UNITED STATES
NUCLEAR REGULATORY COMMISSION
WASHINGTON, D. C. 20555

DESIGNATED ORIGINAL

Certified By

April 30, 1982

Sheldon J. Wolfe, Esq.
Administrative Judge
Chairman, Atomic Safety and
Licensing Board
U.S. Nuclear Regulatory Commission
Washington, D.C. 20555

Dr. Harry Foreman
Administrative Judge
Box 395, Mayo
University of Minnesota
Minneapolis, Minnesota 55455

Dr. Walter H. Jordan
Administrative Judge
881 West Outer Drive
Oak Ridge, Tennessee 37830

In the Matter of
LOUISIANA POWER & LIGHT COMPANY
(Waterford Steam Electric Station, Unit 3)
Docket No. 50-382

Dear Administrative Judges:

Pursuant to my conversations today with Chairman Wolfe and with counsel for the Applicant and Joint Intervenors, please be advised of the following.

1. At the forthcoming hearing session commencing May 3, 1982, the Staff will move to introduce as an exhibit the interim findings prepared by the Federal Emergency Management Agency, under cover of a memorandum from Richard W. Krimm to Brian Grimes, dated March 31, 1982. Counsel for the other parties have advised me that they do not object to the introduction of this proposed exhibit, a copy of which is enclosed herewith.

2. The Applicant indicated, in Mr. Churchill's letter to the Licensing Board of April 20, 1982, that it will seek to introduce as its Exhibit No. 8 a memorandum from Samuel J. Chilk to William J. Dircks dated March 26, 1981, the admissibility of which has not been stipulated to by the parties. The Staff has determined that if the Applicant moves to introduce its proposed Exhibit No. 8, the Staff will move to introduce as a further exhibit SECY 82-77, a memorandum from William J. Dircks to the Commissioners dated February 19, 1982 (with attachments), a copy of which is enclosed herewith. Counsel for Applicant has advised me that he does not object to the introduction of this proposed exhibit; counsel for Joint Intervenors has advised me that he expresses no opinion at this time and reserves the right to object later to the admission of the Staff's proposed exhibit.

3. On April 2, 1982, Judge Foreman requested that any additional studies relating to the issue of synergism in which the doses of ionizing radiation

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were varied showing dose effects or dose rate effects be submitted by the parties within 10 days. The Staff has now identified and obtained copies of six studies in response to the Licensing Board's request, copies of which are enclosed. Counsel for the other parties have advised me that they do not object to the Staff's submission of these studies at this time.

Sincerely,

Sherwin E. Turk

Sherwin E. Turk
Counsel for NRC Staff

Enclosures: As Stated

cc: Service List

DELIVERY BY HAND

Federal Emergency Management Agency

Washington, D.C. 20472

MAR 31 1982

MEMORANDUM FOR: Brian Grimes
Director
Division of Emergency Preparedness
U.S. Nuclear Regulatory Commission

FROM: Richard W. Krimm
Assistant Associate Director
Office of Natural and Technological Hazards

SUBJECT: Interim Findings of Offsite Emergency Preparedness for the Waterford Nuclear Power Plant (Louisiana)

Attached is a copy of the Federal Emergency Management Agency (FEMA) Region VI's A through P interim finding of the adequacy of State and local plans for offsite radiological emergency preparedness for the Waterford nuclear power plant.

Dates for corrective actions are to be established. The joint exercise is now scheduled for April 26, 1982.

The Region VI evaluation of the offsite emergency plans is that there are no serious deficiencies in either the plan or the State's capability to implement the plan.

