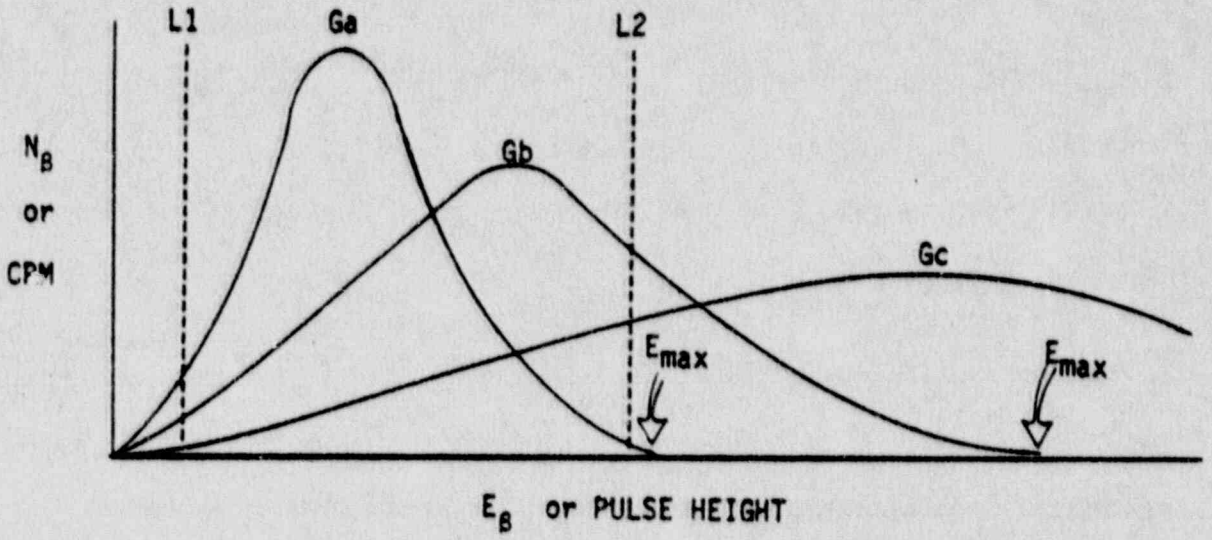
Amino acids, CO_2 - dissolve in 1 ml of 1M hyamine hydroxide in methanol and add 10 ml of toluene containing 0.6% PPO

Gel suspension - Thixin or Cab-O-Sil may be added to suspensions of materials of low solubility to prevent settling.

Beads - plastic phosphor beads may be placed in the vial and covered with any sample in which the plastic is not soluble.

Single Isotope Counting: Balance Point Operation.

For the maximum reduction of background it is desirable to count samples within the window of a pulse height analyzer. The spectrum of a beta emitter is typically as below:



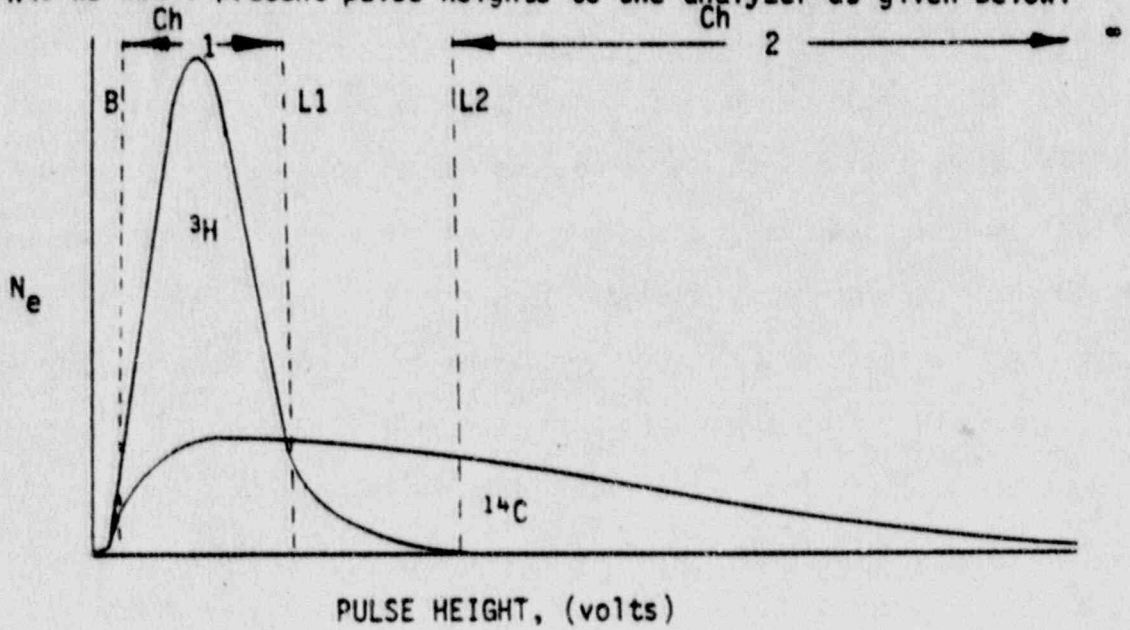
If we adjust the gain such that the pulse heights produced approximately cover the range of the analyzer, as in G_B above, we may set the upper and lower discriminators to look at a considerable portion of the pulse heights produced by the sample. Usually the pulse spectrum is centered between the discriminator settings by adjusting the gain to give a maximum counting rate. At this point the most stable operation of the system is obtained since small changes in parameters which affect gain or pulseheight or shape result in adding about as many pulses above or below levels 1 and 2. If operation is at other than the balance point, as in the above graph, changes in overall gain would change the counting rate; for G_B above an increase in gain would decrease the counting rate, while a decrease in gain would increase the counting rate. G_A would be oppositely affected.

For dual phototube instruments the balance point must be determined by

experiment. For this instrument the gain is determined by the voltage applied to the photomultiplier tubes. The data tube performs this funtion. The gate tube is then optimized to provide suitable pulses to the coincidence circuit. At too low settings of gate voltage no pulses will enter the coincidence circuit so that no counts will be recorded. The gate voltage must be set at an arbitrary value *1400 V) while the data voltage is optimized, then it is adjusted for maximum countrate. Other units accomplish this same result by adjustment of amplifier gain or discriminator setting. Refer to literature of the particular counter for the particular method employed.

Dual Isotope Counting

If we have two beta emitters of sufficiently different energy, we may count them simultaneously in a system such as above. The separation cannot be as great as for gamma counting since the beta spectra overlap to varying extents. If we consider the above two isotopes present in a single sample, for proper voltage settings we would present pulse heights to the analyzer as given below:



by a proper setting of the L1 and L2 levels and recording channel 1 from base to

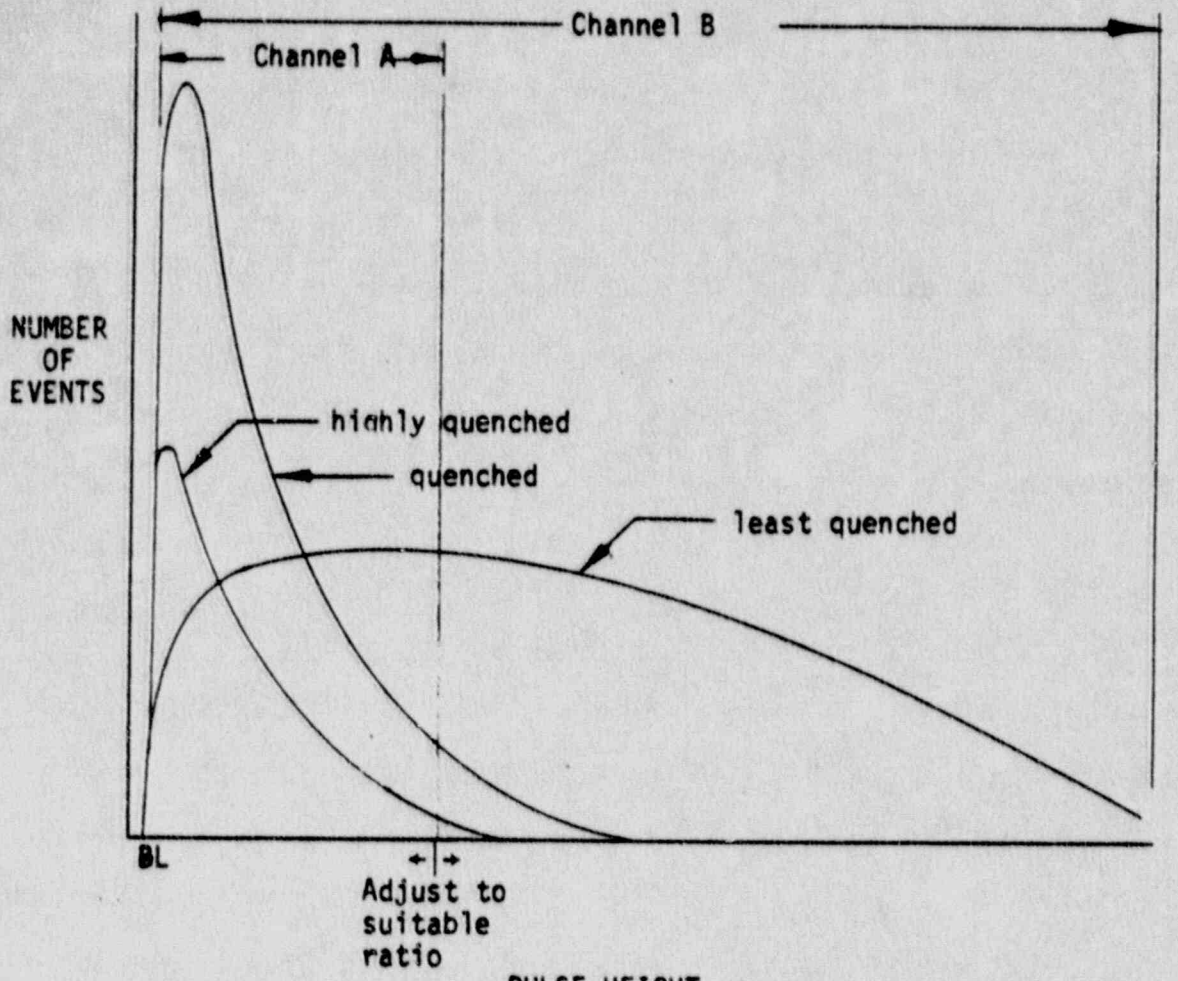
L1 and channel 2 from L2 to ∞, we could theoretically count both isotopes simultaneously with little crosstalk. Of course operation is likely not to be at the balance point for either isotope so that some stability problems may be encountered. It is also likely that considerable efficiency losses would have to be tolerated to prevent counts from both isotopes in the same channel.

With 2 or 3 channel spectrometers, as the Packard or Beckman, a balance point can be set for each channel. A compromise must be made between efficiency and spill-over (or cross-talk) for dual isotope counting.

Quenching.

Variations in counting efficiency often occur as a result of quenching. Either optical or chemical interactions between a sample component and the liquid counting system may result in less light reaching the photomultiplier tube. This gives rise to the measured beta spectrum reaching the electronic stages of the instrument being skewed to lower energy values. For a given setting of a counting channel this would result in fewer than the expected number of counts being recorded. Thus, if different amounts of quenching occurs in a group of samples, errors may result.

Quench correction methods have been developed and are usually applied whenever samples are counted. The specific methods include the use of internal standards, external standards, channels-ratio, and various combinations of these techniques. The choice of method depends on the instrument capabilities and the nature of the samples. They all attempt to determine the amount that a spectrum is shifted and correct for the counts lost by the settings of the instrument which correspond to an unquenched sample. The typical result of such quenching is shown in the figure below:



The channels ratio method of correction counts all samples in two overlaping channels. In the above figure channel B is set for balance point operation of the least quenched sample (or standard) and channel A is set such that the ratio B/A is about 3.5 for this sample. As a quenched sample is counted it is obvious that this ratio will become smaller. By employing a set of standards each containing the same amount of radioactivity and with varying amounts of quench, a curve of channels ratio vs. efficiency may be constructed. If the dpm added is known (and suitably corrected for radioactive decay since calibration) the efficiency for each sample counted (in channel B) is simply (cpm/dpm) x 100. This is plotted against the channels ratio (B/A). Thus, the efficiency of any sample counted at these instrument settings may be read from the graph. This curve may be linear over a

portion of its span, but usually departs from linearity at the more highly quenched end. Some instruments automatically calculate the channels ratio and compute the efficiency by fitting the quench curve by a straight line. Such treatment may be reasonably acceptable for slightly quenched samples, but may lead to error for more highly quenched ones.

Another approach to quench correction now widely used is the external standard method. It makes use of a gamma-emitting source which irradiates the sample container and simulates an emitter within the sample . Light is given off by the Compton electrons and counted in a channel (or channels) for a separate time period for each sample. If the phosphor-solvent system is quenched, less counts are registered in the channel. Several different gamma emitters are used in the various instruments available giving rise to a variety of details in specific function of the system. Often the energy of the electrons are appreciably different from those of the sample being counted. Thus, sample counts do not register in the quench channel. Others of the same emergy range as present in the sample require that sample counts be subtracted from the quench channel count (usually automatically) before ratio calculations or correction is made. Each instrument must be studied to confirm its method of correction--see operating manual. Calibration of the external standard system may be made with the same standard set as for the channels ratio method. It many times requires less calculation. Note that this method is not applicable where solid samples are counted such as bacteria on a membrane filter immersed in phosphor mix, since efficiency in this case is affected by self-absorption in the sample which is not seen by the external method of excitation. Channels ratio correction <u>may</u> be applicable for such counting corrections.

When applying such corrections, do so prior to subtracting background counts (if background is significant). Counts due to background will be quenched by the same processes that quench sample counts, thus the data must be handled mathematically such that this is accounted for. Be cautious of automatic background subtract where sample counts are less than 10X background, unacceptable errors may result.

Sample Preparation and Counting Methods.

The preparation of cells or precipitates for counting is simplified if they may be collected on membrane filters and counted as such. The mass must be small and the size of the cells and the thickness such that the radiation absorption in the sample is minimal. This may be verified by placing different volumes of the same sample on filters and plotting cpm vs. volume applied to establish linearity or lack thereof. The filter and sample may be placed in the bottom of a counting vial and phosphor mix added. Usually 5 cc of mix is sufficient since the beta particles do not travel very far. This may also be experimentally verified for a particular kind of sample. Various mixes for counting cocktails may be employed, mixture A (following) is suitable for samples on membranes. The membrane is transparent to the light in the mix.

Scintillation Counting Cocktail Mixes

Mixture	Components	Application
A	7 g PPO 0.6 g POPOP 1000 ml toluene	moderately quenched non-aqueous
B	4 g PPO 0.05 g POPOP 120 g naphthalene 1000 ml dioxane	aqueous, < 20% water

C	5.5 g PPO 0.1 g POPOP 667 ml toluene 333 ml "Triton X-100"	aqueous < 12%, 23-50% water
D	7 g PPO 0.6 g POPOP 150 g naphthalene 300 ml Cellosolve 700 ml toluene	aqueous < 4% water

Mixtures B,C,D are suitable for samples in solution as long as the limitations on water added is observed. Generally mixtures containing naphthalene are avoided since they tend to clog delivery devices. Many other mixtures may be employed for specific purposes (see references). Changes in mixture may require changes in instrument settings for maximum efficiency.

Use of Liquid Scintillation Counting Systems.

The first step in using any counting system is to verify that the settings are correct for the type samples to be used. A set of quenched standards may be loaded, the method of quench correction for the particular system activated, and each counted along with reference background samples. A comparison of these results with those listed in the instrument operating manual will verify proper operation of the system. If the mixture used for samples differs from the reference set, then a fairly active sample in that mixture should be counted at these settings and at slightly shifted values to see that they are suitable. Always count a reference sample and a background with any set of samples. Identify your samples and keep track of the instrument settings used so that they may be reproduced for a subsequent set of samples. Remove samples from the counter as soon as possible after they have been counted.

Counting statistics for liquid samples are the same as for other types of counting. The standard deviation (σ) is approximated by the square root of the number of counts taken. A suitable time period for counting must be selected

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which is appropriate for the precision required or of the same order of magnitude as other errors inherent in the experiment.

Experimental Procedure

1. Verification of instrument settings.

a. Place an unquenched tritium standard in the counting position and the instrument mode switch in the REPEAT position.

b. With the instrument in MANUAL, vary the gain and discriminator controls to maximize counts from the sample. Use about 10 sec. per counting interval - or such that several thousand counts are registered. Use channel 1 (or RED) for this series. Verify that the maximum efficiency is somewhere near that posted for use of the instrument for tritium.

c. Repeat with a carbon-14 sample using channel 2 (BLUE). In each case the lower discriminator should be high enough to reject noise pulses. This may be checked with the REFERENCE BLANK in position using the same counting techniques.

2. Efficiency and quenching.

a. Load the two sets of reference standards and the blank into the instrument. Set the instrument in the AUTO, AUTO (standardization) and OPERATE modes, preset count 900,000, 10 minutes preset time. The samples will now be counted in sequence to 900,000 in a channel or for 10 minutes, whichever comes first. After each sample is counted, the external standard will be inserted and counted for 1 minute. Three channels of data will be printed for each: 3 with sample only (only the first two of significance) and 3 for the standard (only the last or BLUE channel being of significance).

b. Calculate CPM for channel 1 data for tritium and CPM for channel 2 for carbon-14 data.

c. Calculate the dpm of the reference sources to today (tritium) due to decay losses.

d. Calculate the % efficiency for each source.

e. Plot % efficiency (Y-axis) vs. CPM in quench (BLUE) channel (X-axis) in a manner similar to that posted for each nuclide. Label the curves and give the instrument settings of gain and discriminator setting for all channels.

3. Other nuclides.

a. Check window settings for other nuclides that have been prepared in the same manner as above. Optimize one channel for each in succession and record the instrument settings (gain & window). For those nuclides that have been suspended

in water rather than in the scintillator mix, counting of Cherenkov radiation (like the blue light given off by the reactor) may be performed. Here, a higher efficiency may be obtained by not using the coincidence circuit since each photon is released individually. Compare settings for the various nuclides: ^{3}H, ^{14}C, ^{33}P, ^{32}P and such others that may be available. Determine the efficiency of Cherenkov counting for various energy emitters.

Refer to program QUENCH for data manipulation to obtain efficiency tables as posted on the counter using the quenched source sets.

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6. Gamma Counting

A common method of detecting gamma and X radiations is by using a scintillator coupled to a photomultiplier tube. The most popular scintillation material for this purpose is the sodium iodide (NaI) crystal. Gamma ray interactions within the crystal via the photoelectric effect, compton effect and pair production result in light or scintillations which are amplified and converted into an electrical pulse by the photomultiplier tube.

Sodium iodide crystals can be made in various sizes, some small enough to use in portable survey instruments. Larger crystals (3 inches in diameter by 3 inches deep) are common for most radioisotope counting room applications such as isotope identification by characteristic photopeaks (see Chapter I, Part 5, section B.1). Still others have a hole or "well" in the center, allowing the sample to be surrounded by the crystal, resulting in a very high detection efficiency. This type of detector is found in most laboratory "gamma counters" where a large number of samples can be counted automatically.

Unlike liquid scintillation counting, the sample does not need special preparation. The sample can be counted in any physical form. However, care must be taken to have the material properly contained so as not to contaminate the counting equipment. Gamma emmiting isotopes such as I-125, Cr-51 and those decaying by electron capture are best assayed using a NaI detector.

Problem Set 2

Multiple choice questions may have more than one correct response.

1. When using portable instruments you should:

   a) read the operator's manual
   b) check the batteries and detector operability
   c) extend the probe cord to its fullest length when monitoring
   d) determine the detector's efficiency

2. Ion chamber type instruments are best suited for:

   a) radiation field intensity measurements
   b) radioactive contamination monitoring
   c) determination of radiation energy
   d) identification of radioisotopes

3. GM type instruments are best suited for:

   a) radiation field intensity measurements
   b) radioactive contamination monitoring
   c) determination of radiation energy
   d) identification of radioisotopes

4. What instrument(s) would be most appropriate for detecting the following?

	GM	Ion Chamber	NaI Gamma Counter	Liquid Scintillation Counter
a) non-removable surface contamination	( )	( )	( )	( )
b) X-rays from a dental machine	( )	( )	( )	( )
c) H-3 labelled water	( )	( )	( )	( )
d) a P-32 labelled nucleotide	( )	( )	( )	( )
e) a Cr-51 labellad protein	( )	( )	( )	( )
f) a Mn-54 labelled bacteria	( )	( )	( )	( )
g) a 10 mR/Hr radiation field of beta and gamma rays	( )	( )	( )	( )

5. A 0.05 µCi standard yields 89,200 counts in two minutes. The counter background is 200 cpm. What is the efficiency of the detector?

   a) 80%
   b) 60%
   c) 40%

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6. If a sample was counted for 10 minutes and yielded 20,000 counts, the standard deviation of the countrate would be:

   a) ± 100 cpm

   b) ± 3.16 cpm

   c) ± 141 cpm

   d) ± 14.1 cpm

7. Gamma (NaI) and liquid scintillation detection is based upon what physical property?

   a) radiolysis of an organic solvent

   b) absorption of electromagnetic energy

   c) emission of visible light

   d) ionization of a gas

8. Quenching in a liquid scintillation counting system results in:

   a) a loss in efficiency

   b) less light reaching the photomultiplier tube

   c) shifting of the beta spectrum to lower energy values

   d) an increase in pulse height

9. You have determined that the counting system efficiency for your tracer experiment with I-125 is 25%. You decide that you need a counting rate of 1,000 cpm in your final sample. If 10% of the tracer ends up in the final sample, determine the total dpm of I-125 you must use to get the desired 1,000 cpm.

continued...