Attachment
as stated

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Federal Emergency Management Agency

Region VI Federal Center Denton, Texas 76201

February 4, 1982

MEMORANDUM FOR ASSOCIATE DIRECTOR, STATE AND LOCAL PROGRAMS AND SUPPORT

ATTN: Vern Adler, Technological Hazards Division

FROM: Dell Greer, Acting Chief, Natural and Technological Hazards Division

SUBJECT: Interim Findings

SITE: Waterford 3

STATE: Louisiana

BASIS FOR FINDINGS: Plans as follow:

Enclosures 1 and 2 to Attachment 1 (Parish Plans for Waterford 3)

Annex J, Appendix 7 to Louisiana Preparedness Plan for Emergency Operations, (State of Louisiana Peacetime Radiological Response Plan, Revision 3, September 1981)

INTRODUCTION

The following description of the Waterford 3 site is extracted from Attachment 1:

Waterford 3 is located on the west (right descending) bank of the Mississippi River at River Mile 129.6 between Baton Rouge, Louisiana, and New Orleans, Louisiana. The site is the northwestern section of St. Charles Parish, Louisiana, between the towns of Killona and Taft, as shown in Figure 1-1.

The Waterford property is owned by Louisiana Power and Light Company (LP&L) and includes 3,561.3 acres. The plant area is about 48 acres and is defined as including the fenced-in area immediately adjacent to Waterford 3. The site includes only station structures, and will not include any residential, recreational, or other industrial structures. There are at present no plans for a visitor center or other recreational facilities either within the site or on the LP&L property.

The Mississippi River is the closest prominent natural feature to Waterford 3, while other important natural features include Lac des Allemands, about 5.5 miles southwest of the site, and Lake Ponchartrain, about 7 miles northeast of the site. The land slopes gently from its high points near the Mississippi (10-15 feet above mean sea level) to extensive wetlands located about 1.5 to 2.5 miles inland from the river.

Most of the man-made features are located on the narrow strip of dry land between the Mississippi River and the wetlands. Near the Waterford site are several large industrial facilities, including Waterford 1 and 2 (0.4 miles west-northwest of the site), Little Gypsy Steam Electric Station (0.8 miles north-northwest of the site and across the river from Waterford 3), Beker Industries, a fertilizer manufacturer (0.6 miles east-southeast), and Hooker Chemical Company (0.8 miles east-southeast).

Transportation facilities near the Waterford site include the Mississippi River (0.2 miles north-northeast of the site), Louisiana Highway 18 (0.1 miles north-northeast), Louisiana Highway 3127 (1.1 miles to the south-southwest of the site), Louisiana Highway 628 (0.7 miles north-northeast, across the river) and the Missouri-Pacific Railroad (0.5 miles south-southwest).

Major urban centers in the region of the site include New Orleans (approximately 25 miles east of the site) and Baton Rouge (approximately 50 miles west-northwest). Communities in St. Charles Parish near the site include Killona (0.9 miles west-northwest), Montz (1.0 miles north), Norco (1.9 miles east), and Hahnville (3.7 miles east-southeast). LaPlace (4.7 miles north) is located in St. John the Baptist Parish. Waterford 3 is located approximately 3 miles southeast of the St. John the Baptist Parish boundary.

Other prominent man-made features include the Mississippi River levee system which, at its closest point, is 0.1 miles from Waterford 3, and the Bonnet Carre Spillway, a flood control structure 1.3 miles east-northeast of the site.

The State of Louisiana, Department of Natural Resources, Office of Environmental Affairs, Nuclear Energy Division is the Office of Primary Responsibility for all off-site Radiological Emergency Preparedness Planning and for providing technical guidance and responding to accidents or incidents at a nuclear generating facility. Local site-specific Parish plans are developed by the affected Parishes in cooperation with and assisted by the Louisiana Nuclear Energy Division. Each Parish has submitted its plan to the Office of Primary Responsibility indicating they agree with the content of the plan and accept the plan as a document which provides for appropriate protective measures in the event of an accident/incident at Waterford 3. The State and Parishes were assisted by Energy Consultants, Inc., an Emergency Planning Service, in the deveopment of off-site plans for Waterford 3.

All plans referenced in this report are on display for examination or copying at the FEMA Region VI Office in Denton, TX. (See Federal Register Notice (Docket No. FEMA-REP-6-LA-2) of December 30, 1981, Page #63125.))