10. You are designing a tracer experiment using P-32. You are going to isolate a metabolic product of the labelled compound which you feed your test animals. The best available information indicates that 10% of the fed material leads to the metabolic product. You need to estimate the amount of isotope (in microcuries) to be fed. You elect to use a liquid scintillation counter. You also estimate that you need a minimum count rate of 300 cpm in the counted sample. A 0.01 uCi standard of P-32 has a gross count rate of 15575 cpm. In 10 minutes, the background yields 250 total counts.

a. The net standard count rate is:

15325 cpm ----------( )
15550 cpm ----------( )
15575 cpm ----------( )
15600 cpm ----------( )
15825 cpm ----------( )

b. The counter efficiency, assuming 0.01 uCi at the time of counting, is:

1.43% ----------( )
70% ----------( )
77% ----------( )
0.70% ----------( )
0.77% ----------( )

c. The disintegration rate in a sample necessary to give 300 cpm is:

429 dpm ----------( )
390 dpm ----------( )
400 dpm ----------( )
513 dpm ---------- ( )
210 dpm ---------- ( )

d. The uCi content of the labeled material to be fed to yield the desired dpm in the sample is:

0.019 ----------( )
0.100 ----------( )
0.001 ----------( )
0.010 ----------( )
0.0019 ----------( )

e. If the ESR quench correction factor as printed by the liquid scintillation counter equalled 0.8550, what would be the <u>true</u> standard count rate rather than that as given by item a above?

18235 ----------( )
13317 ---------- ( )
18187 ---------- ( )
13295 ---------- ( )
No difference ---------- ( )

CHAPTER III SOURCES AND EFFECTS OF RADIATION

1. Biological Effects of Radiation

Living organisms are composed of cells. Cells of similar origin and structure are grouped together to form tissues. In the body, there are four main groups of tissues: muscle, nerve, connective and epithelial (lining tissue). Cells and tissues are associated with each other to form organs. A collection of organs which together serve an overall function is called an organ system. The organ systems create within the body the environment required for all cells to function.

Many cells have a limited life span. In order to have their function continue, cells reproduce by a process called mitosis in which the daughter cell takes on the function of the parent. Important to reproduction of the cell are the chromosomes and genes. Changes in these can result either in a mutation or death of the daughter cell when reproduction occurs.

When a cell is injured, many things can happen to alter its ability to produce healthy cells. Radiation in sufficient amounts can damage cells so that they cannot perform their specialized functions. This damage is then reflected in the tissue and organ systems resulting in physiological and morphological changes which can lead to sickness or death.

A) Radiosensitivity of Cells

Radiation passing through living cells will excite or ionize the atoms and molecules that make up the cell structure. This will result in changes in the forces which bind the atoms together into molecules. When the molecules break up, some of the fragments will be charged (become ions) and some of the particles will combine to form free radicals. A free radical is a free atom or molecule that carries an unpaired electron resulting in a high degree of chemical activity. Further damage can be done by the interaction of the free radicals and ions on other cells in the system.

The radiosensitivity of a cell depends on several factors. Since the cells that make up the body differ in function, so does their response to radiation. The "Law of Bergonie and Tribondeau" states that "the radiosensitivity of a tissue is directly proportional to the reproductive activity and inversely proportional to the degree of differentiation". A blood cell that has a short life span and must reproduce rapidly is more sensitive to radiation than a cell which is undifferentiated (not undergone any cell changes during its embryonic growth) and does not undergo mitosis. Other factors involved in a cells sensitivity to radiation are its metabolic state, its state of nourishment, its oxygen level and enzyme levels associated with repair processes.

Damage to the somatic cells (cells of the body) will result in changes in the individual, i.e., damage to or loss of an organ or organ system. Damage to the germ cells (reproductive cells) can result in changes or damage to the offspring of an individual.

The following table gives a summary of how various cells, tissues, organs, and organ systems are affected by radiation. The doses reported are for X or gamma rays only and represent a single acute exposure.

Table of Relative Radiosensitivity of various Cells, Tissues, and Organ Systems

Cell/Tissue/Organ Type	Relative Sensitivity	Biological Effect
Blood Forming Organs: lymph nodes thymus spleen bone marrow	Extremely Radiosensitive	Doses as low as 50 R can affect the white cell population within 15 minutes. Red cells drop off 2-3 weeks later. Result is a feeling of general weakness, anemia, and a lower resistance to infection.
Reproductive Organs: Female Male	Moderately Radiosensitive	Doses below 100 R can reduce fertility. Temporary sterility can occur lasting 12-15 months following doses of 200-300 R. On the average, a larger dose is needed to produce sterility in the male than in the female. Damage to the germ cells can lead to somatic and/or hereditary changes.
Digestive Organs: Small Intestine Lower Intestine Pharynx, Esophagus	Radiosensitive	Degenerative changes occur as soon as 30 minutes after exposure of 500 R - 1000 R. Initial effects are: impaired secretion of necessary fluids; cell breakdown results in failure of food and water absorption leading to infection and dehydration from diarrhea.
Vascular System Small Arteries and Capillaries Heart and Large Arteries and Veins	Moderately Radioresistant	Sensitivity varies for the vascular system - damage is great only for doses in the 600-1500 R range. Damage by radiation to the vascular system contributes to some of the changes in other tissues.
Skin	Radioresistant	Doses between 500-1000 R can produce changes in the skin. However, doses as low as 100 R can cause cell death in the germinal layer of the skin.

continued on following page.....

Cell/Tissue/Organ Type	Relative Sensitivity	Biological Effect
Bone and Teeth (non-growing portions)	Radioresistant	Some parts of bone can be damaged by doses ranging from 700 R - 1500 R. Regeneration can begin 2-6 weeks after exposure.
Respiratory System Lungs Trachea	Relatively Radioresistant	Inflammation of the lungs can occur at large doses of 1000 R to 2000 R; possible hemmorrhaging due to changes produced in blood vessels can also occur.
Urinary System	Relatively Radioresistant	Secondary effects can show up years after exposure in the 500 R - 2000 R range due to changes in the blood vessels.
Muscle and Connective Tissue	Very Radioresistant	Massive doses (greater than 2000 R) are needed to cause slight changes in muscle and connective tissues.
Nervous Tissue	Extremely Radioresistant	Massive doses are required (greater than 3000 R) to bring about morphological changes in nervous tissue.

B) Radiation Damage

The effect that radiation has on the body depends on several factors: The type and energy of the radiation; the amount of radiation absorbed; the time the radiation was distributed, i.e., a single large exposure (acute) or a continuing low level exposure (chronic); and the area to which the radiation was distributed (whole body, single area or organ).

The amount of radiation that is needed to kill an organism is called the $LD_{50/30}$, which is defined as the lethal dose to 50 percent of the exposed individuals within 30 days following an acute whole body exposure. The $LD_{50/30}$ varies widely for different organisms -- 350 rads for dogs, 550 rads for mice, 2300 rads for goldfish. The $LD_{50/30}$ for man is about 450 rads, although this value can vary due to the wide responses of radiation that are present in individuals and lack of experimental data.

When man is exposed to radiation in doses up to the lethal limit, the body will show characteristic changes. These changes are described as "Radiation Sickness". The table on the next page shows some of the symptoms of Radiation Sickness in Man.

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EXPECTED EFFECTS OF ACUTE WHOLE-BODY RADIATION DOSES *

Acute dose (roentgens)	*Probable effect*
0 to 50	No obvious effect, except possibly minor blood changes.
80 to 120	Vomiting and nausea for about 1 day in 5 to 10 percent of exposed personnel. Fatigue but no serious disability.
130 to 170	Vomiting and nausea for about 1 day, followed by other symptoms of radiation sickness in about 25 percent of personnel. No deaths anticipated.
180 to 220	Vomiting and nausea for about 1 day, followed by other symptoms of radiation sickness in about 50 percent of personnel. No deaths anticipated.
270 to 330	Vomiting and nausea in nearly all personnel on first day, followed by other symptoms of radiation sickness. About 20 percent deaths within 2 to 6 weeks after exposure; survivors convalescent for about 3 months.
400 to 500	Vomiting and nausea in all personnel on first day, followed by other symptoms of radiation sickness. About 50 percent deaths within 1 month; survivors convalescent for about 6 months.
550 to 750	Vomiting and nausea in all personnel within 4 hours from exposure, followed by other symptoms of radiation sickness. Up to 100 percent deaths; few survivors convalescent for about 6 months.
1000	Vomiting and nausea in all personnel within 1 to 2 hours. Probably no survivors from radiation sickness.
5000	Incapacitation almost immediately. All personnel will be fatalities within 1 week.

SUMMARY OF CLINICAL SYMPTOMS OF RADIATION SICKNESS *

Time after exposure	Survival improbable (700 r or more)	Survival possible (350 r to 200 r)	Survival probable (250 r to 100 r)
	Nausea, vomiting, and diarrhea in first few hours.	Nausea, vomiting, and diarrhea in first few hours.	Possibly nausea, vomiting, and diarrhea on first day.
1st week	No definite symptoms in some cases (latent period).		
	Diarrhea Hemorrhage Purpura Inflammation of mouth and throat. Fever	No definite symptoms (latent period).	No definite symptoms (latent period).
2nd week	Rapid emaciation Death (Mortality probably 100 percent).	Epilation Loss of appetite and general malaise. Fever	
		Hemorrhage Purpura Petechiae Nosebleeds Pallor Inflammation of mouth and throat. Diarrhea Emaciation	Epilation Loss of appetite and malaise Sore throat Hemorrhage Purpura Petechiae Pallor Diarrhea Moderate emaciation.
3rd week			
4th week			
		Death in most serious cases. (Mortality 50 percent for 450 roentgens.)	Recovery likely in about 3 months unless complicated by poor previous health or superimposed injuries or infections.

* "The Effects of Nuclear Weapons", U. S. Government Printing Office, May 1957

When an organism is exposed to radiation much greater than the $LD_{50/30}$, the organism as a whole will show characteristic syndromes. The composite effect on the organism from the various organ systems is called the "Acute Radiation Syndrome".

Acute Radiation Syndrome

Molecular Death - After a dose in excess of 100,000 rads, death occurs almost immediately. Death is caused by inactivation of substances which are needed for the basic metabolic process of the cells and tissues.

Central Nervous System Syndrome - Doses of 10,000 rads produce death within two days. Within minutes after exposure, the individual becomes disoriented and uncoordinated, followed by semiconsciousness. After 30 hours, the individual lapses into a coma and death occurs from irreparable damage to the central nervous system.

Gastrointestinal Syndrome - Doses between 1000 - 10,000 rads produce death in 4-7 days. Nausea, vomiting and diarrhea are present, normal food and water intake is depressed. Death is due to severe morphological changes in the gastrointestinal tract.

Hematopoetic Syndrome - Doses of 700 - 1000 rads produce death in 10 to 21 days, caused by changes in the blood resulting in infection or hemorrhaging.

Doses below 300 rads can damage other organ systems, but few deaths will occur during the 30 days after exposure.

C) Late Effects of Radiation

Delayed effects of radiation can be due to an acute exposure or a chronic exposure. Radiation has been shown to increase the incidence of the following injuries:

Cancer - No one knows why exposure to radiation (or other carcinogenic agents) causes cancer. Some theories are: radiation may actuate a latent cancer forming virus infection; radiation causes genetic or somatic mutations to initiate tumorous formations; generalized tissue disorder following irradiation promotes a formation of a cancerous growth. The latent period can be long for radiation induced cancers - on the order of years. The most likely to develop are: skin, lung, and bone cancer, and leukemia.

Tissue Effects - Of most concern are radiation induced cataracts and sterility. Cataracts induced by radiation slowly progress over a period of time, but can stop or even regress. Sterility can be either permanent or temporary.

Hereditary Effects - Since the time between generations is so long, the effects of radiation on future generations is incomplete. More work in this area is needed to fully answer these questions.

Life Span Shortening - Data on life span shortening is also incomplete. It is believed that the levels of radiation legally allowed are not high enough to shorten a worker's life span significantly.

D) Comparison of Health Risks

Studies have compared the projected loss of life expectancy resulting from exposure to radiation with other health risks. Estimates are calculated by looking at large numbers of individuals, recording the age when death occurs from apparent causes, and estimating the number of days of life lost as a result of these early deaths. The total number of days of life lost is then averaged over the total group observed:

Estimated Loss of Life Expectancy from Health Risks

Health Risk	Estimates of Days of Life Expectancy Lost
Smoking 20 cigarettes/day	2370 (6.5 years)
Overweight by 20%	985 (2.7 years)
Auto Accidents	200
5 Rems/Yr for 30 years, calculated	150
Alcohol Consumption	130
Home Accidents	95
Safest Jobs, such as teaching	30
1 Rem/Yr for 30 years, calculated	30
Natural Background Radiation, calculated	8
Medical X-Rays, calculated	6
Natural Disasters	3.5
1 Rem Occupational Dose, calculated	1

Adapted from US NRC Regulatory Guide 8.29

These estimates indicate that health risks from occupational radiation exposure are not greater than risks associated with events we encounter in normal day to day activities.

2. Radiation Exposure Limits

A) Historical Review

Soon after the discovery of X-Rays and radium, the dangers of radiation exposure became well known. Standard setting organizations like the International Council on Radiation Protection (ICRP) and the National Council on Radiation Protection and Measurements (NCRP) were formed to recommend limits on

the exposure of radiation. Prior to 1928, the radiation exposure limit was based on the amount of radiation needed to produce reddening of the skin (erythema). When the Roentgen was defined in 1928, this "erythema exposure" was calculated to range from 0.04 R to 2R per day. In 1935, the NCRP's first recommendation for exposure limitation was 0.1 R/Day (31 R/Year). This was an arbitrary limit, based on no observable effects on three technicians' exposure to radium gamma rays. In 1949, the NCRP reduced the limit to 0.05 R/Day (0.3 R/Week; 15 R/Year) because radiations then being used were more penetrating. The last major revision adopted by both the NCRP and ICRP took place in 1957 and is still in effect today. This limit allows an individual to receive up to 3 Rem in 13 consecutive weeks, provided that the accumulated dose does not exceed 5(N-18) Rem, where N is the individual's age. It is apparent, then, that radiation workers must be at least 18 years old.

B) Basis for the Current Radiation Exposure Limits

Occupationally exposed individuals are allowed higher radiation exposures than the general population for the following reasons:

1) The radiation worker accepts some small risk balanced against some benefit (through employment);

2) There is a conscious selection of occupationally exposed individuals: minors are excluded, medical histories can be obtained and maintained. Fertile women may be excluded. Preferential treatment is possible to those beyond the reproductive age.

3) There is a limit on the percentage of radiation workers in the total population.

C) Derivation of the Age-Proration Formula 5(N-18)Rem

Geneticists felt that radiation workers should not receive a total cumulative dose to the reproductive cells of more than 50 Rem by age 30, since statistically at that age, over 50% of their children would have been born. They should be limited to an additional 50 Rem by 40 since by then over 90% of their children would have been born. Practically, this meant limiting the annual dose to about 5 Rem per year:

$$\frac{50 \text{ Rem}}{30y - 18y} = 4.17 \text{ Rem/y} \; ; \qquad \frac{100 \text{ Rem}}{40y - 18y} = 4.55 \text{ Rem/y}$$

Prior experience with the 15 R/y level had shown that radiation doses could be maintained to 1/10 of that limit. However, there was a reluctance to make a too restrictive level of 5 R/y. Thus, an averaging concept based on the age of the worker was recommended.

The 5(N-18) Rem formula was responsive to the genetic limit since 5(30y-18) Rem = 60 Rems which was sufficiently compatible to the 50 Rems recommended. The weekly limit of 0.3R/wk was basically retained, reduced to 3 Rem/13 weeks (0.3R/wk = 3.9 Rem/13 wk $\approx$ 3 Rem/13 wks). This had the effect

of providing a prospective limit of 5 Rems/y averaged over several years with an allowance to receive up to 12 Rem/y. The 12 Rem/y was meant for unusual circumstances, but only when adequate past records of radiation exposure had been maintained. The 3 Rem in 13 weeks should be distributed in time and uniformly if possible. It was not meant to be received within a short time interval.

D) Current Radiation Exposure Limits

Current State and Federal guidelines describe the radiation exposure limits to an occupational radiation worker as follows:

Area of Body	Rems per Calendar Quarter
Whole Body, defined as any of the following: head and trunk; active blood forming organs; lens of eye; or gonads	1.25*
Skin of the whole body, (any portion)	7.50
Extremities, (hands and forearms; feet and ankles)	18.75

* The whole body can receive up to 3 Rems/quarter as long as the dose to the whole body, when added to the accumulated occupational dose to the whole body, does not exceed 5(N-18) Rems. Thus, the maximum allowable dose to the whole body can be as high as 12 Rem per year.

Other Radiation Exposure Limits are as Follows:

Major Organs and Thyroid Gland: 15 Rem/Yr (0.3 Rem/Wk)

Fetus: 0.5 Rem

The dose limit to the whole body for non-radiation workers, in addition to natural and medical sources is 0.5 Rem/year.

The dose limit to the whole body for the U.S. population from all sources of radiation other than natural and medical sources is 0.17 Rem/year per person.

3. Radiation from Background, Consumer Products, and Medical Exposures

The population as a whole is exposed to radiation whether it be from naturally occurring radioactivity present in the earth, from interstellar space, from medical sources, or from radioactivity contained in consumer products.

A) Naturally Occurring Radiation

Naturally occurring radiation arises from three sources: cosmic rays entering the earth's atmosphere, naturally occurring radioactive materials in the earth's crust; and naturally occurring radioactive materials within the body.

i) Cosmic Radiation

Primary cosmic rays are of galactic origin and consist of high energy protons, ^4He ions, electrons, and photons (X and gamma rays). When these particles enter the atmosphere, they interact with the nuclei of the atoms in the air, giving rise to neutrons, electrons, protons, gamma rays, and other particles which are responsible for most of the observed cosmic ray dose. Because of the earth's magnetic field, the cosmic ray intensity varies with latitude, the lowest value at the geomagnetic equator. The intensity also varies with elevation, the highest levels being in the upper atmosphere.

Cosmic rays from solar flares consist of X-Rays, protons, and alpha particles. Because these solar cosmic rays are relatively low in energy, they usually do not contribute significantly to increases in the radiation dose at ground level.

ii) Terrestrial Radiation

Naturally occurring radionuclides in the environment are classed as either cosmogenic or primordial. Cosmogenic nuclides are those nuclides produced in the atmosphere when primary and secondary cosmic rays undergo nuclear reactions with nuclei of atoms in the air. The main contributors to external exposure from cosmogenic nuclides are Be-7, Na-22, and Na-24.

Primordial nuclides are those that are long lived and have existed in the earth's crust throughout history. The main contributors to external exposure from primordial nuclides are K-40, U-238, and Th-232, and their decay products. The concentrations of primordial nuclides in soil are dependent on the process by which the soil was formed. The table below shows the typical activity of these nuclides in various types of rocks:

Type of Rock	Typical Activity Concentration ($pCig^{-1}$) K-40	U-238	Th-232	Absorbed dose rate in air ($\mu rad\ h^{-1}$)
Igneous				
Acidic (e.g. granite)	27	1.6	2.2	12
Intermediate (e.g. diorite)	19	0.62	0.88	6.2
Mafic (e.g. basalt)	6.5	0.31	0.30	2.3
Ultrabasic (e.g. durite)	4.0	0.01	0.66	2.3
Sedimentary				
Limestone	2.4	0.75	0.19	2.0
Carbonate	---	0.72	0.21	1.7
Sandstone	10	0.5	0.3	3.2
Shale	19	1.2	1.2	7.9

Source: UNSCEAR 1977 Report

In various parts of the world, there are areas with high natural radiation levels. At the Beach of the Black Sands in Guarppari, State of Espirto Santos, Brazil, it is possible to receive a radiation exposure of 5 mR/Hr due to the monazite (Thorium bearing minerals) sands. At Pocos de Caldas, State of Gerais, Brazil, the average range of radiation exposure is from 0.1 - 3 mR/Hr.

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Naturally occurring radionuclides can give rise to external doses when contained in raw materials used to construct roads and buildings. Uranium and thorium are commonly found in cement, concrete blocks, and masonry products. For example, the possible annual dose near a granite wall at the "Redcap Stand" in Grand Central Station, New York is 200 mRem (assuming an occupancy of 8 hrs/day).

iii) Internal Radiation

Naturally occurring radionuclides enter the body through inhalation and ingestion. Of the cosmogenic nuclides only H-3, C-14, and Na-22 contribute to internal exposure. The major contribution to internal exposure from primordial nuclides are K-40 and the decay products of the uranium and thorium series.

a) Tritium

Tritium is produced in the atmosphere by secondary cosmic ray neutrons interacting with N-14 nuclides. The global inventory of tritium is calculated to be 34 Mega Curies*. Most (99%) of the H-3 inventory is converted to tritiated water and takes part in the normal water cycle. Approximately 65% of the inventory is in the oceans as a result of transport by rain. About 30% of the inventory is in land surfaces with the remaining in the atmosphere.

b) Carbon-14

Carbon-14 is also produced by cosmic ray neutrons. The global inventory of C-14 is about 300 M Curies, with 94% distributed in the ocean, 4% in the land surfaces and biosphere and the remaining in the atmosphere. The natural specific activity of C-14 is 6.1 pCi/gm of carbon.

c) Potassium-40

Potassium is an essential element of the body and enters via the food chain. The amount of potassium in the body varies with age and sex. The average whole body activity concentration of K-40 is 1600 pCi/kg. Potassium-40 emits beta and gamma radiations and is, therefore, a source of both internal and external radiation exposure.

d) Uranium and Thorium Series

The radionuclides that contribute to internal exposure from the uranium series are: U-238, Ra-226, Rn-222, and its decay products (Pb-210, Bi-210, and Po-210). The major nuclides that contribute to internal exposure from the thorium series are: Th-232, Ra-228, Ra-220, and its decay products (Pb-212, Bi-212, and Po-212).

The major contribution to the natural internal dose is from the decay products of Rn-222. The major source of these alpha emitting nuclides is through emanation of Rn-222 from the ground. The decay products form in clusters with water, oxygen, and other gases and attach themselves to aerosol particles.

---

* Mega Curie = 1,000,000 Curies (MCi)

They then can be inhaled, ingested, and through direct deposition on plant leaves and root absorption enter the food chain. Cigarettes are estimated to contain 0.6 pCi of Pb-210 and 0.4 pCi of Po-210. Brazil nuts and Pacific salmon have been found to contain larger concentrations (> 5 pCi Kg^{-1}) of radium-226. There are areas in the world in which water concentrations of uranium and radium are high due to isolated deposits. Reindeer and caribou contain elevated levels of Pb-210 and Po-210 mainly because they feed on lichens in the winter which accululate these isotopes. The Pb-210 in fish and molluscs range between 20-500 pCi Kg^{-1}.

The main source of radon indoors is from building materials such as by-product gypsum, used for internal walls and ceilings, and concrete. Increasing the ventilation of the room will significantly reduce the radon levels. The highest levels are found in poorly ventilated areas, such as basements where radon diffuses out of the concrete walls and through cracks in the floor. Sealing the walls and floor with epoxy paint can reduce the emanation rate by a factor of four. Three layers of oil paint can reduce the emanation rate by an order of magnitude.

iv) Summary

The following table summarizes the estimated annual tissue absorbed dose from natural sources:

Source of Irradiation	mrad Gonads	Lungs
External Irradiation		
Cosmic Rays:		
Ionizing component	28	28
Neutron component	0.35	0.35
Terrestrial radiation: (γ)	32	32
Internal Irradiation		
Cosmogenic radionuclides:		
H-3 (β)	0.001	0.001
Be-7 (γ)	-----	0.002
C-14 (β)	0.5	0.6
Na-22 ($\beta+\gamma$)	0.02	0.02
Primordial radionuclides:		
K-40 ($\beta+\gamma$)	15	17
Rb-37 (β)	0.8	0.4
U-238, U-234 (α)	0.04	0.04
Th-230 (α)	0.004	0.04
Ra-226, Po-214 (α)	0.03	0.03
Pb-210, Po-210 ($\alpha+\beta$)	0.6	0.3
Rn-222, Po-214 (α) inhalation	0.2	30
Th-232 (α)	0.004	0.04
Ra-228, Tl-208 (α)	0.06	0.06
Rn-220, Tl-208(α) inhalation	0.008	4
TOTAL (rounded)	78	110

Source: UNSCEAR 1977 Report

B) Technologically Enhanced Exposures to Natural Radiation

Technologically enhanced exposure to natural radiation is defined as exposure to natural radiation to which man would not be exposed if some kind of technology had not been developed. For example, travel by air, using natural gas for cooking or heating, and living near a coal fired power plant increase an individual's exposure to naturally occurring radiations.

Air travel increases the exposure due to cosmic rays and solar flares when flying at high altitudes. The following table shows calculated doses for various routes:

Comparison of Calculated Cosmic-Ray Doses to a Person Flying in Subsonic and Supersonic Aircraft

Average Solar Conditions

	Subsonic flight at 11 km		Supersonic flight at 19 km	
Route	Flight duration (h)	Dose per round trip (mrad)	Flight duration (h)	Dose per round trip (mrad)
Los Angles-Paris	11.1	4.8	3.8	3.7
Chicago-Paris	8.3	3.6	2.8	2.6
New York-Paris	7.4	3.1	2.6	2.4
New York-London	7.0	2.9	2.4	2.2
Los Angeles-New York	5.2	1.9	1.9	1.3
Sydney-Acapulco	17.4	4.4	6.2	2.1

Source: UNSCEAR 1977 Report

The table below shows the doses received by astronauts on various space missions. The largest part of the dose was received when the spacecraft passed through the earth's radiation belts. These belts contain protons, electrons, and alpha particles trapped by the earth's magnetic fields.

Absorbed Dose in the Chests of Astronauts on Space Missions

Mission or Mission Series	Launch Date (year-month-day)	Duration of Mission (h)	Type of Orbit	Dose (mrad)
Apollo VII	1968-08-11	260	Earth Orbital	157
Apollo VIII	1968-12-21	147	Circumlunar	150
Apollo IX	1969-02-03	241	Earth Orbital	196
Apollo X	1969-05-18	192	Circumlunar	480
Apollo XI	1969-07-16	195	Lunar Landing	179
Vostok 1-6			Earth Orbital	2-80
Voskhad1, 2			Earth Orbital	30, 70
Soyuz 3-9			Earth Orbital	62-234

Source: UNSCEAR 1977 Report

Individuals living around coal-fired power plants are exposed to enhanced levels of Ra-226, Ra-228, U-238, Pb-210, Th-228, Th-232, and K-40 from gaseous and particulate combustion products of coal. The major contribution to the dose is from the alpha radiation of Pb-210, Th-228, and Th-232.

Phosphate products contain high concentrations of the nuclides in the U-238 decay series. About 1/2 of the phosphate rock that is mined is converted into fertilizer, the rest goes into commodities such as phosphoric acid, gypsum, and land fills. Thus, the use of phosphate fertilizers result in radiation exposures from the following: 1) absorption of radionuclides by food crops, 2) external radiation from fertilizer storehouses and production plants, 3) airborne radon decay products over land reclaimed after phosphate mining, and 4) radiation from gypsum used in building products.

Natural gas used for heating and cooking contains small amounts of radon. Radium contained in the geological formations gives rise to the radon which diffuses into the gas wells. The concentration of radon in natural gas varies widely, depending upon processing, storage and transmission time.

C) Consumer Products

Radiation exposures from consumer products are not considered "Technologically Enhanced" since the radioactive material is deliberately incorporated into the product to serve a specific purpose.

i) Radioluminous Products

Products such as time pieces, aircraft instruments, signs, indicators, etc. contain various amounts of Ra-226, Pm-147, or H-3 to provide illumination. Light is generated when the radiations from these nuclides interact with a scintillator, usually zinc sulfide. The scintillator can be in the form of a paint (watch hands) or a coating inside of glass tubes (exit markers) to make the product "glow in the dark". With the exception of Ra-226, the low energy radiations are unable to penetrate watch crystals, glass tubes, etc. Because of the more energetic radiations from Ra-226, it is now rarely used.

ii) Electronic and Electrical Equipment

Radioactive materials are used in lamps and electronic tubes to provide pre-ionization in gases for the purposes of passing an electrical current. This allows the equipment to respond faster and more reliably. Smoke detectors use alpha radiation from Am-241 to provide an ionization current. Smoke or combustion products entering the detection chamber cause a change in resistance (the alpha particles being stopped or absorbed by the smoke) triggering an alarm. Anti-static devices use Po-210 to ionize the air around a charged object, thereby allowing the charge to be neutralized.

iii) Miscellaneous

Porcelains used in dentistry contain uranium in combination with cerium in order to simulate the natural fluorescence of teeth. Certain glazes used in ceramics contain uranium oxides and sodium uranite as pigments. Glazes ranging from black, brown, green, and the spectrum from yellow to red are used primarily to decorate pottery and tableware. Mantles in gas lanterns

and yard lights consist mainly of thorium oxides. Major radiation exposure occurs during the first few hours that a new mantle is used, primarly from the inhalation of the thorium. Color TV's generate X-Rays (via Bremsstrahlung) as a result of high speed electrons striking the phosphor screen of the picture tube. Most TV's today have high voltage controls and a sufficient thickness of glass to absorb most of these low energy X-Rays.

The following tables describes various consumer products containing radioactive material and some annual population dose rates:

Selected Products Containing Radioactive Materials

Product	Nuclide(s)	Amount
Radioactive Material Contained in Paint or Plastic:		
Time pieces	H-3	1-25 mCi
	Pm-147	65-200 uCi
	Ra-226	0.1-3 uCi
Compasses	H-3	5-50 mCi
	Pm-147	10 uCi
Thermostat Dials and Pointers	H-3	25 mCi
Automobile Shift Quadrants	H-3	25 mCi
Speedometers	Pm-147	0.1 mCi
Radioactive Material Contained in Sealed Tubes:		
Time pieces, Marine navigational instruments	H-3	0.2-2 Ci
Exit signs, stepmarkers, public telephone dials, light switch markers	H-3	0.2-30 Ci
Electronic and Electrical Devices:		
Fluorescent lamp starters	Ra-226	1 uCi
Vacuum tubes, electric lamps, germicidal lamps	Natural Thorium	50 mg
Glow lamps	H-3	0.01 mCi
High voltage protection devices	Pm-147	3 uCi
Low voltage fuses	Pm-147	3 uCi
Miscellaneous:		
Smoke and fire detectors	Am-241	1-100 uCi
	Ra-226	0.01-15 uCi
	Kr-85	7 mCi
Incadescent gas mantles	Natural Thorium	0.5 gm
Ceramic Tableware glaze	Natural Uranium or Thorium	20% by weight of the glaze

Adapted from UNSCEAR 1977 Report

Average Annual Population Dose Equivalents from Selected Consumer Products and Miscellaneous Sources

Product	mRem
TV Receivers	0.5
Airport X-Ray	0.001
Luminous Watches	0.05
Tobacco Products	2,000.00
Coal Combustion	1.0
Natural Gas	5
Uranium in Dentures	10,000.00

Adapted from NCRP Report No. 56

D) Medical Exposures

The population receives an exposure of radiation as part of planned medical procedures. This type of exposure is dependent on individual's health needs and is not considered as part of the individual's occupational exposure. Typical radiation exposures for various radiographic techniques are shown below:

Patient Skin Entrance Exposure, per Film

Technique	mR
Sacral Spine	2180
Barium Enema	1320
Upper GI Series	710
Dental Bite-Wing	400
Skull	330
Chest	44

Source: Bureau of Radiological Health

E) Summary

The table below summarizes the annual dose rates received from natural background, medical and other sources of radiation. The values indicated below are averages and may vary slightly with other reported values:

Annual Dose Rates to Population in USA	BEIR III (1980)
Natural Background	
Cosmic	28 mRem/y
Terrestrial	26
Internal- C-14, Ra-226, Pm-222, K-40	28
	82
Medical	
Diagnosis	77
Dental	1.4
Radiopharmaceuticals	13.6
	92
Other	
Weapon Tests (fallout)	5
Power Plant and Nuclear Industry	< 1
Building Materials (brick, masonary)	5
TV Receivers	0.5
Airline Travel	0.5
	12
TOTAL	186 mRem/y

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Problem Set 3

Multiple choice questions may have more than one correct response.

1. The primary indirect effect of ionizing radiation upon biological target is:

   a) erythema response

   b) free radical formation

   c) leukegenic response

   d) target absorption of the radiation

2. The $LD_{50/30}$ for humans is approximately

   a) 100 mRem

   b) 1 Rem

   c) 25 Rem

   d) 450 Rem

   e) 850 Rem

3. The primary cause of death following an $LD_{50/30}$ in humans is directly associated with irrepairable and irreversible damage to:

   a) the nervous system

   b) the heart, liver, and kidneys

   c) the hematopoietic organs (blood tissue producing)

   d) the skeletal bone

4. Which of the following cells are correctly grouped from radiosensitive to radioresistant?

   a) lymphocytes (white blood cells), endothelial cells (cells lining the GI tract), nerve cells

   b) nerve cells, lymphocytes, endothelial cells

   c) endothelial cells, lymphocytes, nerve cells

   d) endothelial cells, nerve cells, lymphocytes

5. Late effects (5 - 20 years) of a large exposure to ionizing radiation may result in:

   a) deaths as predicted by the LD_{50} concept

   b) carcinogenesis

   c) a change in skin pigmentation

   d) significant blood changes

6. Immediate effects (within 30 days) of a large exposure to ionizing radiation may result in:

   a) bacterial infections

   b) deaths

   c) development of tumors

   d) erythema

7. Radiation damage to the body depends on:

   a) the type and energy of the radiation

   b) the absorbed dose

   c) the time the radiation was distributed

   d) the area of the body affected

8. An acute dose of 1 Rem to the whole body may result in:

   a) significant blood changes

   b) nausea, vomiting

   c) sterility

   d) no observable effects

9. The "Law of Bergionie and Tribondeau" explains the radiosensitivity of tissues as:

   a) directly proportional to the growth rate and inversely proportional to the degree of specialization

   b) directly proportional to the degree of specialization and inversely proportional to the growth rate

   c) directly proportional to the growth rate and directly proportional to the degree of specialization

10. Name five factors that determine a given tissue's radiosensitivity:

continued....

Problem Set 3 continued.....

11. What are the allowed Federal Exposure Limits for radiation workers? Fill in the table.

Rems per Calendar Quarter

	Quarterly Average	Quarterly Maximum
Whole Body		
Skin		
Extremities		

12. _____ is the formula used to compute the maximum allowable accumulated lifetime exposure to ionizing radiation to the whole body for radiation workers.

13. Under what conditions can the maximum quarterly whole body exposure limits be applied?

14. The yearly Federal whole body exposure limits for individual non-radiation workers is _____ Rem.

15. The yearly whole body exposure limit for the U.S. population is _____Rem per person.

16. Occupational radiation workers must be at least _____years of age.

17. A 24 year old person becomes a radiation worker (with no previous radiation exposure history). What would be the maximum allowable whole body dose this person could receive in the next five years?

18. What are three major sources of natural background radiation?

continued....

19. Why may the levels of natural radiation exposure be greater inside of some buildings than in open spaces?

20. What naturally occurring isotopes contribute to external radiation exposure? Internal radiation exposure?

21. If the natural specific activity of C-14 is 6.1 pCi/gm of Carbon, how much naturally produced Carbon-14 will be recycled to the environment during incineration of MSU's 3100 ton of paper waste in 1980?
Cellulose = $(C_6H_{10}O_5)_x$; MW = 162.14

CHAPTER IV RADIATION PROTECTION TECHNIQUES

1. External Radiation Protection

The three basic methods used to reduce the external radiation hazard are time, distance, and shielding. Good radiation protection practices requires optimization of these fundamental techniques.

A) Time

The amount of radiation an individual accumulates will depend on how long the individual stays in the radiation field:

Dose = Dose Rate x Time

mRem = mRem/Hr x Hr

Therefore, to limit a persons dose, one can restrict the time spent in the area. How long a person can stay in an area without exceeding a prescribed limit is called the "stay time" and is calculated from the simple relationship:

$$\text{Stay Time} = \frac{\text{Limit (mRem)}}{\text{Dose Rate (mRem/Hr)}}$$

Example: How long can a radiation worker stay in a 1.5 Rem/Hr radiation field if we wish to limit his dose to 100 mRem?

$$\text{Stay Time} = \frac{100 \text{ mRem}}{1500 \text{ mRem/Hr}} = 0.0667 \text{ Hr} = 4 \text{ minutes}$$

B) Distance

The amount of radiation an individual receives will also depend on how close the person is to the source.

i) The Inverse Square Law - Point sources of X and gamma radiation follow the inverse square law, which states that the intensity of the radiation decreases in proportion to the inverse of the distance squared:

$$I \propto \frac{1}{d^2}$$

To represent this in a more useful formula:

$$I \propto \frac{1}{d^2} \quad \text{or} \quad I = K = \left(\frac{1}{d^2}\right)$$

$$I_1 = K\left(\frac{1}{d_1^2}\right) \; ; \; I_2 = K\left(\frac{1}{d_2^2}\right)$$

continued on following page

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Formula continued....

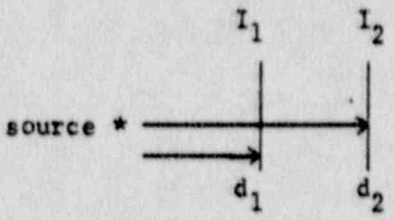
$$\frac{I_1}{I_2} = \frac{\frac{K}{d_1^2}}{\frac{K}{d_2^2}}$$

$$\frac{I_1}{I_2} = \frac{d_2^2}{d_1^2}$$

OR

$$I_1 d_1^2 = I_2 d_2^2$$

Where:



And Where:

I_1 = the radiation intensity at distance d_1 from the radiation source

d_1 = the shorter distance from the source where the radiation intensity is I_1

I_2 = the radiation intensity at distance d_2 from the radiation source

d_2 = the longer distance from the source where the radiation intensity is I_2

Therefore, by knowing the intensity at one distance, one can find the intensity at any given distance.

Example on the following page.

Example: The exposure rate one foot from a source is 500 mR/Hr. What would be the exposure rate three feet from the source?

I_1 = 500 mR/Hr

d_1 = 1 foot

d_2 = 3 feet

$$I_2 = \frac{I_1 d_1^2}{d_2^2} = \frac{(500 \text{ mR/Hr}) (1 \text{Foot})^2}{(3 \text{ Ft})^2} = \frac{500 \text{ mR/Hr}}{9} = 55.6 \text{ mR/Hr}$$

ii) Gamma Constants

Gamma radiation levels (in R/Hr) for one Curie of many radionuclides at a distance of one meter have been measured. These "gamma constants" can be used to determine 1) the expected exposure rate at a given distance (using inverse square) for a known quantity of a radionuclide or 2) the number of Curies of a radionuclide from a measured exposure rate. Gamma constants (Γ) for selected radionuclides appear in Appendix IV.

Example No. 1: What is the radiation exposure rate one foot from a 100 mCi point source of Cs-137? (Γ = 0.33).

Γ = 0.33 R/Hr at 1 meter/Curie or 0.33 mR/Hr at 1 meter/mCi

$I_1 d_1^2 = I_2 d_2^2$

I_1 = ?

I_2 = 0.33 mR/Hr/mCi x 100 mCi = 33 mR/Hr

d_2 = 1 meter

d_1 = 1 foot = 0.3048 m

$$I_1 = \frac{I_2 d_2^2}{d_1^2} = \frac{(33 \text{ mR/Hr}) (1 \text{ m})^2}{(0.3048 \text{ m})^2} = 355 \text{ mR/hr}$$

Example No. 2: If the exposure rate from Cs-137 at one meter is 250 mR/Hr, how many Curies are present? (Γ = 0.33)

$$\frac{0.25 \text{ R/Hr/meter}}{0.33 \text{ R/Hr/meter/Curie}} = 0.76 \text{ Curies}$$

iii) Gamma Exposure Rate Formula

The exposure rate from a gamma point source can be approximated from the following expression:

R/Hr at 1 foot = 6 C E n

Where: C is the number of Curies of the gamma emitter

E is the gamma ray energy in MeV

n is the number of gamma's per disintegration

This expression holds only for gamma emitters with energies ranging from 0.07 MeV to 4 MeV.

Example: What would the exposure rate be one foot away from 100 mCi of I-131?