The FEMA Region VI Regional Assistance Committee has reviewed the written off-site plans for Waterford 3 and submitted their comments to FEMA Region VI for consolidation. The following comments represent a condensed version of findings relative to NUREG-0654, FEMA-REP-1, Rev. 1.

EVALUATION

GENERAL:

The Louisiana State Plan for Peacetime Radiological Response was received, reviewed and exercised in accordance with 44 CFR, Part 350 for the Grand Gulf Nuclear Facility in Mississippi which impacts on Louisiana within the 10 mile Emergency Planning Zone. There were no serious deficiencies noted in either the plan nor the States' capability to implement the plan as written. Implementing procedures have not been incorporated as an integral part of the plan to date but have been provided for review and will be incorporated prior to FEMA Regional submission to FEMA Headquarters for final approval/disapproval.

Attachment 1 and Enclosures 1 and 2 thereto (site-specific Parish Plans) generally meet the NUREG-0654 planning criteria with the following comments being forwarded to the State/Local governments for consideration for incorporation prior to or immediately after exercising. Individual RAC member and FEMA comments from which this condensation is made is also being forwarded to the State/Local Governments and may be obtained from the FEMA Region VI REP Office.

Attachment A - Consolidated and condensed review of Louisiana State Plan by NUREG 0654 element.

Attachment B - Consolidated and condensed exercise evaluation comments relative to the Louisiana State plan as submitted to the State after exercising the Grand Gulf Facility.

Attachment C - Consolidated and condensed plan review/evaluation comments relative to Enclosures 1 and 2 of Attachment 1 (Waterford 3 site-specific plans for St. Charles and St. John the Baptist Parishes)

ATTACHMENT A

Consolidated and Condensed Review of

Louisiana State Plan by NUREG 0654 Element

CONSOLIDATED SYNOPSIS OF RAC COMMENTS

LOUISIANA PEACETIME RADIOLOGICAL RESPONSE PLAN

GENERAL COMMENTS

The Table of Contents of the State Plan should be expanded to show the various tables, tabs, and enclosures to tabs. This is important, as much of the important information is in these places and is difficult to locate. This is especially critical with Chapters 8 and 9.

State Plan contains both pg. 5-1 as well as its revision, pg. 5-1.

A. ASSIGNMENT OF RESPONSIBILITY

1.e. Twenty-four hour per day emergency response capability, including manning of communications.

Page 3-2 indicates at the state level, LNED will provide coverage of the dedicated land-line telephone during normal office hours and the Louisiana State Police will provide coverage through an extension at other hours (as indicated during RAC meeting, a plan change is required since OEP has been added).

2.a. Functions and responsibility both primary and support.

Many functional assignments are made to organizational entities rather than to individuals by title as specified (e.g., in Section 7, III. A-D and V. D-F, etc.).

Section VI. A. 3. assigns responsibility to each "State Department" (again, not to an individual) to designate an individual, by title to be in charge of remergency response. This step should be accomplished in the Plan rather than given as a responsibility to be accomplished.

3. Written agreements as needed with federal, state, and local support organizations.

The State Plan does not include letters of agreement nor a signature page where functions are covered by laws as is the case in the State of Louisiana.

Section VII, A.4. refers to the Federal Radiological Monitoring and Assessment Plan and to letters of agreement which outline specific Federal resources, etc., but the only agreement that has been executed is apparently between the State and utilities (Section 14). EPA is not listed among the organizations to support/implement the plan (Table 1). If EPA support is anticipated, a written instrument needs to be prepared which details specific EPA resources which are relied upon. Without such written instruments, EPA may not be able to justify the resources needed to maintain the necessary capability.

The Agreement should list any specific EPA resources relied upon and designate the channel(s) of communication to obtain the resources.