From the reference data for selected radionuclides (Appendix IV):

I-131 : γ_1 = 0.364 MeV, 81.2% γ/d

γ_2 = 0.636 MeV, 7.3% γ/d

R/Hr at 1 foot = 6(0.1Ci)[(0.364 x 0.812) + (0.636 x 0.073)]

= 0.21

or 210 mR/Hr at one foot

C) Shielding

When reducing the time or increasing the distance may not be possible, one can choose shielding material to reduce the external radiation hazard. The proper material to use depends on the type of radiation and its energy.

i) Alpha and Beta Radiation

As discussed in Chapter I, alpha particles are easily shielded. A thin piece of paper or several cm of air is usually sufficient to stop them. Thus, alpha particles present no external radiation hazard. Beta particles are more penetrating than alpha particles. Beta shields are usually made of aluminum, brass, plastic, or other materials of low atomic number to reduce the production of bremsstrahlung radiation. Appendix IV gives the range of beta radiation for selected radionuclides in air and plastic. (see Appendix II).

ii) X and Gamma Radiation

Monoenergetic X or gamma rays collimated into a narrow beam are attenuated exponentially through a shield according to the following equation

$$I = I_o e^{-\mu x}$$

continued on following page .....

Where: I is the intensity outside of a shield of thickness x

I_o is the unshielded intensity

μ is the linear attenuation coefficient

x is the thickness of shielding material

The linear attenuation coefficient is the sum of the probabilities of interaction per unit path length by each of the three scattering and absorption processes - photoelectric effect, compton effect, and pair production. Note that μ has dimensions of inverse length. The reciprocal of μ is defined as the mean free path which is the average distance the photon travels in an absorber before an interaction takes place.

Because linear attenuation coefficients are proportional to the absorber density, which usually does not have a unique value but depends somewhat on the physical state of the material, it is customary to use "mass attenuation coefficients" which removes density dependence:

Mass attenuation coefficient $\mu_m = \frac{\mu}{\rho}$ ρ = density (gm/cm^3)

For a given photon energy, μ_m does not change with the physical state of a given absorber. For example, it is the same for water whether present in liquid or vapor form. If the absorber thickness is in cm, then μ_m will have units of cm^2/gm $\left[\frac{cm^{-1}}{gm/cm^3}\right]$.

Values of the mass attenuation coefficient for lead are given in Appendix IV.

Example: The intensity of an unshielded Cs-137 source is 1R/Hr. If the source is put into a lead shield two (2) inches thick, what would be the intensity on the outside of the shield? Density of lead = $11.35 gm/cm^3$.

$I = I_o e^{-\mu x}$

$I_o = 1$ R/Hr

$\mu = \mu_m \cdot \rho = (0.114\ cm^2/gm)\ (11.35 gm/cm^3) = 1.29\ cm^{-1}$

$x = 2$ inches $\cdot$ 2.54 cm/inch $= 5.08$ cm

$I = (1\ R/Hr) \cdot e^{-[(1.29\ cm^{-1})\ (5.08\ cm)]} = 0.0014$ R/Hr

$= 1.4$ mR/Hr

iii) Half Value Layer

The half value layer (HVL) is the thickness of the shielding material required to reduce the intensity to one half of its original intensity and can be calculated from:

$$\frac{I}{I_o} = 0.5 = e^{-\mu x_{\frac{1}{2}}}$$

$$x_{\frac{1}{2}} = \frac{0.693}{\mu} = \text{HVL}$$

Half value layers (for lead) are given for selected radioisotopes in Appendix IV.

Example: How much lead shielding must be used to reduce the exposure rate from an I-131 source from 32 mR/Hr to 2 mR/Hr? HVL of lead for I-131 is 0.178 cm.

$$\frac{32 \text{ mR/Hr}}{2 \text{ mR/Hr}} = 16 = 4 \text{ HVL} \quad (2 \times 2 \times 2 \times 2 = 16)$$

$$4 \times 0.178 \text{ cm} = 0.71 \text{ cm} .$$

D) Personnel Monitoring

External radiation exposure is measured by personnel monitoring devices. Three major types of monitoring devices in use today are the pocket dosimeter, the film badge, and the Thermoluminescent Dosimeter (TLD). Personnel Monitoring is required when it is likely that an individual will be exposed to in any calendar quarter a dose of 300 mRems to the whole body (head and trunk, active blood forming organs, gonads, lens of eye); 5 Rems to the extremities (hands, forearms, feet, ankles); 2 Rems to the skin of the whole body or in any area where you can receive 100 mRems in any hour. Personnel monitoring provides a permanent, legal record of an individual's occupational exposure to radiation.

i) Pocket Dosimeters

Pocket Dosimeters are small devices (about the size of a fountain pen) one can carry in a shirt or lab coat pocket to record exposure to radiation. They come in two types, direct reading and indirect reading. Each type of dosimeter is "zeroed" prior to use by a battery or AC line operated charger. When radiation passes through the sensitive volume of the dosimeter, the charge is dissipated in proportion to the amount of radiation received. In the direct reading type, the amount of exposure in mR is read off a calibrated scale by looking through the dosimeter much like looking through a telescope. The indirect reading type requires a separate readout device that also serves as the dosimeter charger.

The advantages of pocket dosimeters are that they can provide an on-the-spot result of an individual's exposure to radiation. Disadvantages are that they are susceptible to erroneous readings from excessive moisture, dust, or physical abuse (dropping, etc.) In each case, the readings will be high due to these events. For this reason, two or more dosimeters are usually worn at the same time. The lower reading dosimeter is considered to be the most accurate result. Also, if an individual is exposed to radiation in excess of the range of the dosimeter or to beta radiation, no information can be derived.

ii) Film Badges

The film badge consists of a film packet and holder. Radiation causes the film to turn black - the degree of film blackening is then related to the amount of radiation exposure. The film packet usually contains two pieces of film, ranging in sensitivity from 15 KeV to 3 MeV for X or gamma radiation, and 200 KeV to 1 MeV for beta radiation. Radisotopes with energies below these values cannot be detected. This is why users of nuclides such as H-3, C-14, S-35 are not issued film badges.

Filters in the badge holder allow different radiation types (β, γ, X, n) and energies to interact with the film: An "open" window allows all radiations to pass and expose the film. A plastic filter absorbs most low energy beta radiation. An alumina filter absorbs most high energy beta radiation. A tin/cadmium filter absorbs all but high energy gamma rays. Fast neutrons interact with the cadmium to produce film blackening. Slow neutrons interact with the nitrogen atoms in the film's gelatin layer, and the resulting proton tracks are counted.

Advantages of film badges are: 1) they are relatively inexpensive compared to other dosimeter types; 2) they provide a permanent record of an individual's dose (films are kept on file); 3) films are processed and results reported by a disinterested third party. Disadvantages are: 1) films are susceptible to extremes of heat, pressure and moisture; 2) film processing and receipt of exposure results may take several weeks. To eliminate this latter disadvantage, pocket dosimeters can be worn along with film badges. If the pocket dosimeter indicates a possible high exposure, the film badge can be evaluated on an emergency basis, usually within twenty-four hours after receipt by the Vendor.

iii) Thermoluminescent Dosimeters (TLD's)

TLD's are small chips (1/8" x 1/8" x 1/32") of lithium flouride or calcium fluoride. The chips absorb energy from radiation which excites atoms to higher energy levels within the crystal lattice. Heating the chip releases the excitation energy as light, proportional to the amount of radiation received. Chips are placed in badge holders containing filters to distinguish between radiation energy and type.

Advantages of TLD's are: 1) they are small and can be used as extremity monitors; 2) they can be read on-site or through a disinterested third party; and 3) they are reuseable. Disadvantages are: 1) once the chips are analyzed, the exposure information is lost - exposures cannot be re-verified at a later date; 2) chips are relatively expensive; and 3) chips are subject to physical damage (cracking, breaking, etc.).

iv) Proper Use of Personnel Dosimeters

a) Personnel dosimeters must be worn only by the person to whom it was issued. Any exposure information will then become a part of that person's exposure history record.

b) Dosimeters should be worn on the body, where exposure to radiation is likely. Usually, they are worn between the neck and waist, care being taken to prevent items like pens, buttons, lab benches, hood aprons, etc. from shielding the badge holder.

c) Store dosimeters along with the "control"dosimeter in a designated area, away from extremes in temperature and radiation. The purpose of the control is to record any non-occupational exposure while the badge is not being worn (i.e., during transit to and from the vendor).

d) Do not wear personnel dosimeters during non-occupational exposures of radiation, i.e., chest or dental X-Rays.

E) Posting and Labeling of Radioactive Materials

i) Cautionary Signs

Cautionary signs are required to be posted under certain conditions as described below to warn other individuals in the area that radioactive materials or radiation is present:

Caution - Radiation Area: In areas where the level of radiation could cause a major portion of an individual's body to receive an exposure from external radiation in any hour that exceeds 5 millirems; or in a period of five (5) consecutive days that exceeds 100 millirems.

Caution - High Radiation Area: In areas where the level of radiation could cause a major portion of an individual's body to receive an exposure from external radiation in any hour that exceeds 100 mRem.

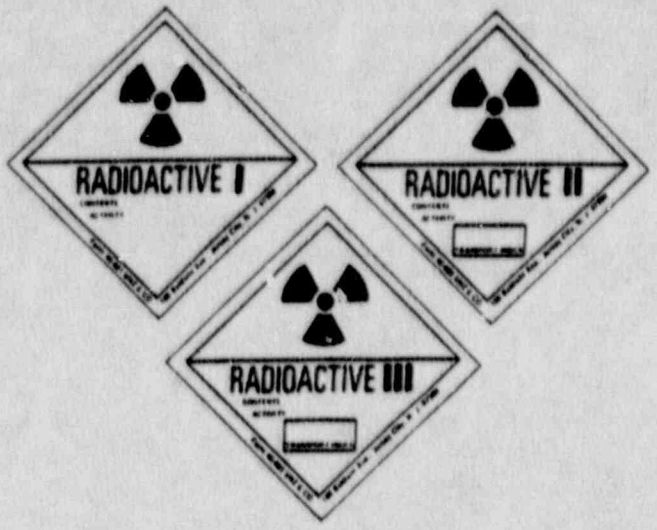
Posting must be at the point at which an area measures 5 mR/Hr and/or 100 mR per 40 Hr/week for a Radiation Area, and at the point at which an area measures 100 mR/Hr for a High Radiation Area.

In addition, individuals posting such signs should provide information on the sign to aid others to minimize their exposure. Information may include: 1) the source of radiation; 2) the exposure rate in mR/Hr or R/Hr on contact, or at the highest spot; 3) the name of the person posting the sign; and 4) the date the sign was posted.

Caution - Radioactive Materials: In areas or on items where radioactive material is used or stored. Each label shall provide sufficient information to permit individuals handling or using containers or working in the general vicinity to take precautions to avoid or minimize exposures. Such information should include: 1) the type of radioactive material; 2) the estimated activity; 3) assay date; 4) the name of the individual responsible for the material.

ii) Department of Transportation (DOT) Warning Labels

Each package of radioactive material offered for transportation, unless exempted, must be labeled on two sides with one of the three labels shown below:



DOT Warning Labels for Radioactive Materials Packages

The purpose of these labels is to alert individuals handling packages that special handling may be required. When the background color of the label is all white (Radioactive White-I), the external radiation level from the package is minimal and no special handling is necessary. If however, the background of the upper half of the label is yellow (Radioactive Yellow II or III), a radiation level may exist at the outside of the package, and precautions should be taken to minimize radiation exposures when handling the package. The radiation level in mR/Hr at three feet from the external surface of the package is known as the transport index, and is written in the space provided on the warning label. Furthermore, if the package bears a Radioactive Yellow III, the rail or highway vehicle in which it is carried must be placarded. The table below defines the label criteria for radioactive materials packages:

Label	Dose Rate Limits	
	At Any Point On Accessible Surface of Package	At Three Feet From External Surface of Package (Transport Index)
"RADIOACTIVE-WHITE I"	0.5 mR/Hr	0
"RADIOACTIVE-YELLOW II"	50 mR/Hr	1.0 mR/Hr
"RADIOACTIVE -YELLOW III" (requires vehicle placarding)	200 mR/Hr	10 mR/Hr

The cautionary signs and warning labels described in these sections must be removed or defaced when they are no longer serving their function.

2. Internal Radiation Protection

Internal radiation exposure results when the body is contaminated internally with a radioisotope. When radioactive materials enter the body, they are metabolized and distributed to the tissues according to the chemical properties of the elements and compounds in which they are contained. For example, consider a complex molecule which can be equally satisfied with a C-12 (stable) atom or a C-14 (radioactive) atom at its regular carbon position. If the C-14 decays to nitrogen; the molecular structure is affected. If the molecule were DNA, this might be equivalent to gene mutation. Once radioactive material is in the body, little can be done to speed its removal. Thus, internal radiation protection is concerned with preventing or minimizing the deposition of radioactive substances in personnel.

A) Methods of Entry

Radioactive substances, like other toxic agents, may gain entry into the body by four processes:

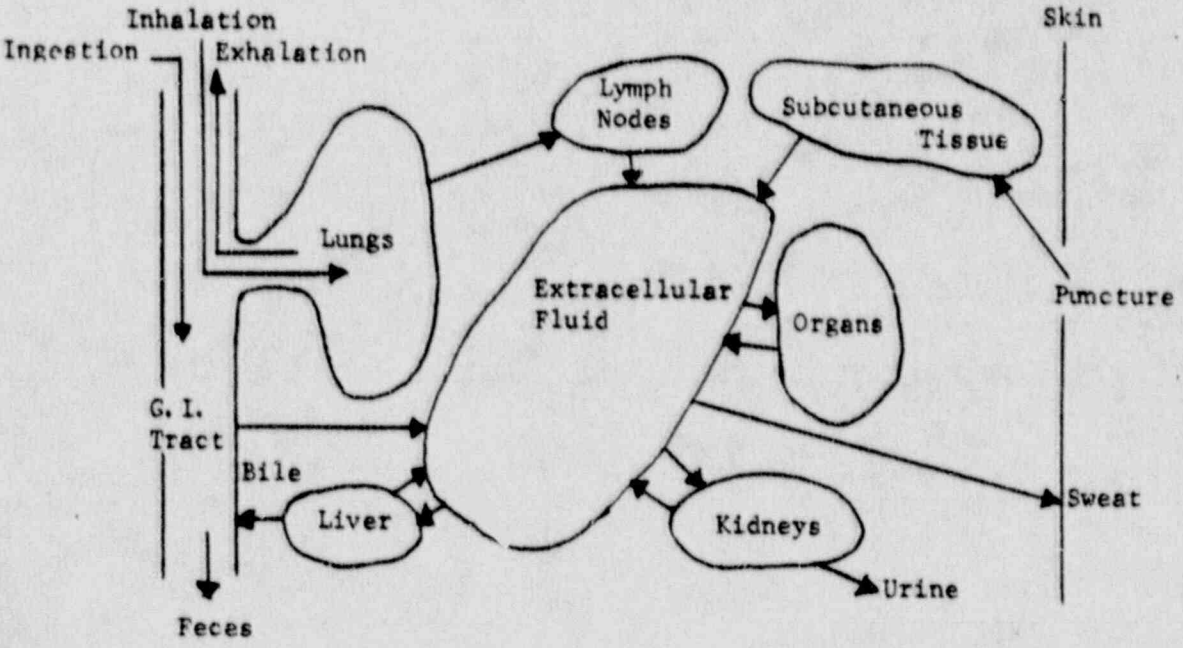
i) Inhalation - breathing radioactive aerosols or dust

ii) Ingestion - drinking contaminated water, or transferring radioactivity to the mouth.

iii) Absorption - through intact skin.

iv) Injection - puncture of skin with an object bearing radioactive materials.

The following diagram is a summary of radionuclide entry, transfer, and exit within the body:



B) Guidelines

Three basic methods to control radioactive contamination which can lead to internal radiation exposures are:

1) Minimize the amount of radioactive material being handled. Use only as much activity that is needed.

2) Contain the radioactive material. Different physical states (gas, liquid, solid) require different containment techniques. Generally, two levels of containment should be provided. For example, a vial containing a stock solution of radioactive material should be properly capped and placed or transported using a drip tray or other similar devices lined with absorbent paper.

3) Follow established laboratory procedures. Proper protective clothing, designated work areas, surface contamination monitoring, etc. are required in all laboratories that use radioactive material (see Chapter 5, "Radioisotope Laboratory Techniques").

C) Limits

Limits pertaining to internal emitters are set up for particular radionuclides. These limits are called body burdens. A permissible constant body burden of a radionuclide is a quantity (in uCi) which when present continuously in the body will deliver a dose rate not exceeding the maximum permissible dose rate. Thus, if the whole body is the critical organ, the constant body burden must not deliver more than 5 Rem per year. The concentrations of radionuclides in air and water required to yield a body burden are called Maximum Permissible Concentrations (MPC's). An MPC is the concentration of a radionuclide which when taken into the body on an occupational exposure basis results in an organ burden which produces a maximum permissible dose rate to the organ of interest. Some of the factors which are considered in calculating MPC's are:

1) the type and energy of the radiation emitted;
2) its distribution in the body;
3) the solubility of the compound containing the isotope; and
4) the effective half-life of the isotope.

The effective half-life (T_{eff}) is defined as the rate of removal of a radioactive substance from the body and is calculated from the following equation:

$$T_{eff} = \frac{T_R \times T_B}{T_R + T_B}$$

where: T_R = the radiological half-life

T_B = the biological half-life (how long it takes for 1/2 of a material to be eliminated from the body)

Values of T_{eff} for selected radioisotopes are listed in Appendix IV.

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MPC's are commonly calculated for occupational exposure situations, assuming a 40 hour per week exposure, 50 weeks per year. The longest exposure duration is taken as 50 years.

Value for MPC's can be found in the Code of Federal Regulations, Title 10, Part 20

D) Monitoring

Monitoring for internal contamination is accomplished by whole body or partial body counting in the case of gamma emitters and by urine or feces samples in the case of beta emitters. Such monitoring techniques are called "Bioassays".

Bioassays are required when surveys or calculations indicate that an individual has been exposed to radioactive materials in excess of established limits (i.e., MPC's, removable contamination limits) or when required by State or Federal regulations.

Problem Set 4

Multiple choice questions may have more than one correct response. Refer to Appendix IV for reference data.

1. Film badge results are reported in units of:

   a) Rads
   b) mR/Hr
   c) Rems
   d) mCi

2. Film badges cannot detect H-3, C-14, or S-35 because:

   a) They are pure beta minus emitters
   b) They have beta energies below the sensitivity of the film
   c) They have beta energies above the sensitivity of the film
   d) The specific ionization of the beta particles is too low

3. The purpose of filters in a film badge holder is to:

   a) Help in identifying the type and energy of radiation
   b) Determine the amount of radiation exposure
   c) Shield the film from radiation exposure
   d) Determine the identity of radioisotopes the badge was exposed to

4. Film badges and other personnel dosimeters should be worn:

   a) Generally, between the neck and waist
   b) On the area of the body where exposure to radiation is likely
   c) On only the person to whom it was issued
   d) For extremity monitors, on the inside of protective gloves.

5. A radioactive package displaying a DOT "Radioactive Yellow II" warning label with a Transport Index of 0.2 means that:

   a) The transport vehicle requires placarding
   b) The radiation level at the surface of the package is 0.2 mR/hr
   c) The radiation level at 3 feet from the package is 0.2 mR/hr

Problem Set 4 continued.....

6. If you have a source of radiation which emits high energy beta particles only, what is the most appropriate shielding material to use?

   a) a container of lead

   b) a container of plastic

   c) a container of plastic inside a container of lead

   d) a container of lead inside a container of plastic

7. If you have a source of radiation which emits <u>both</u> high energy beta particles and gamma rays, what is the most appropriate shielding material to use?

   a) a container of lead

   b) a container of plastic

   c) a container of plastic inside a container of lead

   d) a container of lead inside a container of plastic

8. What is Body Burden? How does it relate to a maximum permissible concentration?

9. Name four (4) factors which are considered in evaluating the potential internal hazards from a radioisotope?

10. If the intensity of a gamma source is 5 mR/Hr at a distance of one meter, what is the intensity one foot away from the source?

Problem Set 4 Continued.....

11. The intensity of a source measured outside a 2 cm thick lead pig is 2 mR/Hr. If the pig is known to contain Cr-51, what would the intensity of the source be without the pig? (density of lead = 11.35 gm/cm^3)

12. A 20 mCi Cs-137 source (calibrated on 9/10/79) is to be used for the calibration of pocket dosimeters. The source is to be placed at the center of a board with the dosimeters distributed around a circle of a radius of 22 inches. What dose rate will the chambers receive? Where, how and at what distance should such a facility be posted?