B. ONSITE EMERGENCY ORGANIZATION

2. Re utility's designated emergency coordinator who would initiate emergency actions.

While not a state or local function, off-site plans should indicate the utility's designated emergency coordinator who would initiate emergency actions.

C. EMERGENCY RESPONSE SUPPORT AND RESOURCES

1.a. Specific person by title authorized to request federal assistance.

The assistant Secretary or his designated alternate of the Office of Environmental Affairs is listed as the authorized requestor but there should be an alternate named.

1.c. Specific State and local resources needed to support federal response.

Responsibilities are assigned (common) for implementing support responsibilities (VI, A.1.). Implementing procedures are required to be prepared (Section VIII, D.) but are not included in the plan. No specific provisions are made to support EPA, although FRMAP is relied upon (Section VII, A.4.). No airfields specified, no telephone lines or radio frequencies assigned, no telecommunications centers arranged for EPA.

3. Availability and capability of radiological laboratories.

Incomplete. The LNED laboratory is adequately described in the plan. However, other laboratories, such as LSU and local laboratories, are briefly mentioned but no details are provided as to expected availability, or capability.

No specific mention is made of reliance upon EPA laboratory facilities, but such reliance is implied since laboratory support may be requested from DOE. The plan does not, but should, detail specifically what kinds of support may be needed, as well as "turnaround times" required. Requirements should be coordinated with laboratory capabilities, and letters of agreement should also be considered.

4. Availability of support from nuclear and other organizations.

Section VII. A. and B. (pages 36-37) of the plan describes support and resources available from Federal, State, and local agencies. However, letters of agreement are not presented. In particular, L.S.U. is indicated as an organization having a capability which might be used. There should, therefore, be a letter of agreement with the institution so noting the capability and its willingness to respond.

D. EMERGENCY CLASSIFICATION SYSTEM

3. An emergency classification system consistent with that of utility.

Chapter I of the State plan establishes emergency action levels consistent with NUREG-0654, Appendix 1. Assume they are consistent with licensee's.

E. NOTIFICATION METHODS AND PROCEDURES

1. Mutual agreeable procedures for notification of emergency response organizations.

Chapter 2 of the plan indicates the general concept for notifying response organizations of an accident at a nuclear facility including an accident notification from which requires verification. Paragraph III, N. page 2-3 indicates implementing procedures will contain detailed procedures for notifying the various affected entities. (Implementing procedures to be published.)

It is not at all clear from the Plan whether EPA assistance is anticipated. Reference is made to technical federal aupport (Section VIII, A.3. & 4.), so EPA would likely be involved in event federal support is ever requested. However, EPA is omitted from the distribution list for the state plan (Table 1). Implementing procedures which might detail such plans (Table 2) are omitted.

While notification procedures are outlined in Chapter 2, verification requirements are referenced to State and Parish Plan implementing procedures, which are missing. The only reference to verification requirements that could be found is the blank on Tab 1 of Chapter 2 in both plans and a space for noting verification is included on the "Accident Notification Form".

2. Procedures for alerting, notifying and mobilizing emergency response personnel.

Paragraph VI, A. 5. page 23 indicates each state department will be responsible for developing procedures for notification and mobilization of its personnel assigned emergency functions. NOT included in plan.

3. For licensee (emergency messages content).

The plan in Chapter 2, III. A.C., page 2-2 indicates forms for notification will be used. The accident notification form covers areas of consideration noted in criteria with exception of potentially affected population.

5. System for dissemination to public of appropriate information received from licensee.

Notification of the public initially and with following messages is thoroughly addressed. It may be advisable to designate a single source of information. Chapter 5, IV.B. 3. 5. and 6. appear to provide several spokespersons. This could lead to some confusion and perhaps some embarrassment. All those mentioned in the references may well take part in preparation of information but there should be a single source for clarity.

6. Procedures for notification and prompt instruction to the public in the plume exposure pathway.

Incomplete. A means for providing prompt instructions to the public is partially addressed. The state plan states that EBS messages for public protective actions are to be developed. However, the Tensas Parish Plan contains an EBS sheltering and evacuation message.