13. What would be the approximate dose to an operator using this calibration facility, assuming the operator spent a total of 30 minutes near or adjacent to the dosimeters?

14. A radiation worker begins work six (6) feet away from a source determined to be 5 mR/Hr at that point. The worker's daily dose limit is 50 mRem. If this person worked in the first area for five (5) hours and then moved to an area three (3) feet from the source, what would be the new exposure rate in this area and how long could the person remain there until the daily limit has been expended? (Assume 1 mR = 1 mRem).

15. If commercially available lead blocks are 1 inch thick, how many blocks are needed to reduce the unshielded exposure rate from a vial of Co-60 to 1/8 its original value?

CHAPTER V RADIOISOTOPE LABORATORY TECHNIQUES

All laboratories authorized to use radioactive materials require special precautions to minimize the external and internal hazard from radiation and radioactive contamination. This chapter deals with general regulations and techniques that should be followed in the Radioisotpe Lab.

1. Protective Clothing

a) Lab coats should be worn when manipulating radioactive materials to prevent contamination of street clothes.

b) Disposable plastic gloves should always be worn when using radioactive materials. Personnel with breaks in the skin should use waterproof tape to seal such breaks or not use radioactive material.

c) Care should be exercised not to transfer contamination from the hands or lab coat by reflex actions such as wiping one's brow or scratching an itch.

2. The Workplace

a) Areas in which radioactive material are used should be covered with plastic backed absorbent paper to contain spills and prevent contamination of the working surface.

b) Drip trays can be used to transfer beakers, test tubes, etc. from one location to another.

c) Change absorbent paper at regular intervals to prevent cross contamination.

d) Label all containers used for radioactive materials work. Keep the work areas neat and clean to prevent accidents as well as making it easier to decontaminate if accidental spills do occur.

e) Secure all radioactive materials from unauthorized removal. Close or lock the lab door when materials must be left unattended. Most refrigerator/freezers can be equipped with locks and make an ideal place for storage.

f) There must be no eating, drinking, smoking, or storage of food in areas in which radioactive materials are used.

3. Manipulations of Radioactive Materials

a) No mouth pipetting of anything in a radioisotope work area. Assume all pipettes and glassware in the work area are contaminated. For standard laboratory pipettes, use a safety pipetting aid. Eppendorf or other precision pipettes can be used for smaller dispensing. Contaminated glass pipettes can be placed in a pipette jar for washing. Disposable pipettes and contaminated Eppendorf tips should be placed in radioactive waste containers.

b) Containers used in vortexing, mixing, shaking, or centrifuging operations should be sealed with parafilm or stoppers.

c) Prepare samples carefully. Heating, drying, distilling, and other operations which could result in volatilization of the material should be performed in a fume hood or glove box.

d) Provide proper shielding to reduce exposure, but not so that you hinder the safe execution of the experiment.

e) Whenever possible, rehearse operations with non-radioactive materials to ensure that the technique will be reasonably free of incidents.

f) Accurate records of radioactive material inventory on hand should be maintained. Record withdrawals from the stock vial on inventory control forms received with the isotope.

4. Emergency Procedures

A) Radioactive Material Spills

i) Contain the spill: If the material is a liquid, place an absorbent material such as paper towels, tissues, cloth, etc. over the spill to prevent its spread. If the material is a powdered solid, attempt to contain spread of the material by covering the area with a protective barrier such as a drip tray, empty beaker, section of kraft paper, etc. If appropriate, close doors and windows; turn off room ventilation fans.

ii) Inform others of the spill: Adjust your response to the seriousness of the spill. Instruct those personnel present in the room at the time of the spill to remain in an evacuation area to prevent contamination spread. Report the spill to the Office of Radiation, Chemical and Biological Safety. Evacuated personnel should not eat, drink, or smoke until they are monitored and found free of contamination.

iii) Decontaminate the area: Plan ahead. Provide adequate protection and supplies for personnel involved in the cleanup. Specific decontamination procedures are outlined in Part 8.

iv) Monitor the area: Using appropriate survey techniques, monitor the area for contamination (see Part 6 "Surveys"). Monitor all personnel and materials before releasing them to clean areas. Radioactive contamination limits are outlined in Part 7.

B) Other Emergencies:

Emergencies such as fire, accidental uptake of radioactive material, radiation injury, etc. require the same basic responses as described above: Containment-Notification-Corrective Action and Monitoring. The ORCBS shall be notified at once (through Campus Police after normal work hours) of such incidents and will act with other authorities to control emergencies of this nature.

5. Radioactive Waste Disposal

a) Waste disposal areas should be managed with close attention to cleanliness. Housekeeping employees should be instructed not to move or empty radioactive waste containers during the course of their duties.

b) Care should be exercised when disposing of waste in different physical or chemical forms:

i) Liquid wastes containing acids should not be mixed with liquids containing bases. Aqueous liquids should not be mixed with organic liquids.

ii) Pipettes and other sharp objects should be bundled together in order to prevent them from puncturing the inner plastic liner of a dry waste container. Needle disposal boxes are recommended for the safe disposal of contaminated needles. Waste in powder form should be sealed in an air tight wrap before placing into a dry waste container.

iii) Biological waste should contain sufficient amounts of a preservative and absorbent to prevent decomposition.

iv) Radioactive biohazard waste must be treated in the same manner as regular biohazard waste (i.e. autoclaving) before being placed into radioactive waste containers.

c) Waste should be segregated according to its radiologic half-life. Short lived waste can be stored until decayed and then be disposed of as regular waste.

d) Non-radioactive waste must not be mixed with radioactive waste as this adds to the cost of disposal. A simple wipe survey or instrument survey of the item can determine if it is radioactive. If only a portion of an item (i.e., lab bench soaker) is contaminated, just that portion should be disposed of into radioactive waste.

6. Surveys

Surveys for radiation and removable radioactive contamination must be performed in all radioisotope laboratories, preferably after each use of radioactive materials. Appropriate radiation survey equipment should be available to users for the type of surveys required for the laboratory. For example, H-3 and C-14 contamination can be detected by wiping the work surface with a piece of filter paper and analyzing the "wipe" in a liquid scintillation counter. A geiger-mueller (GM) counter can be used to detect higher energy beta emitting nuclides (i.e., P-32). Survey equipment must be properly calibrated for energy and type of radionuclide in use at least every six months.

To perform a survey, choose sites in the lab where the radioactive material has been used. Areas or equipment such as benchtops, floors near the waste containers, fraction collectors, water baths, storage areas, hoods, etc. are good places to start. Each site chosen should be labeled on a diagram or floor plan of the lab for later referral when interpreting results.

A) Survey for Fixed Contamination

In laboratories where gamma or high energy beta/gamma radioisotope(s) are in use, an initial survey for fixed or gross contamination with a GM survey instrument should be performed. In order to prevent contamination of the GM probe during the survey, take care not to touch the probe to the surface of that which is being examined. As each site is surveyed, record the results on the lab diagram for future reference and to identify any long range trends. Note that results from a GM survey should be expressed in terms of counts per minute (cpm) and yield an estimate of how much radioactivity may be present. Radiation exposure rates in units of mR/Hr can be determined using a GM counter only as long as the detector is calibrated for the energy of the nuclide being measured or by using an Ion Chamber type survey instrument (see Chapter II).

As a general rule, sites in which the results are twice the "background" rate (the level of radiation that the instrument responds to from natural sources) are considered to be radioactively contaminated. Limits for fixed contamination are given in part seven of this chapter. Any fixed contamination found should be tested for removable activity as described in the next section.

B) Survey for Removable Contamination

Contamination not fixed to a surface can be transferred to hands, clothing, notebooks, etc. leading to internal exposure or contamination of clean areas. In laboratories where low energy beta emitting isotopes such as H-3, C-14, and S-35 are in use, a "wipe test" is the most practical method of detecting removable contamination. To perform the survey, the site is wiped with a cotton swab or small piece of coarse filter paper. Wetting the wipe first usually enhances the pick up of any contaminated particles. A single wipe can be used to determine contamination over a large surface area such as a floor or bench top. If activity is found, a series of wipes covering smaller areas should be performed to localize the contamination. Wipes taken can be placed in a numbered scintillation vial or other carrier to organize and prevent cross contamination of the samples. Wipes should also be treated as potentially contaminated until analyzed.

Prepare the wipes for analysis as you would a regular sample. In most labs, a liquid scintillation counter (or gamma counter) is already optimized for the particular nuclides in use. Verify counter settings before analyzing any samples. A control vial consisting of a "clean" wipe and counting solution must be counted with your samples to determine the machine background. A set of quenched standards should be used to determine the counters efficiency for various degree's of quench (see Chapter II - Liquid Scintillation Counting Techniques).

Report results on your diagram in terms of disintegrations per minute (dpm) per area surveyed according to the following formula:

$$\text{Sample dpm} = \frac{\text{Sample Gross cpm - Background cpm}}{\text{Counter Efficiency (counts/disintegration)}}$$

Any wipe indicating a gross cpm greater than twice the background cpm should be cleaned up. Limits for removable contamination are given on the following page.

7. Radioactive Contamination Limits

The limits in the table below set the maximum allowable quantity of radioactive contamination on surfaces and areas. Following good radioisotope laboratory techniques should keep contamination levels to less than 10% of these values. Contamination greater than the maximum values should be reported to the lab supervisor and cleaned up right away. Corrective steps should be taken to prevent reoccurances.

RADIOACTIVE SURFACE CONTAMINATION LIMITS

APPLICATION	ALPHA	BETA/GAMMA	
	Removable (dpm/100 cm^2)	Total(Fixed) at 1 cm from surface (mR/Hr)	Removable (dpm/100 cm^2)
Controlled Area			
Maximum hot spot on a surface	500	1.0	5,000
Clean Area	100	0.5	1,000
Non-Controlled Area			
Skin, personal clothing	no detectable activity allowed	0.1	no detectable activity allowed
Release of material or facilities	100	0.2	1,000

Adapted from 10NYCRR16

8. Decontamination Procedures

The following Personnel, Area, and Material Decontaminating Procedures were taken from the 1970 edition of the Radiological Health Handbook.

PERSONNEL DECONTAMINATION

Method*	Surface	Action	Technique	Advantages	Disadvantages
Soap and water	Skin and hands	Emulsifies and dissolves contaminate.	Wash 2-3 minutes and monitor. Do not wash more than 3-4 times.	Readily available and effective for most radioactive contamination.	Continued washing will defat the skin. Indiscriminate washing of other than affected parts may spread contamination.
Soap and water	Hair	Same as above.	Wash several times. If contamination is not lowered to acceptable levels, shave the head and apply skin decontamination methods.		
Lava soap, soft brush, and water	Skin and hands	Emulsifies, dissolves, and erodes.	Use light pressure with heavy lather. Wash for 2 minutes, 3 times. Rinse and monitor. Use care not to scratch or erode the skin. Apply lanolin or hand cream to prevent chapping.	Same as above.	Continued washing will abrade the skin.
Tide or other detergent (plain)	Same as above.	Same as above.	Make into a paste. Use with additional water with a mild scrubbing action. Use care not to erode the skin.	Slightly more effective than washing with soap.	Will defat and abrade skin and must be used with care.

*Begin with the first listed method and then proceed step by step to the more severe methods, as necessary.

PERSONNEL DECONTAMINATION--Continued

Method*	Surface	Action	Technique	Advantages	Disadvantages
Mixture of 50% Tide and 50% cornmeal	Skin and hands	Emulsifies, dissolves, and erodes.	Make into a paste. Use with additional water with a mild scrubbing action. Use care not to erode the skin.	Slightly more effective than washing with soap.	Will defat and abrade skin and must be used with care.
5% water solution of a mixture of 30% Tide, 65% Calgon, 5% Carbose (carboxymethyl cellulose)	Same as above.	Same as above.	Use with water. Rub for a minute and rinse.	Same as above.	Same as above.
A preparation of 8% Carbose, 3% Tide, 1% Versene, and 88% water homogenized into a cream.	Same as above.	Same as above.	Use with additional water. Rub for 1 minute and wipe off. Follow with lanolin or hand cream.	Same as above.	Same as above.
Titanium dioxide paste. Prepare paste by mixing precipitated titanium dioxide (a very thick slurry, never permitted to dry) with a small amount of lanolin. If not successful, go on to next step.	Skin, hands, and extremities. Do not use near face or other body openings.	Same as above.	Work the paste into the affected area for 2 minutes. Rinse and wash with soap and warm water. Monitor.	Removes contamination lodged under scaly surface of skin. Good for heavy surface contamination of skin.	If left on too long will remove skin.

*Begin with the first listed method and then proceed step by step to the more severe methods, as necessary.

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PERSONNEL DECONTAMINATION--Continued

Method*	Surface	Action	Technique	Advantages	Disadvantages
Mix equal volumes of a saturated solution of potassium permanganate and 0.2 N sulfuric acid. (Saturated solution of $KMnO_4$ is 6.4 grams per 100 ml of H_2O.) Continue with next step.	Skin, hands, and extremities. Do not use near face or other body openings.	Dissolves contaminant absorbed in the epidermis.	Pour over wet hands, rubbing the surface and using hand brush for not more than 2 minutes. Rinse with water.	Superior for skin contamination. May be used in conjunction with titanium oxide.	Will remove a layer of skin if in contact with the skin for more than 2 minutes.
Apply a freshly prepared 5% solution of sodium acid sulfite. (Solution made by dissolving 5 gm of $NaHSO_3$ crystals in 100 ml distilled water.)	Same as above.	Removes the permanganate stain.	Apply in same manner as above. Apply for not more than 2 minutes. The above procedure may be repeated. Apply lanolin or hand cream when completed.		Same as above.
Flushing	Eyes, ears, nose, and mouth	Physical removal by flushing.	Roll back the eyelid as far as possible, flush with large amounts of water. If isotonic irrigants are available, obtain them without delay. Apply to eye continually and then flush with large amounts of water.	If used immediately will remove contamination. May also be used for ears, nose, and throat.	When using for nose and mouth, contaminated individual should be warned not to swallow the rinses.

*Begin with the first listed method and then proceed step by step to the more severe methods, as necessary.

Method*	Surface	Action	Technique	Advantages	Disadvantages
Flushing (Cont'd)			(Isotonic irrigant [0.9% NaCl solution]: 9 grams NaCl in beaker, fill to 1000 cc with water.) Can be purchased from drug suppliers, etc. Further decontamination should be done under medical supervision.		
Flushing	Wounds	Physical removal by flushing.	Wash would with large amounts of water and spread edges to stimulate bleeding, if not profuse. If profuse, stop bleeding first, clean edges of wound, bandage, and if any contamination remains, it may be removed by normal cleaning methods, as above.	Quick and efficient if wound not severe.	May spread contamination to other areas of body if not done carefully.
Sweating	Skin of hands and feet	Physical removal by sweating.	Place hand or foot in plastic glove or booty. Tape shut. Place near source of heat for 10-15 minutes or	Cleansing action is from inside out. Hand does not dry out.	If glove or booty is not removed shortly after profuse sweating starts and part washed with soap

*Begin with the first listed method and then proceed step by step to the more severe methods, as necessary.

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PERSONNEL DECONTAMINATION--Continued

Method*	Surface	Action	Technique	Advantages	Disadvantages
Sweating (Cont'd)			until hand or foot is sweating profusely. Remove glove and then wash using standard techniques. Or gloves can be worn for several hours using only body heat.		and water immediately, contamination may seep into the pores.

AREA AND MATERIAL DECONTAMINATION

Method*	Surface	Action	Technique	Advantages	Disadvantages
Vacuum cleaning	Dry surfaces	Removes contaminated dust by suction.	Use conventional vacuum technique with efficient filter.	Good on dry, porous surfaces. Avoids water reactions.	All dust must be filtered out of exhaust. Machine is contaminated.
Water	All nonporous surfaces (metal, painted, plastic, etc.).	Dissolves and erodes.	For large surfaces Hose with high-pressure water at an optimum distance of 15 to 20 feet. Spray vertical surfaces at an angle of incidence of 30° to 40°; work from top to bottom to avoid recontamination. Work upwind to avoid spray.	All water equipment may be utilized. Allows operation to be carried out from a distance. Contamination may be reduced by 50%. Water equipment may be used for solutions of other decontaminating agents.	Drainage must be controlled. Not suitable for porous materials. Oiled surfaces cannot be decontaminated. Not applicable on dry contaminated surfaces (use vacuum); not applicable on porour surfaces such as wood, concrete,

*Begin with the first listed method and then proceed step by step to the more severe methods, as necessary.

AREA AND MATERIAL DECONTAMINATION--Continued

Method*	Surface	Action	Technique	Advantages	Disadvantages
Water (Cont'd)			Determine cleaning rate experimentally, if possible; otherwise, use a rate of 4 square feet per minute.		canvas, etc. Spray will be contaminated.
	All surfaces	Dissolves and erodes.	For small surfaces Blot up liquid and handwipe with water and appropriate commercial detergent.	Extremely effective if done immediately after spill and on nonporous surfaces.	Of little value in the decontamination of large areas, longstanding contaminants and porous surfaces.
Steam	Nonporous surfaces (especially painted or oiled surfaces).	Dissolves and erodes.	Work from top to bottom and from upwind. Clean surface at a rate of 4 square feet per minute. The cleaning efficiency of steam will be greatly increased by using detergents.	Contamination may be reduced approximately 90% on painted surfaces.	Steam subject to same limitations as water. Spray hazard makes the wearing of waterproof outfits necessary.
Detergents	Nonporous surfaces (metal, painted, glass, plastic, etc.).	Emulsifies contaminant and increases wetting power of water and cleaning efficiency of steam.	Rub surface 1 minute with a rag moistened with detergent solution then wipe with dry rag; use clean surface of the rag for each application. Use a power rotary brush with	Dissolves industrial film and other materials which hold contamination. Contamination may be reduced by 90%.	May require personal contact with surface. May not be efficient on longstanding contamination.

*Begin with the first listed method and then proceed step by step to the more severe methods, as necessary.

AREA AND MATERIAL DECONTAMINATION--Continued

Method*	Surface	Action	Technique	Advantages	Disadvantages
Detergents (Cont'd)			pressure feed for more efficient cleaning. Apply solution from a distance with a pressure proportioner. Do not allow solution to drip onto other surfaces. Mist application is all that is necessary.		
Complexing agents	Nonporous surfaces (especially unweathered surfaces; i.e., no rust or calcareous growth).	Forms soluble complexes with contaminated material.	Complexing agent solution should contain 3% (by weight) of agent. Spray surface with solution. Keep surface moist 30 minutes by spraying with solution periodically. After 30 minutes, flush material off with water. Complexing agents may be used on vertical and overhead surfaces by adding chemical foam (sodium carbonate or aluminum sulfate).	Holds contamination in solution. Contamination may be reduced by 75% in 4 minutes on unweathered surfaces. Easily stored; carbonates and citrates are nontoxic, noncorrosive.	Requires application for 5 to 30 minutes. Little penetrating power; of small value on weathered surfaces.

*Begin with the first listed method and then proceed step by step to the more severe methods, as necessary.

AREA AND MATERIAL DECONTAMINATION--Continued

Method*	Surface	Action	Technique	Advantages	Disadvantages
Organic solvents	Nonporous surfaces (greasy or waxed surfaces, paint or plastic finishes, etc.).	Dissolves organic materials (oil, paint, etc.).	Immerse entire unit in solvent or apply by wiping procedure (see Detergents).	Quick dissolving action. Recovery of sovlent possible by distillation.	Requires good ventilation and fire precautions. Toxic to personnel. Material bulky.
Inorganic acids	Metal surfaces (especially with porous deposits; i.e., rust or calcareous growth); circulatory pipe systems.	Dissolves porous deposits.	Use dip-bath procedure for movable items. Acid should be kept at a concentration of 1 to 2 normal (9 to 18% hydrochloric, 3 to 6% sulfuric acid). Leave on weathered surfaces for 1 hour. Flush surface with water, scrub with a water-detergent solution, and rinse. Leave in pipe circulatory system 2 to 4 hours; flush with plain water, a water-detergent solution, then again with plain water.	Corrosive action on metal and porous deposits. Corrosive action may be moderated by addition of corrosion inhibitors to solution.	Personal hazard. Wear goggles, rubber boots, gloves, and aprons. Good ventilation required because of toxicity and explosive gases. Acid mixtures should not be heated. Possibilty of excessive corrosion if used without inhibitors. Sulfuric acid not effective on calcareous deposits.
Acid mixtures: hydrochloric, sulfuric, acetic, citric acids, acetates, citrates	Nonporous surfaces (especially with porous deposits); circulatory pipe systems.	Dissolves porous deposits.	Same as for inorganic acids. A typical mixture consist of 0.1 gal. hydrochloric acid, 0.2 lb sodium acetate and 1	Contamination may reduced by 90% in 1 hour (unweathered surfaces). More easily handled than inorganic acid solution.	Weathered surfaces may require prolonged treatment. Same safety precautions as required for inorganic acids.

*Begin with the first listed method and then proceed step by step to the more severe methods, as necessary.

AREA AND MATERIAL DECONTAMINATION--Continued

Method*	Surface	Action	Technique	Advantages	Disadvantages
Acid mixtures (Cont'd)			gal. water		
Caustics: lye (sodium hydroxide) calcium hydroxide potassium hydroxide	Painted surfaces (horizontal).	Softens paint (harsh method).	Allow paint-remover solution to remain on surface until paint is softened to the point where it may be washed off with water. Remove remaining paint with long-handled scrapers. Typical paint remover solution: 10 gal. water, 4 lb lye, 6 lb boiler compound, 0.75 lb cornstarch.	Minimum contact with contaminated surfaces. Easily stored.	Personal hazard (will cause burns). Reaction slow; thus, it is not efficient on vertical or overhead surfaces. Should not be used on aluminum or magnesium.
Trisodium phosphate	Painted surfaces (vertical, overhead).	Softens paint (mild method).	Apply hot 10% solution by rubbing and wiping procedure (see Detergent).	Contamination may be reduced to tolerance in one or two applications.	Destructive effect on paint. Should not be used on aluminum or magnesium.
Abrasion	Nonporous surfaces.	Removes surface.	Use conventional procedures, such as sanding, filing, and chipping; keep surface damp to avoid dust hazard.	Contamination may be reduced to as low a level as desired.	Impracticable for porous surfaces because of penetration by moisture.
Sandblasting	Nonporus surfaces.	Removes surfaces.	Keep sand wet to lessen spread of contamination.	Practical for large surface areas.	Contamination spread over area must be removed.

*Begin with the first listed method and then proceed step by step to the more severe methods, as necessary.

AREA AND MATERIAL DECONTAMINATION--Continued

Method*	Surface	Action	Technique	Advantages	Disadvantages
Sandblasting (Cont'd)			Collect used abrasive or flush away with water.		Contaminated dust is personnel hazard.
Vacuum blasting	Porous and non-porous surfaces.	Removes surface; traps and controls contaminated waste.	Hold tool flush to surface to prevent escape of contamination.	Contaminated waste ready for disposal. Safest abrasion method.	Contamination of equipment.

*Begin with the first listed method and then proceed step by step to the more severe methods, as necessary.

PROBLEM SET 5

You have completed a survey for radiation levels and removable contamination for a radioisotope laboratory that uses H-3 and P-32. From the laboratory floor plan, raw data sheet, and quench curves supplied, answer the following questions:

1. For each of the areas surveyed, what is the level of removable contamination in dpm? (Fill in the bottom section labelled "Removable Contamination Survey" on laboratory floor diagram).

2. Which of the contaminated areas are due to H-3? Which are due to P-32?

3. Which of the gross counts can be ignored due to statistical fluctuations? (Hint: $\sigma = \sqrt{N}$)

4. Which of the areas exceed the allowable removable contamination limits?

5. What areas require posting with a "Caution-Radiation Area" sign? A "Caution-High Radiation Area" sign?

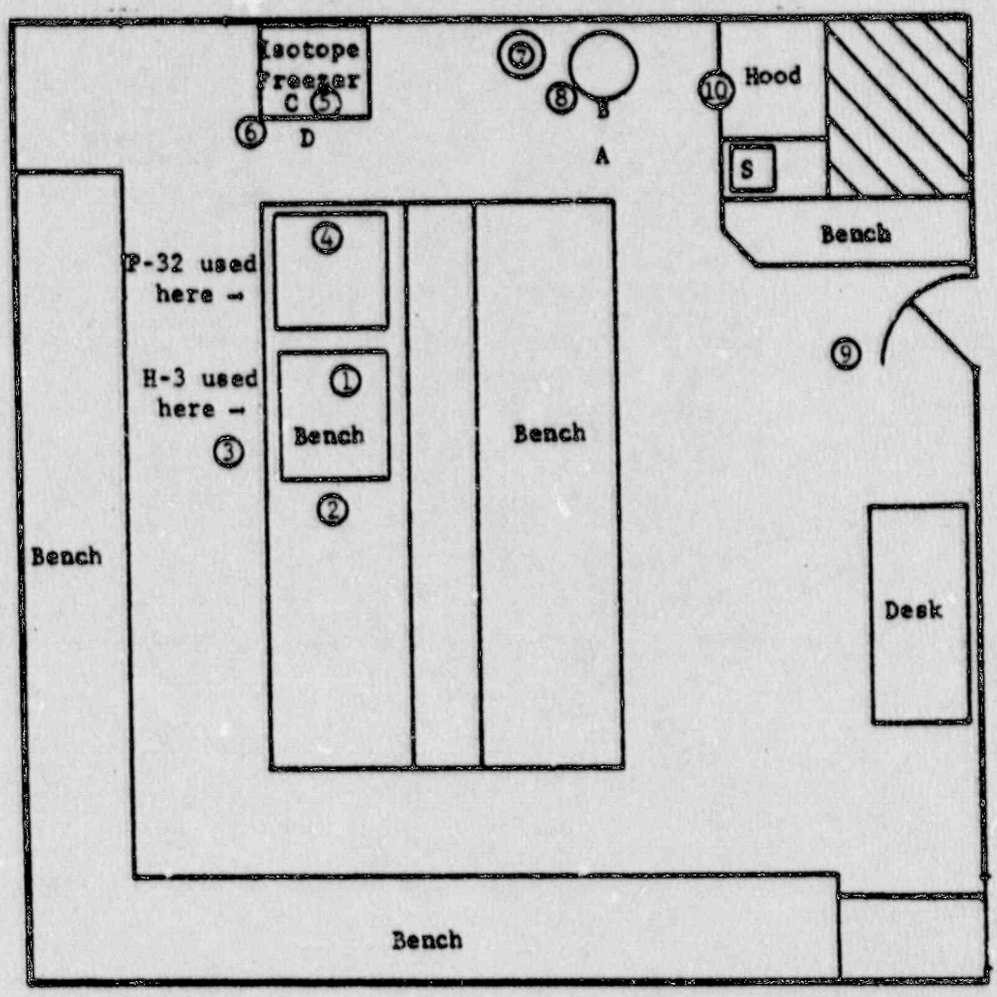
6. What is the most probable cause for contamination at site # 6? What methods of decontamination would you recommend?

7. Which of the users is responsible for the contamination on the floor?

8. What recommendations would you give to the supervisor of this laboratory in order to improve radiation safety?

PROBLEM SET 5

Routine Radiation and Contamination Survey
Room 112 - 114: Teaching Lab



Radiation Survey			Removable Contamination Survey			
	β-γ (mR/Hr)	Remarks		Net cpm	÷ Eff	= dpm
A.	1	1 ft above floor, 1 ft away from waste cont.	1.	____	____	____
B.	5	On contact with side of waste container	2.	____	____	____
C.	125	Unshielded isotope containers in freezer	3.	____	____	____
D.	0.1	On contact with freezer door closed	4.	____	____	____
			5.	____	____	____
			6.	____	____	____
			7.	____	____	____
			8.	____	____	____
			9.	____	____	____
			10.	____	____	____

Instrument(s) used: Victoreen Panoramic with Beta shield off, neglecting air absorbtion.

Background 0.1 mR/Hr.

PROBLEM SET 5

RAW COUNTING DATA SHEET

Room 112-114: Teaching Lab

Counter Used: Packard Tricarb Liquid Scintillation Counter

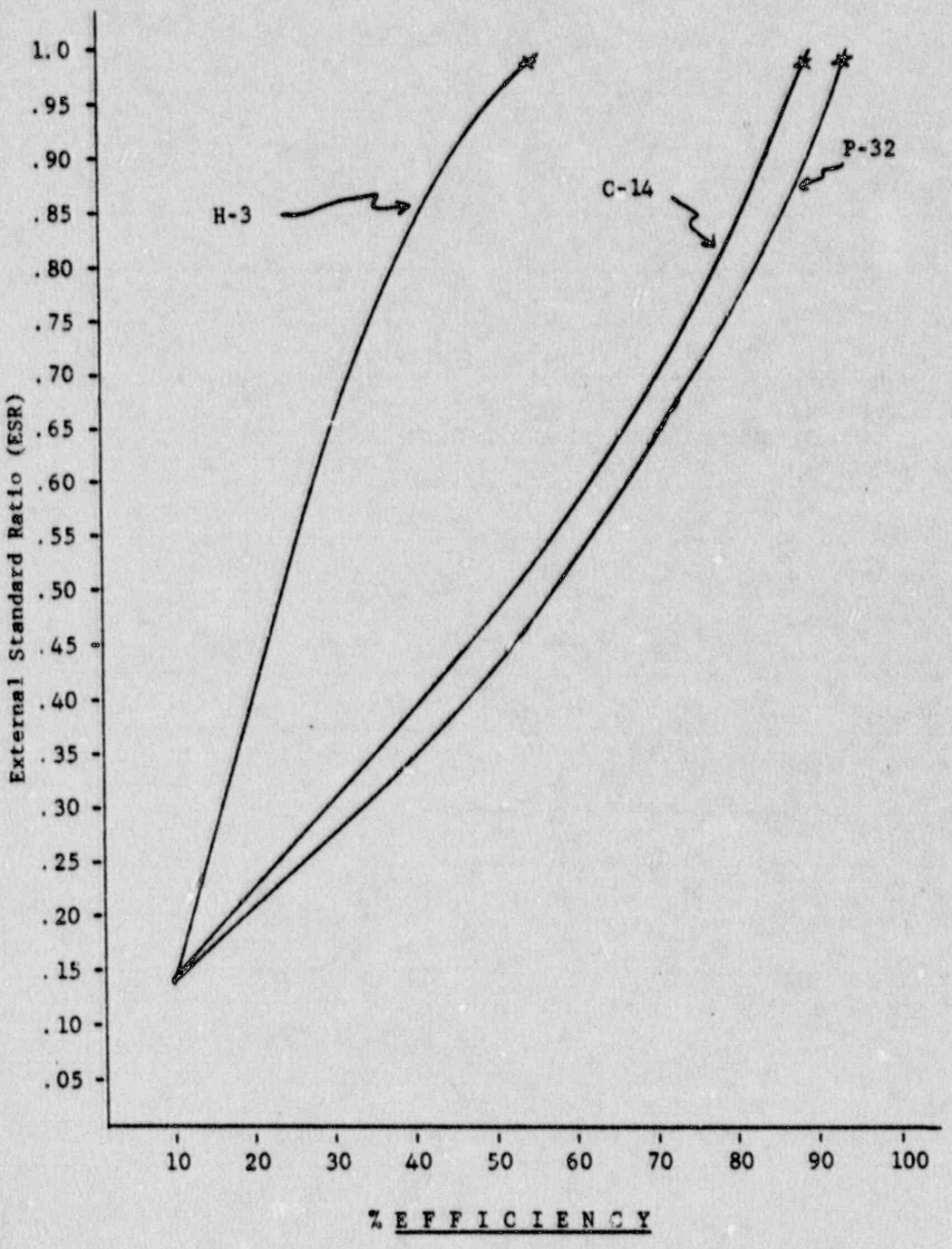
Counting Time: 1 Minute

Sampling Technique: Filter Paper Smear Covering an Area of 100 cm^2

Survey Site	Identification	Red Channel Counts	Green Channel Counts	External Standard Ratio
-	H-3 Standard	154,000	77,000	0.99
-	C-14 Standard	17,000	87,000	0.99
-	P-32 Standard	7,000	70,000	0.99
-	Background	50	25	0.98
1	Absorbent Paper	54	27	0.95
2	Laboratory Notebook	101	65	0.95
3	Floor	71	237	0.80
4	Pipetter Bulb	350	75	0.98
5	Isotope Storage Shelf Inside Freezer	120	60	0.45
6	Freezer Handle	450	4,025	0.80
7	Liquid Waste Container Cover	150	925	0.90
8	Floor	110	525	0.85
9	Floor	70	225	0.80
10	Hood Apron	47	24	0.75

PROBLEM SET 5

Standard Quench for
Packard Tricarb Liquid Scintillation Counter



Note: This graph is to be used only with Problem Set 5. It represents the theoretical quench for a typical liquid scintillation counter. You must generate your own quench curves for the particular counter you are using to determine what efficiencies apply to your data.

CHAPTER VI

Radiation Safety and Use of Radioactive Materials

Approval to Obtain and Use Radioisotopes

The MSU Radioactive Isotopes Committee is in part the subcommittee in radiation safety of the Committee on Radiation, Chemical and Biological Safety. It evaluates the radiological aspects of all proposed investigations. Each project leader shall present, in writing, his request to obtain and use radioactive isotopes to the Committee for its evaluation. The Committee's primary concern is radiation safety and thus considers (a) the nature of the isotopes requested, (b) quantity to be used, and (c) overall experimental procedures. Application forms may be obtained from the Office of Radiation, Chemical and Biological Safety Office (phone 355-0153).

MSU Ordering Procedures

All requestions for radioactive isotopes must be approved by the Office of Radiation, Chemical and Biological Safety. Authorization is based on the prior approval by the Radioactive Isotopes Committee for the project leader to obtain and use radioisotopes. All requisitions should be sent by the Project Leader directly to the Purchasing Department.

Transfer of Radioactive Isotopes

On-campus transfer of material between investigators on different projects shall be reported to the Office of Radiation, Chemical and Biological Safety.

Shipment of any byproduct material off the MSU campus must have the prior consent and approval of the Office of Radiation, Chemical and Biological Safety. Federal and state laws require that the shipper must obtain, through the MSU Office of Radiation, Chemical and Biological Safety the recipient's Nuclear Regulatory Commission or State license number prior to shipment of the material.

"Byproduct material" means any radioactive material yielded in or made radioactive by the exposure incident to the process of producing or utilizing special nuclear (fissionable) material. All radioactive shipments, including cyclotron produced material, must be checked through the Office of Radiation, Chemical and Biological Safety for compliance with Nuclear Regulatory Commission and State regulations covering the receipt and transfer of such materials.

Radioisotope Inventories

A radioisotope inventory will be mailed routinely to each project leader. The corrected forms must be returned promptly to the Office of Radiation, Chemical and Biological Safety to assure continued authorization for acquisition and use of radioactive materials.

Responsibilities of the Radiation Safety Officer (RSO)

The institution's radiation safety program is conducted under the authority of a Radioisotopes Committee and is implemented by the Radiation Safety Officer (RSO) within the Office of Radiation, Chemical and Biological Safety. The duties of the RSO include preparing regulations, developing training programs, advising on matters of radiation protection, maintaining a system of accountability for all radioactive materials from procurement to disposal, inspecting work spaces and handling procedures, determining personnel radiation exposures, monitoring environmental radiation levels, and instituting corrective action in the event of accidents or emergencies.

Responsibilities of Users of Radionuclides

When a user receives authorization to work with radionuclides, she/he becomes directly responsible for 1) compliance with all regulations governing the use of radionuclides in his/her possession, and 2) the safe use of his/her radionuclides by other investigators or technicians who work with the material under his/her supervision. She/he has the obligation to:

(a) Insure that individuals working with radionuclides under his/her control are properly supervised and have obtained training and indoctrination required to enable safe working habits and prevention of exposure to others or contamination of the surroundings. (Inadequate supervision and lack of training have been cited in radiation lawsuits as indicative of negligence).

(b) Avoid any unnecessary exposure, either to himself/herself or to others working under him/her.

(c) Limit the use of radionuclides charged to him/her to individuals over whom he/she has supervision and to specified locations.

(d) Keep current working records of the receipt and disposition of radionuclides in his/her possession

including use in research, waste disposal, transfer, storage, etc.

(e) Notify the appropriate administrative department of any personnel changes and changes in rooms or areas in which radioactive materials may be used or stored.

(f) Keep an adequate inventory of the amount of radioactive material possessed and be prepared to submit this inventory to inspectors upon request.

(g) Insure that functional survey instrumentation is available to enable personnel to monitor for radiation exposure and surface contamination.

(h) Inform the ORCBS when he/she cannot fulfill his/her responsibilities because of absence and designate another qualified individual to supervise the work.

(i) Inform the ORCBS when a woman who is or will be working with a source of radiation under his/her supervision is known to be pregnant. This is required by state law.

The importance of proper record keeping by the individual users as well as by the institution under whose auspices the work is being performed cannot be overemphasized. Records of personnel exposure, radiation surveys, instrument calibration, waste disposal, radiation incidents, and all the other radiation activities discussed in this section represent the main proof of compliance with protection regulations, and are important for legal purposes as well as for effective administration of the radiation protection program.

Training Required for Working with Radionuclides

Training is required in basic radionuclide handling techniques. If the application is for medical uses of the radionuclides, clinical training is required. Information on training and experience criteria for specific procedures should be obtained from the Office of Radiation, Chemical and Biological Safety.

Standards for Radiation Exposure

The basic radiation protection standards formulated by the NRC for radionuclide users are published in the Code of Federal Regulations, Title 10, Part 10. Every user of radionuclides should obtain and study these standards, which cover many topics including permissible doses, permissible levels, permissible concentrations, precautionary procedures,

CONTROL NO. 87285

waste disposal, and records. It should be emphasized that regardless of limits that are set for allowable radiation exposures, the general policy is to avoid all unnecessary exposure to ionizing radiation. Copies of the regulations may be obtained from the Office of Radiation, Chemical and Biological Safety.

Personnel Monitoring

Personnel monitoring devices are required by law, and records must be kept for workers over 18 years of age if they receive or are liable to receive a dose in any calendar quarter in excess of 25 percent of the occupational dose limits.

Laboratory Design and Equipment

Successful work with radioisotopes, other than in tracer amounts, requires the use of laboratories and equipment specially designed for the purpose. Consult ORCBS for assistance.

Warning Signs

Areas in which radioactive materials are stored, or are being used, shall be posted with appropriate radiation warning signs. Signs may be obtained from the Office of Radiation, Chemical and Biological Safety. The symbol prescribed by this section is the conventional 3-bladed design.

Monitoring Instruments

Every laboratory using radioactive materials must possess or have available for immediate use some type of radiation monitoring instrument satisfactory to the Radiation Safety Officer. Each person in charge of a laboratory using radioactive materials shall be responsible for making surveys or having surveys made of all suspected radiation hazards in the area. It is impossible to assign the frequency at which these surveys should be made, but they should be made at least after every use of the laboratory that could result in fresh contamination. The Office of Radiation, Chemical and Biological Safety is to be immediately informed whenever hazardous conditions exist (e.g., if a serious spill occurs or a potentially hazardous condition exists). The Department of Public Safety will also be informed of serious problems.

Surveys by the Office of Radiation, Chemical and Biological Safety

The Radiation Safety Officer and his staff will make independent routine surveys and pass pertinent information on to those responsible for keeping the laboratory in a safe

condition. Records will be kept by the Office of Radiation, Chemical and Biological Safety showing the results of these surveys. Records are maintained for inspection by the U.S. Nuclear Regulatory Commission and the Michigan Department of Public Health.

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APPENDIX I

Solutions to Problem Sets

Solutions to Problem Set 1

1) d 2) c 3) a,c 4) a,b 5) b,c,d 6) c 7) b 8) d

9) c

10) alpha, U-238; Ra-226
beta, C-14, H-3; P-32
positron, Na-22; Zn-65

---

11) nuclear transition (gamma), Co-57; Mn-54;
electron capture, I-125; Cr-51

---

12) a. $\lambda = \frac{0.693}{T_{1/2}\ \text{min}} = \frac{0.693}{30\ \text{min.}} = 0.0231\ \text{min}^{-1}$

b. $A = \lambda N$, therefore $N = \frac{A}{\lambda} = \frac{10,000\ \text{dpm}}{0.0231\ \text{min}^{-1}} = 432,900$ atoms that disintegrate

c. $A = A_o e^{-\lambda t}$, where t = 90 minutes and A_o = 10,000 dpm.

$= (10,000\ \text{dpm})\ e^{-[(0.0231\ \text{min}^{-1})\ \cdot\ (90\ \text{min})]}$

$= (10,000)\ (0.125) = 1,250$ dpm

---

13) $\frac{A}{A_o} = e^{-\lambda t}$; $\frac{3885\ \text{dpm}}{15,000\ \text{dpm}} = 0.259 = e^{-\lambda t}$

$\ln 0.259 = \ln e^{-\lambda t}$; $(\ln e^{-\lambda t} = -\lambda t)$

$\ln 0.259 = -\lambda t$

$1.35 = \frac{0.693}{T_{1/2}}\ (t)$, $T_{1/2} = \frac{0.693}{1.35}\ (24\ \text{Hrs}) = 12.32$ hours

The isotope is potassium-42, determined by the half-life from Appendix IV. Chart of Nuclides, etc.