7. Prescribed written instructional messages for public in affected areas.

State plan references parish attachments which have prescripted messages as Tab 1 to Chapter 4 on pp. 4-4 and 4-5 (parish plan). However, the State plan also has a Tab 1 to its Chapter 4 which indicates EBS messages are to be developed.

F. EMERGENCY COMMUNICATIONS

1.a. An emergency response communications network with manning on a 24-hour basis.

Plan indicates in Chapter 3, III. A. 1. and 2. that dedicated phone circuits will serve as primary communications between the licensee and LNED with commercial telephone as the backup. This does not appear to meet the criteria in that if you lose the primary link you have also lost the secondary link.

..c. Communications as appropriate with federal emergency response organizations.

LOEP will use NACOM land line and radio to communicate with FEMA with NAWAS being used as an alternate. Plan does not mention notification of other federal response organizations (DOE) if to be coordinated by FEMA, it should be so indicated and should be consistent with the utility scheme for notification.

1.d. Communications between nuclear facility and licensee's EOF, state/local EOC, and RAD monitoring teams.

Do not find provisions for communications between utility and field response teams. A communications schematic or block diagram would help in showing communications capability, systems and flow.

G. PUBLIC EDUCATION AND INFORMATION

3.a. Designated points of contact and physical locations for use by news media.

Inadequate. News media points of contact and specific media reception facilities are not identified. The plan merely says that facilities will be activated as necessary.

4.a. Designated media spokesperson with access to all necessary information.

LNED will designate a spokesperson to release state-wide information with parish governments designating spokespersons for releasing EPI to the parish populace. While the plan reads "spokespersons", it is suggested only one spokesperson being responsible for EPI news releases and this after coordination with spokesperson responsible for state-wide releases. A specific spokesperson is not identified by the state nor is any position (e.g., Public Information Officer).

H. EMERGENCY FACILITIES AND EQUIPMENT

3. Adequacy of emergency operating center.

Paragraph IV, H. and J., page 16 addresses the location and function of the State EOCs. Part N, p. 17 indicates each parish will activate and staff an EOC. Parish EOC here should be classified to read those parishes falling wholly or partially within the 10 mile EPZ.

4. Activation and staffing of EOCs and other facilities.

Timely activation and staffing of EOCs are to be addressed in the implementing procedures which are not included in the plan.

7. Provisions for offsite RAD monitoring equipment.

Chapter 6, Tab 3, enclosure 1 lists the needed equipment and the office where it is available. There is no indication, however, as to how readily the equipment can be made available. Some prior arrangements should be made, and reflected in the Plan, for having the equipment ready on short notice. Maintenance of "kits", or storage of some of the equipment in the mobile laboratory (Chapter 6, Tab 3, Item G.2) are two ways to maintain a state of readiness.

10. Provisions for management of emergency equipment/instruments including inventory and inspection.

Inspection, inventory, and checking of all emergency equipment and instrumentation on an assigned schedule is addressed. There is, however no indication as to "sufficient reserves" as replacements during calibration or repair.

11. Identification of emergency kits by general category.

No kits are specified. It is not sufficient to merely have the response equipment "available". It must be available in one place in kit form, or in a configuration such that an undue amount of time is not required to collect it.

Equipment not specifically itemized:

1. Instructions for monitoring instruments.
2. Check sources for portable instruments.
3. Instructions for emergency site monitoring and control (DCFs and procedures for projecting dose).

Policy for use of radio-protective drugs is provided (Chapter 9, Section IV. A. V. B. 2. and Tab 1) but no provisions could be found for their supply. These drugs should be considered for incorporation in the emergency kits, especially for use by emergency workers.

I. ACCIDENT ASSESSMENT

8. Rapid assessment of magnitude and location of liquid and gaseous radiological hazards.

This element is thoroughly written in the plan and is well done. The only exception was the absence of estimated deployment times.