---

14) $\frac{A}{A_o} = e^{-\lambda t}$; $\frac{6\ \text{dpm}}{9\ \text{dpm}} = 0.6667 = e^{-\lambda t}$

$0.4054 = \frac{0.693}{5730y}\ (t)$, $t = \frac{0.4054}{0.000121y^{-1}} = 3,352$ years old

---

15) $A_o = \frac{A}{e^{-\lambda t}} = \frac{2\ \text{mCi}}{e^{-\left[\frac{0.693}{12.71\ \text{hrs}} \times 72\ \text{hrs}\right]}} = 101.4$ mCi

Solutions To Problem Set 2

1) a, b, d 2) a 3) b 4) a) GM
b) Ion Chamber
5) c 6) d 7) c 8) a, b, c c) Liquid Scintillation
d) Liquid Scintillation
9) $\frac{1000 \text{ cpm}}{0.1} = \frac{10000 \text{cpm}}{0.25 \text{ c/d}} = 40{,}000$ dpm e) NaI
f) NaI g) Ion Chamber

---

10)

a) $S_{gross} - S_{bkg} = S_{net}$, $15575 - \frac{250 \text{ cpm}}{10 \text{ min}} = 15550$ cpm

b) $(.01 \text{ uCi})(2.22 \times 10^6 \text{ dpm/uCi}) = 2.22 \times 10^4$ dpm

$\frac{15550 \text{ cpm}}{2.22 \times 10^4 \text{ dpm}} \times 100 = E = .70 \times 100 = 70\%$

c) $\frac{300 \text{ cpm}}{0.70} = 429$ dpm

d) (429 dpm) (10) = 4290 dpm, $\frac{4290 \text{ dpm}}{2.22 \times 10^6 \text{ dpm/uCi}} = 0.0019$ uCi

e) $\frac{15550 \text{ cpm}}{\text{ESR}} = \frac{15550}{0.8550} = 18187$

Solutions To Problem Set 3

1) b 2) d 3) c 4) a 5) b 6) a, b, d 7) a, b, c, d, 8) d
9) a

10) Rate of cell division
State of cell division
Metabolic rate
State of nourishment
Oxygen levels
Enzyme levels associated with the repair process

11) Rems per Calendar Quarter

	Quarterly Average	Quarterly Maximum
Whole body	1.25	3
Skin	7.5	
Extremities	18.75	

12) 5 (N-18) Rem

13) As long as the total accumulated whole body dose does not exceed 5 (N-18) Rem

14) 0.5 Rem

15) 0.17 Rem

16) 18 years old

17)

Age	5 (N-18) - Previous Dose (Rem)				"Bankroll"	Dose Allowed for the year (Rem)
24	30	-	0	=	30	12
25	35	-	12	=	23	12
26	40	-	24	=	16	12
27	45	-	36	=	9	9
28	50	-	45	=	5	5*
29	55	-	50	=	5	5

* From this point on, the "Bankroll" is used up as long as he continues to receive the maximum dose.

18) Primary and secondary cosmic rays; naturally occuring radioactive materials in the earths crust; naturally occurring radioactive materials in the body.

19) Buildings made from materials containing Uranium, Thorium, Radium, and Potassium-40 can increase the natural background external exposure. If the ventilation rate is poor, radon gas and its decay products can build up and increase internal exposures.

20) External exposure from K-40, U-238, and Th-232 decay series; Internal exposure from K-40, Radon, and Thoron.

---

21) % of Carbon in paper (cellulose) $= \frac{72}{162.14} = 0.444$

$$6.1 \frac{\text{pCi}}{\text{gm Carbon}} \times 0.444 \frac{\text{Carbon}}{\text{Paper}} = 2.71 \frac{\text{pCi}}{\text{gm paper}}$$

$$2.71 \frac{\text{pCi}}{\text{gm paper}} \times \frac{454 \text{ gm}}{\text{lb.}} \times \frac{2000 \text{ lb.}}{\text{ton}} = 2.46 \times 10^6 \frac{\text{pCi}}{\text{ton of paper}}$$

$$2.46 \times 10^6 \frac{\text{pCi}}{\text{ton of paper}} \times 3100 \text{ tons} = 7.62 \times 10^9 \text{ pCi}$$

$$= 7.62 \text{ mCi of C-14 released}$$

Solutions To Problem Set 4

1) c 2) b 3) a 4) a, b, c, d 5) c 6) b 7) c

8) A body burden is a quantity in uCi when present continuously in the body will deliver a dose rate not in excess of a maximum permissible dose rate. Maximum permissible concentrations are concentrations in air or water required to yield a body burden.

9) 1. The type and energy of the radiation emitted;
2. The radiological half-life of the isotope;
3. The biological half-life of the isotope; } effective half-life
4. The isotope's distribution in the body;
5. The solubility of the compound containing the isotope.

---

10) $I_1 = \frac{I_2 d_2^2}{d_1^2} = \frac{(5\text{mR/Hr})\ (100\text{ cm})^2}{(30.48\text{ cm})^2} = 53.82\text{ mR/Hr}$

---

11) $I = I_o e^{-\mu x}$, Where $\mu = \mu_m \times \rho$

= mass attenuation coefficient for Cr-51 gamma (from Appendix IV) in lead, multiplied by the density of lead

= $(0.369\text{ cm}^2/\text{gm}) \times (11.35\text{ gm/cm}^3) = 4.19\text{ cm}^{-1}$

$I_o = \frac{I}{e^{-\mu x}}$, $I_o = \frac{2\text{ mR/Hr}}{e^{-(4.19\text{ cm}^{-1} \times 2\text{ cm})}} = 8,718\text{ mR/Hr}$

---

12) For Cs-137, $\Gamma = 0.33$ R/h/meter/Ci (from Appendix IV)

For 20 mCi: $0.33 \times 0.02\text{Ci} = 0.0066$ R/h/m (Note: the source must be decay corrected to obtain the true dose rate at present time).

$I_1 = \frac{I_2 d_2^2}{d_1^2} = \frac{(0.0066\text{ R/Hr})\ (39.37\text{ inches})^2}{(22\text{ inches})^2} = 0.021\text{ R/Hr}$

A "Caution-Radiation Area" sign must be posted at the point where the radiation field is 5 mR/Hr.

---

13) 21 mR/Hr x 0.5 Hr = 10.5 mR $\approx$ 11 mRem

14) $I_2 = \frac{I_1 d_1^2}{d_2^2} = \frac{(5\ mR/Hr)\ (6\ ft)^2}{(3\ ft)^2} = 20\ mR/Hr$

Exposure = 5mR/Hr x 5 Hrs. = 25 mR; Stay time = $\frac{50\ mR - 25\ mR}{20\ mR/Hr} = 1.25\ Hr$

(Note: 1 mR ≈ 1 mRem)

---

15) Two lead blocks are required to shield the vial:

$\frac{I}{I_o} = 1/8 = 0.125 = e^{-\mu x}$

$-2.0794 = -\mu x$

$x = \frac{2.0794}{(0.059\ cm^2/gm \times 11.35\ gm/cm^3)} = 3.1\ cm$

OR

3 HVL = 1/8 reduction (2x2x2 = 8)

HVL for Co-60 = 1.035 cm x 3 = 3.1 cm

Since 3.1 cm x $\frac{1\ inch}{2.54\ cm}$ = 1.22 inches, one block isn't enough shielding, therefore, two blocks are required.

Solutions to Problem Set 5

1)

#	gross cpm	- Bkg	= net cpm	÷ Eff*	= dpm
1	54	50	4	---	---
2	101	50	51	.50	102
3	237	25	212	.85	250
4	350	50	300	.55	546
5	120	50	70	.20	350
6	4025	25	4000	.85	4706
7	925	25	900	.90	1000
8	525	25	500	.88	569
9	225	25	200	.85	236
10	47	50	---	---	---

* Determined off the quench curves for the particular ESR and isotope in question.

2) By observing the distribution of the standard counts in the red and green channels, you can determine which isotopes contributed to the contamination:

Sites 2, 4, 5, are contaminated with H-3.

Sites 3,6, 7, 8, 9 are contaminated with P-32.

3) Sites 1 and 10 are within the statistical flucuation of the background counts ($\sqrt{50}$ = 7.07; $\sqrt{25}$ = 5). Thus, any gross counts that fall between 43 and 57 in the red channel, or 20 and 30 in the green channel can be considered to be due to background. As a general rule of thumb, counts that are twice background counts can be considered to be from radioactivity.

4) Sites 6 and 7 exceed the removable contamination limits of 1000 dpm/100 cm^2.

5) The waste container should be posted with a "Caution-Radiation Area" sign since an individual could receive an exposure of 5 mR in one hour.

There are no areas in which an individual is likely to receive 100 mR in one hour. Even though the exposure rate is 125 mR/Hr inside the freezer, one is not likely to stand there for one hour. Therefore, a "Caution-High Radiation Area" sign is not required anywhere in the lab.

6) Opening the freezer with contaminated gloves is the most probable way the handle became contaminated. Since the handle is metal, soap and water should remove contamination. Other commercially prepared decontaminating solutions such as "count-off" or "lift away" can be used if soap and water fail.

7) The person or persons using P-32 have contaminated the floor.

8) Recommendations:

a) Keep articles like laboratory notebooks out of radioactive materials work areas; areas should be maintained as neat and as clean as possible to aid in controlling the spread of any contamination.

b) Shield the radioactive waste container by placing it in another container made out of plastic or steel or move it to an area where there is little or no traffic.

c) Shield the isotope storage area in the freezer by using plastic bins or shields since the high exposure rate is due to the P-32 beta radiation.

d) A GM survey meter should be available whenever P-32 is being used so that areas of contamination can be instantly identified, limited, and decontaminated as appropriate. For areas of H-3 use, smears should be taken and evaluated as soon as possible after the manipulations have occurred. Equipment, surfaces, etc. should be treated as potentially contaminated until proven otherwise.

e) More attention should be afforded to changing gloves given the probable instance by which the refrigerator handle and the cover of the liquid waste container were contaminated.

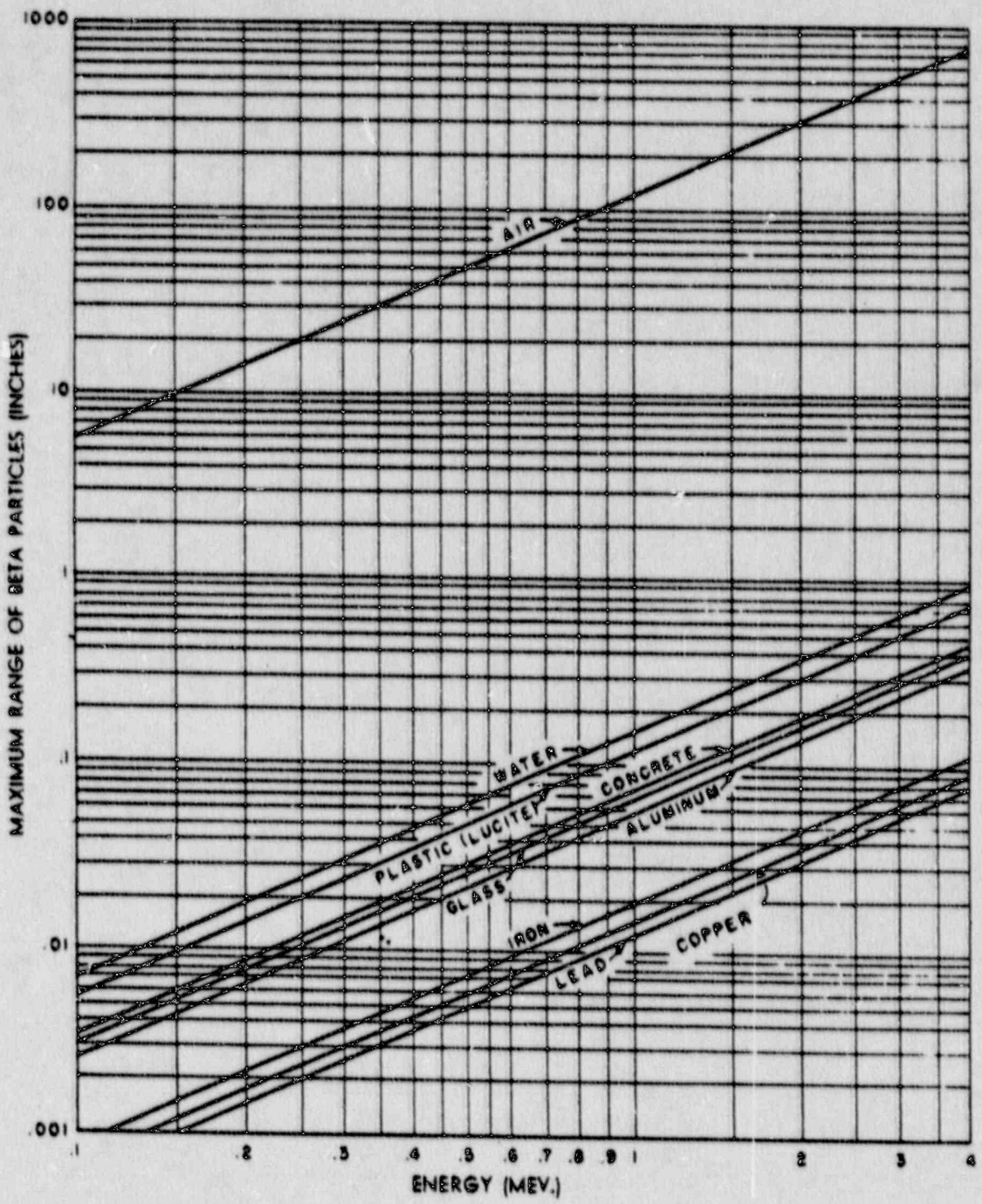
f) Minimize and localize all items which may be used in procedures involving the use of radioactive materials. Contamination control effectiveness can be of crucial importance to the success of experimental data and results.

g) Persons manipulating materials should be directly responsible for the monitoring and cleanup after they have worked, rather than having someone else find out how careless they may have been days after the occurrance. This is especially true in laboratory settings in which space is limited, people are numerous, and much time is spent in close quarters.

h) The person working with P-32 received a pipette bulb from the person working with H-3 which was contaminated. This is not only poor safety practice, but could interfere with accuracy of the P-32 worker's experimental results.

APPENDIX II

PENETRATION ABILITY OF BETA RADIATION



The maximum range of beta particles as a function of energy in the various materials indicated. (From SRI Report No. 361, "The Industrial Uses of Radioactive Fission Products". With permission of the Stanford Research Institute and the U. S. Atomic Energy Commission.)

APPENDIX III

Rules of Thumb

Beta Particles

a. Beta particles of at least 70 keV energy are required to penetrate the nominal protective layer of the skin (7 mg/cm² or 0.07 mm).

b. The average energy of a beta-ray spectrum is approximately one-third the maximum energy.

c. The range of beta particles in air is ~ 12 ft/MeV. (Maximum range of ^{32}P beta is 1.71 MeV × 12 ft/Mev ≈ 20 ft).

d. The dose rate in rads per hour in a solution by a beta emitter is 1.12 EC/ρ, where E is the average beta energy per disintegration in MeV, C is the concentration in microcuries per cubic centimeter, and ρ is the density of the medium in grams per cubic centimeter. The dose rate at the surface of the solution is one-half the value given by this relation. (For ^{32}P average energy of approximately 0.7 MeV, the dose rate from 1 μCi/cm³ (in water) is 1.48 rads/hr).

e. The surface dose rate through the nominal protective layer of skin (7 mg/cm²) from a uniform thin deposition of 1 μCi/cm² is about 9 rads/hour for energies above about 0.6 MeV. Note that in a thin layer, the beta dose rate exceeds the gamma dose rate, for equal energies released, by about a factor of 100.

f. For a point source of beta radiation (neglecting self and air absorption) of strength mCi millicuries, the dose rate at 1 cm is approximately equal to 200 × mCi rads/hour and varies only slowly with beta energy. Dose rate for 1 mCi ^{32}P at 1 cm is approximately 200 rads/hour.

Gamma Rays

a. For a point source gamma emitter with energies between 0.07 and 4 MeV, the exposure rate (mR/hr) within ± 20% at 1 foot is 6 × mCi × E × n, where mCi is the number of millicuries; E, the energy in MeV; and n, the number of gammas per disintegration.

b. The dose rate to tissue in rads per hour in an infinite medium uniformly contaminated by a gamma emitter is 2.12 EC/ρ, where C is the number of microcuries per cubic centimeter, E is the average gamma energy per disintegration in MeV, and ρ is the density of the medium. At the surface of a large body, the dose rate is about half of this.

X-Ray

a. The exposure rate at 2 feet from diagnostic x-ray equipment operated at 100 kVp and 100 milliamperes is approximately 2.3 roentgens/second.

b. Exposure rate at the fluoroscopy table with tube potential at 80 kVp and tube current of 1 milliampere should not exceed 2.1 roentgens/minute.

c. Scattered radiation can be as penetrating as the primary beam.

X-Ray Diffraction

a. The x-ray beam intensities from the primary beam can be as much as 400,000 R/min.

b. Scattered radiation 10 cm from the points of scatter about the x-ray tube head has been measured in the order of 150 R/hr.

c. The threshold dose sufficient to produce skin erythema is 300 to 400 roentgens.

d. The minimum cataractogenic single dose is 200 rads, while a dose of 750 rads exhibits a high incidence of cataract formation.

Miscellaneous

a. The activity of any radionuclide is reduced to less than 1% after 7 half-lives (i.e., 2^{-7} = 0.8%).

b. For material with a half-life greater than six days, the change in activity in 24 hours will be less than 10%.

(from NIH Publication 79-18, DHEW)

APPENDIX IV

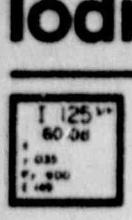
REFERENCE DATA FOR SELECTED RADIOISOTOPES

NUCLIDE	HALF LIFE	BETA: MAX ENERGY MeV	BETA: I %	BETA: RANGE IN INCHES AIR	BETA: RANGE IN INCHES PLASTIC	GAMMA: ENERGY MeV	GAMMA: I %	GAMMA: Γ	GAMMA: HVL (cm Pb)	GAMMA: ATTN. COEF. cm^2/gm Pb	CRITICAL ORGAN, BODY BURDEN (uCi)	EFFECTIVE HALF-LIFE (d)
Calcium-45	163 d	0.257	(100)	20	.02	---		---	---	---	Bone, 30	17
Carbon-14	5730 y	0.156	(100)	10	.01	---		---	---	---	Whole Body, 400	10
Cesium-137	30.17 y	1.173	(5.4)	150	.15	0.6616	(89.9)	0.33	0.536	0.114	Whole Body, 30	113.8
Chromium-51	27.7 d	---		---	---	0.3201	(9.8)	0.016	0.165	0.369	Lower Large Intestine, 800	26.6
Cobalt-60	5.27 y	0.318	(99.9)	25	.03	1.17 1.33	(99.9) (99.98)	1.32	1.035	0.059	Whole Body, 10	9.5
Copper-64	12.71 h	0.578	(37.2)	60	.06	1.346	(0.49)	0.12	1.11	0.055	Whole Body, 80	0.529
Hydrogen-3	12.33 y	0.0186	(100)	0.5	0.00	---		---	---	---	Whole Body, 1000	10
Iodine-125	60.14 d	---		---	---	0.0355	(6.67)	0.07	0.0029	21.0	Thyroid, 0.325	42
Iodine-131	8.04 d	0.606	(89.4)	60	.06	0.364 0.636	(81.2) (7.27)	0.22	0.178	0.342	Thyroid, 0.140	7.6
Potassium-42	12.36 h	3.521 1.996	(82) (17.5)	600 300	0.6 0.3	1.524	(17.9)	0.14	1.174	0.052	*	*
Phosphorous-32	14.28 d	1.71	(100)	250	.25	---		---	---	---	Bone, 6	13.5
Sodium-22	2.60 y	0.546 β^+	(89.8)	55	0.05	1.274	(99.9)	1.2	1.00	0.061	Whole Body, 10	11
Sulfur-35	87.4 d	0.1675	(100)	11	.01	---		---	---	---	Whole Body, 400 Testis, 90	44.3
Zinc-65	243.9 d	0.329 β^+	(1.5)	30	.03	1.115	(50.8)	0.27	0.925	0.066	Whole Body, 60	193.2

I = Intensity h = hours Γ = Roentgens per hour at one meter per Curie

d = days y = years * Data not availab'

Iodine-125: Handling and Hazards



The widespread use of Iodine-125 for radioiodination, and its relative biological uniqueness, requires that proper caution be exercised in handling and storage of this radioisotope.

PRINCIPAL RADIATIONS AND DECAY TABLE

Half-life: 60.0 days

Decay Mode: Electron capture

Radiations: Gamma 35.5 keV (7%)
X-rays K_α 27 keV (112%)
K_β 31 keV (24%)

Specific Gamma Ray Constant: $\frac{0.6 \text{ rad}}{\text{mCi-hr}}$ at 1cm[a]

Maximum Permissible Air Concentration: (Occupational limit based on 40 hour work week) 5×10^{-9} μCi/ml[b]

Dose Rate for Iodine-125 measured using a 0.1ml solution in a typical glass vial: 400mR/hr-mCi at contact
150mR/hr-mCi at 1cm

Days	0	2	4	6	8	10	12	14	16	18
0		977	955	933	912	891	871	851	831	812
20	794	776	758	741	724	707	691	675	660	645
40	630	616	602	588	574	561	548	536	524	512
60	500	489	477	467	456	445	435	425	416	406
80	397	388	379	370	362	354	345	338	330	322
100	315	308	301	294	287	281	274	268	262	256
120	250	244	238	233	228	223	218	213	208	203
140	198	194	189	185	181	177	173	169	165	161
160	157	154	150	147	144	140	137	134	131	128
180	125	122	119	117	114	111	109	106	104	102
200	099	097	095	093	090	088	086	084	082	081
220	079	077	075	073	072	070	069	067	065	064
240	063	061	060	058	057	056	054	053	052	051

BODY DOSE

The critical organ for iodine uptake is the thyroid, which may accumulate 30% or more of the total iodine ingested. The total dose to the thyroid from 1μCi of ingested Iodine-125 is ~2.27 Rem. The maximum permissible thyroid burden is 650nCi (30 Rem/yr)[c]

PRECAUTIONS

Conduct routine thyroid scans.

Establish action levels at a percentage of maximum limits.

Employ gloves, shielding and tools to avoid direct handling. Almost all radiations from Iodine-125 are absorbed by 1mm of lead. Note that some iodinated compounds will penetrate laboratory gloves.

Conduct periodic breathing zone and environmental air sampling using commercially available activated charcoal filters or absorbent solutions.

Use activated charcoal vents on waste containers.

Wipe test work areas to assure contamination-free results.

Store NaI solutions at room temperature. Studies show that freezing results in instability of the iodine form and volatilization.

Avoid acidic solutions which result in volatile iodine.

Personnel internal exposure is best determined by routine sodium iodide crystal monitoring of the thyroid. Any increase should be reviewed for cause. An increase of 52nCi in the thyroid over seven consecutive days suggests that the individual may have worked in an air concentration of 5×10^{-9} μCi/ml for forty hours.

[a] Wagner, Henry N., Jr., Ed. Principles of Nuclear Medicine, W. G. Saunders Co., Phil., Pa. 1969, Pg. 863.
[b] 10 CFR Part 20 — Standards for Protection Against Radiation
[c] Report of Committee II on Permissible Dose for Internal Radiation (1959) Pergamon Press, London, 1959.

DETECTION

An understanding of how to detect and assay trace quantities of radioiodine is essential for safe handling of the isotope.

Detecting greater than 1 μCi - Levels of contamination down to about 1 μCi can be detected on surfaces by a suitable Geiger-Mueller tube detector and scaler...an essential apparatus for the iodination laboratory. This detector can also be used to determine the location of activity on chromatographic purification columns, in collection tubes, or on paper or thin-layer plates. The detection efficiency for this type of instrument is approximately 0.1 to 0.5% at contact.

Detecting less than 1 μCi - The maximum permissible levels of contamination in non-ventilated areas (see Table I) are orders of magnitude below the detection limit for a typical Geiger-Mueller detector. Therefore, a thin, thallium-activated sodium iodide detector shielded by a lead sleeve of about 1/8 inch thickness is recommended. The counting efficiency for this detector is about 20% at contact. One such instrument, useful for detecting extremely low levels of ^{125}I contamination, is the Model 44-3 Gamma Scintillator and the Model 177 Scaler, manufactured by Ludlum Measurements, Inc., Sweetwater, Texas. Similar equipment is available from other manufacturers. Care should be taken that the detector probe be protected from contamination by use of a frequently-changed plastic covering.

TABLE 1

Maximum allowable limits
for Iodine-125 (soluble)

Maximum permissible air concentration (Room)	5×10^{-9} μCi/ml, based on 40 hour work week
Maximum permissible water concentration (Sewerage)	2×10^{-7} μCi/ml
Maximum permissible air concentration (Stack release)	8×10^{-11} μCi/ml, based on 168 hour week
Bench tops, floors and other surfaces in non-ventilated areas	4.5×10^{-3} μCi/100 cm^2 on smear (10,000 dpm)

CONTROL NO. 8728

RECOMMENDED GUIDELINES FOR THE SAFE HANDLING OF IODINE-125

As with any other radionuclide, standard rules for safe handling of radioiodine must be followed. These laboratory rules must conform to federal, state and local regulations regarding exposure and contamination limits, disposal of radioactive waste, and posting of laboratories.

In General

1. Use forceps fitted with rubber sleeves to ensure a secure grip on containers. One-inch sections of 1/3 inch O.D. latex surgical tubing are ideal, and can be replaced easily when contaminated.
2. Employ appropriate shielding when required.
3. A lab coat, safety glasses and gloves should be worn.
4. If hands or skin become contaminated, remove activity by washing with commercially available decontamination solutions, such as NEN's COUNT-OFF Decontaminant. Rescue soap pads (3M) are particularly useful for removing activity. Rub gently so that the skin is not injured.
5. Film badges or thermoluminescent dosimeters (TLD's) should be used by all personnel. Wrist or finger badges are optional when working with ^{125}I; significant extremity dose occurs only if the user handles the vial or container directly. (See Figure 1)
6. Frequent wipe surveys should be performed on fume hood lips and other work surfaces where radioactivity is handled.
7. Pipetting of any radioactivity by mouth is forbidden. Devices such as Propipet pipet fillers and transfer pipettors with disposable tips should be used.
8. Containers must be properly labeled with a tag stating the radionuclide, the amount of radioactivity, and the date.

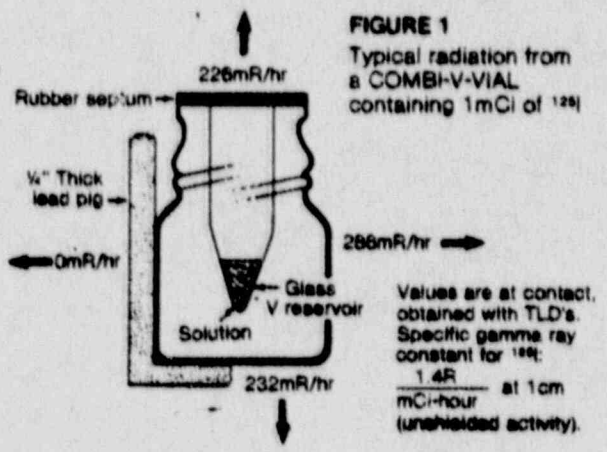


FIGURE 1
Typical radiation from a COMBI-V-VIAL containing 1mCi of ^{125}I

When Handling Radioiodine

1. Always work in a well-ventilated fume hood. Preplenum filters, lined with a charcoal impregnated filter material are recommended. The stack filter should be of the activated charcoal type, available from Filtek Filtration Technology, Inc. Wakefield, MA, or equivalent.

2. Use two pairs of gloves, as radioiodine can diffuse through rubber or plastic. The inner pair must be kept uncontaminated. We do not recommend handling contaminated vials or items directly. Rule 1, page 6.

3. Vials containing radioiodine should be opened for as brief a time as necessary and capped tightly when not in use.

4. Dispose of, or clean, contaminated apparatus and glassware as quickly as possible to minimize release of volatile iodine into the hood or room air. Contaminated items, such as vials, syringes, pipet tips, etc., should be wrapped in a double polyethylene bag, taped securely and placed immediately in containers designated "radioactive waste".

5. If a spill has occurred, or equipment must be decontaminated, immediately wipe the surfaces with a solution consisting of 0.1 M NaI, 0.1 M NaOH and 0.1 M $Na_2S_2O_3$. This helps to stabilize the material and minimize evolution of volatile species. Proceed with a detergent or decontaminant to complete the clean-up.

6. Use the above described solution in liquid waste containers to stabilize any iodine waste.

7. In addition to routine studies, thyroid measurements and urinalyses should be performed whenever significant contamination has occurred.

8. Do not add acids to radioiodine solutions. The volatility of ^{125}I is significantly enhanced at low pH.

Phosphorus-32: Handling and Hazards

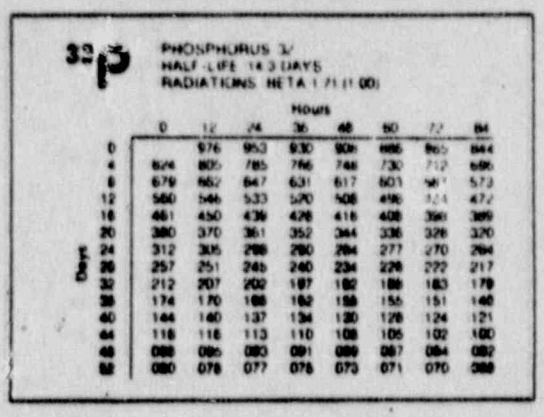
The marked increase in the use of Phosphorus-32 and the relatively large shipments have prompted the following reminder concerning the hazards associated with this hard beta emitter:

Maximum Energy: 1.71 MeV (beta particle)

Maximum Range in Air: Approximately 18 feet

Maximum Range in Water: Approximately 0.3 inches

Critical Organ: Soluble forms — Bone
Insoluble forms — Lung, G.I.



DOSE RATES

Dose rates from 1mCi Phosphorus-32 over 1cm² of skin are:

At surface of the skin	2,000 rads/hr
At 1cm	200 rads/hr
At 10cm	22 rads/hr

Dose rate for 1mCi Phosphorus-32 in 1ml of water

At surface	780 rads/hr

BODY DOSE

The maximum permissible body burden of Phosphorus-32 is 30μCi. *However, the maximum permissible burden for the bone critical organ is 6μCi.* Although about 60% of Phosphorus-32 that is ingested is excreted within the first 24 hours, only about 1% per day is excreted after the second or third day following ingestion. Therefore, regular urine samples should be submitted, followed by rapid analysis. Dose evaluations also require knowledge of the approximate date and time the isotope was handled.

PRECAUTIONS

Employ both low and high density shielding.
Avoid direct contact with skin. Use gloves.
Protect eyes from chemical splash and unnecessary radiation.
Use remote handling tools.
Prevent ingestion.
Isolate waste.

Film badges should be used by all personnel working with Phosphorus-32. If millicurie quantities are manipulated, wrist and finger badges should also be used.

Wrist badge readings serve ONLY as an indication of exposure problems in the laboratory. Relatively low wrist badge readings may fail to reveal high exposure to the fingers.

One of the **greatest hazards** associated with beta emitters of this energy exists in handling uncovered vessels containing the material. For example, the surface dose rate of 1mCi of Phosphorus-32 in 1ml of solution is approximately 13 rads/minute.

This dose rate will not be appreciably reduced by attenuation in a few centimeters of air, nor will there be much reduction by inverse square law from a source of this kind. It is obvious that a hand or face over such an open container may receive a considerable dose of radiation in a short period of time.

DETERMINING WEIGHT PER MICROCURIE

The following equation can be used to calculate the amount of a radionuclide required to obtain a specific number of microcuries.

$$W = C(N)(T_{\frac{1}{2}})(MW)$$

Where: W = weight in micrograms
N = number of microcuries
$T_{\frac{1}{2}}$ = half-life
MW = molecular weight
C = constant (this value of C is given to the right for different units of time of $T_{\frac{1}{2}}$)

C	$T_{\frac{1}{2}}$
8.87×10^{-14}	seconds
5.32×10^{-12}	minutes
3.19×10^{-10}	hours
7.66×10^{-9}	days
2.80×10^{-6}	years

H — HYDROGEN-3 (TRITIUM)

HALF-LIFE 12.3 YEARS
RADIATIONS: BETA 0.018 (1.00)

Years \ Months	0	1	2	3	4	5	6	7	8	9	10	11
0	—	995	991	986	981	977	972	968	963	959	954	950
1	945	941	936	932	928	923	919	915	910	906	902	898
2	893	889	885	881	877	873	869	865	860	856	852	848
3	844	841	837	833	829	825	821	817	813	810	806	802
4	798	794	791	787	783	780	776	772	768	765	762	758
5	754	751	747	744	740	737	733	730	727	723	720	716
6	713	710	706	703	700	697	693	690	687	684	680	677
7	674	671	668	665	661	658	655	652	649	646	643	640
8	637	634	631	628	625	622	619	616	614	611	608	605
9	602	599	597	594	591	588	585	583	580	577	575	572
10	569	567	564	561	559	556	553	551	548	546	543	541
11	538	535	533	530	528	526	523	521	518	516	513	511
12	509	507	504	501	499	497	494	492	490	487	485	483

I — IODINE-125

HALF-LIFE 60 DAYS
RADIATIONS: GAMMA 0.035 (0.07)
X-RAY (FROM E.C. & I.C.): 0.027 (1.36)

Days \ Days	0	2	4	6	8	10	12	14	16	18
0	—	977	955	933	912	891	871	851	831	812
20	794	776	758	741	724	707	691	675	660	645
40	630	616	602	588	574	561	548	536	524	512
60	500	489	477	467	456	445	435	425	416	406
80	397	388	379	370	362	354	345	338	330	322
100	315	308	301	294	287	281	274	268	262	256
120	250	244	239	233	228	223	218	213	208	203
140	198	194	189	185	181	177	173	169	165	161
160	157	154	150	147	144	140	137	134	131	128
	125	122	119	117	114	111	109	106	104	102
	099	097	095	093	090	088	086	084	082	081
	079	077	075	073	072	070	069	067	065	064
	063	061	060	058	057	056	054	053	052	051

I — IODINE-131

HALF-LIFE 8.06 DAYS
RADIATIONS: BETA 0.812 (.007) 0.608 (.875)
0.335 (.091) 0.250 (.028)
GAMMA 0.724 (.028) 0.639 (.091)
0.3645 (.81) 0.2843 (.055)
0.0802 (.055) 0.156 (.01) 0.210 (.01)

Days \ Hours	0	6	12	18	24	30	36	42	48	54	60	66
0	—	979	958	938	918	898	879	860	842	824	807	789
3	773	756	740	724	709	694	679	665	651	637	623	610
6	597	584	572	560	548	536	525	514	503	492	481	471
9	461	451	441	432	423	414	405	397	388	380	372	364
12	356	349	341	334	327	320	313	307	300	294	287	281
15	275	269	264	258	253	247	242	237	232	227	222	217
18	213	208	204	200	195	191	187	183	179	175	172	168
21	164	161	157	154	151	148	144	141	138	135	133	130
24	127	124	122	119	116	114	112	109	107	105	102	100
27	098	096	094	092	090	088	086	084	083	081	079	077
30	076	074	073	071	070	068	067	065	064	062	061	060
33	059	057	056	055	054	053	051	050	049	048	047	046
36	045	044	043	042	041	040	039	038	037	036	036	035

P — PHOSPHORUS-32

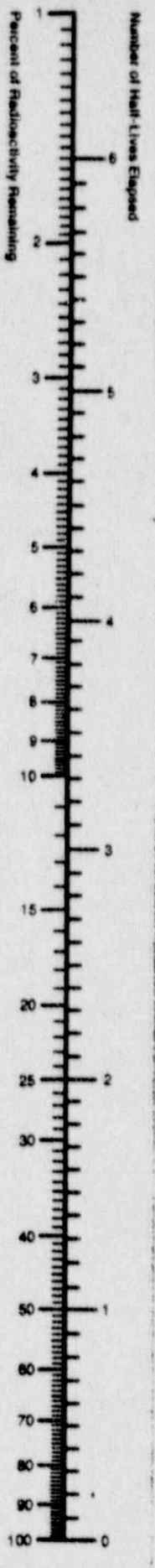
HALF-LIFE 14.3 DAYS
RADIATIONS: BETA 1.71 (1.00)

Days \ Hours	0	12	24	36	48	60	72	84
0	—	976	953	930	908	886	865	844
4	824	805	785	766	748	730	712	695
8	679	662	647	631	617	603	587	573
12	560	546	533	520	508	496	584	472
16	461	450	439	428	418	408	398	389
20	380	370	361	352	344	336	326	320
24	312	305	298	290	284	277	270	264
28	257	251	245	240	234	228	222	217
32	212	207	202	197	192	188	183	179
36	174	170	166	162	158	155	151	148
40	144	140	137	134	130	128	124	121
	118	116	113	110	108	105	102	100
	098	095	093	091	089	087	084	082
	080	078	077	075	073	071	070	068

At the end of 7 half-lives, less than 1% of activity remains, for *any* radionuclide, or: $0.8\% = 2^{-7}$.

Where the half-life of an isotope is longer than [illegible] days, less than 10% of activity is lost after 24 hours.

HALF-LIFE DECAY CHART

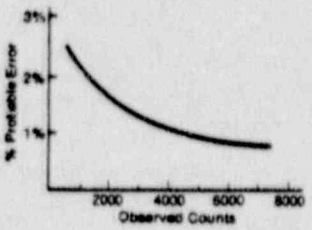


DETERMINING COUNTING ERROR

Radioactive decomposition is a random phenomenon. For this reason counts taken from radionuclides are intrinsically imprecise. Two uncomplicated formulae can be applied to calculate the extent of inaccuracy of your count. In each the single variable is the number of "observed" counts, and in all instances, the statistical error is inversely proportional to the number of counts.

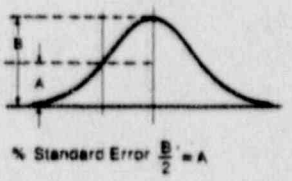
Probable Error:
Where N = number of observed counts.

$\frac{67.45}{\sqrt{N}}$ = % of Probable Error.



Standard Error:

$\frac{100}{\sqrt{N}}$ = % Standard Error



% Standard Error $\frac{B}{2}$ = A

Standard error is determined where the value of A is exactly one-half the value of the apex of the normal bell curve.

1000 millicuries (mCi) = 1 curie (Ci)
1000 microcuries (μCi) = 1 millicurie (mCi)
250 microcuries (μCi) = 0.25 millicurie (mCi)
100 microcuries (μCi) = 0.10 millicurie (mCi)
50 microcuries (μCi) = 0.05 millicurie (mCi)
1 microcurie (μCi) = 0.001 millicurie (mCi)

1 curie = 2.22×10^{12} dpm = 3.7×10^{10} dps
1 millicurie = 2.22×10^{9} dpm = 3.7×10^{7} dps
1 microcurie = 2.22×10^{6} dpm = 3.7×10^{4} dps

TIME CONVERSION FACTORS
1 day = 1.44×10^{3} minutes = 8.64×10^{4} seconds
1 year = 5.26×10^{5} minutes = 3.16×10^{7} seconds

SPECIFIC ACTIVITY AT 100% ISOTOPIC ENRICHMENT FOR SOME COMMON RADIOISOTOPES

Carbon-14: ~62 mCi/milliatom = ~4.4 mCi/milligram
Chlorine-36: ~1.2 mCi/milliatom = ~0.03 mCi/milligram
Iodine-125: ~2200 curies/milliatom = 17.3 curies/milligram
Iodine-131: ~16,100 curies/milliatom = 123 curies/milligram
Phosphorus-32: ~9120 curies/milliatom = ~285 curies/milligram
Phosphorus-33: ~5,200 curies/milliatom = ~158 curies/milligram
Sulfur-35: ~1488 curies/milliatom = 42.8 curies/milligram
Tritium: ~29 curies/milliatom = ~9.6 curies/milligram

FORMULA FOR CALCULATION OF SPECIFIC ACTIVITY AT 100% ISOTOPIC ENRICHMENT

$$\text{Specific Activity} = \frac{1.3 \times 10^{9}}{t_{1/2}\,(\text{days})} \text{ millicuries/milliatom}$$

$$= \frac{1.3 \times 10^{9}}{(\text{Mass. No})\, t_{1/2}\,(\text{days})} \text{ millicuries/milligram}$$

MAXIMUM ENERGY RANGES IN AIR FOR SOME COMMON RADIOISOTOPES

Carbon-14: E_{max} = 0.155 MeV $\beta-$; range: ~28 mg/cm²
Iodine-125: E_{max} = 0.035 MeV X-Ray; range: ~350 mg/cm²
Iodine-131: E_{max} = 0.81 MeV $\beta-$; range ~234 mg/cm²
Phosphorus-32: E_{max} = 1.71 MeV $\beta-$; range: ~800 mg/cm²
Phosphorus-33: E_{max} = 0.25 MeV $\beta-$; range: ~60.0 mg/cm²
Sulfur-35: E_{max} = 0.167 MeV $\beta-$; range: ~34 mg/cm²
Tritium: E_{max} = 0.018 MeV $\beta-$; range: ~0.56 mg/cm²

DESIGNED FOR CONVENIENT REFERENCE