No specific provisions were found for notification of monitoring teams at home or at work, although the general procedures are outlined in Chapter 6, Table 3. No call lists, no telephone numbers (or reference to lists), no response times, etc. Chapter 6, Tab 3, Item C refers to "LNED procedures" which may contain such details.

All State departments are responsible for designating an individual to be in charge, but the actual assignments have not been made in general. Instead, responsibilities are listed for organizational entities.

Transportation arrangements are covered in Chapter 6, Tab 3, Item D, but more details would be helpful, e.g., driver assignments, staging procedures, etc.

10. Relating measured parameters to dose rates and estimated integrated doses.

This element is also well described in the plan. However, a section of the plan entitled "Implementing Procedures" was not completed. This should be reviewed before passing final judgement on accident assessment. The provisions for assessing dose rates, estimating integrated dose from the projected and actual dose rates and for comparing these estimates with the protective action guides, will be contained in the implementing procedures when developed.

Chapter 6, Tab 3, enclosure 4, calls for concentration of radioactivity in units of uCi/cm^3. Since EPA PAGs use the different (although equivalent) units Ci/m^3, this table should be modified, or the equivalency of the units noted, to avoid possible confusion.

11. Location and tracking of airborne radioactive plume with aid or federal and/or state resources.

No specific provisions were found for locating or tracking the plume.

J. PROTECTIVE RESPONSE

9. Implementation of protective measures based on protective action guides.

This appears to be complete and adequate except for an "Access Control Map" to be developed and contained in Attachment 2, Chapter 6.

Limits and criteria are given in Chapter 7, Section IV. A. 6. b. and IV. B. 2. for workers VI. B. 1. for general public, IV. B. 3. for institutionalized persons, IV. B. 4. for school children. Chapter 8, Section IV. F. 3. .. 1. cites EPA drinking water standard, IV. F. 3. c. 2. allows 12 x MPC for short term, IV. F. 3. c. 3. allows 1000 x MPC for crisis conditions.

In estimating doses for purposes of Chapter 8, Section IV. F. 3. c. it should be noted that all doses are for "standard man" and doses to children or other population groups may be higher or lower, depending on the particular radio-nuclides involved.

10.d. Procedures for protecting mobility impaired including institutionally confined persons.

Cross reference indicates Chapter 4, II. F. p. 4-2. This is incorrect. Chapter 7, III. E. 4. indicates provisions for transport of persons having impaired mobility has been arranged for.

10.f. Methods used by State Health Department in decisions administering KI to central population.

Chapter 9, Tab 1, p. 9 is given as the cross reference. Should read p. 9-12 which indicates the ASOEA will make the recommendations for the administering of KI to emergency workers or institutionalized people with an established criteria which is printed in the plan on p. 9-12.

10.i. Projection of traffic capacities of evacuation routes under emergency conditions.

Chapter 7, Tab 1, indicates evacuation time studies have been prepared and can be found as supportive documentation to the plan. The criteria indicates the organizations plan shall include projected traffic capacities of evacuation routes under emergency conditions. This aspect of the plan does not meet the criteria as literally interpreted.

10.j. Organization and control of access to evacuated areas.

Chapter 7- IV. A. 3. is given as the reference to the plan satisfying this criteria. While the plan defines what is meant by access control, it does not address how, who, or under what circumstances such an activity might be initiated.

10.k. Identification and means for dealing with potential impediments to evacuation.

Chapter 7, III. E. 3. references the parish plans and Tab 3; Tab 3 of what? There is no Tab 3 to chapter 7 of the state plan. Chapter 6, III. D. 3. p. 6-2 of the Grand Gulf attachment indicates procedures for dealing with potential impediments will be implemented in accordance with highway department operating procedures. Potential impediments (such as flooding) have not been identified and highway department SOPs are not included as part of plan.

10.l. Time estimates for evacuation based on dynamic analysis.

Cross reference notes Chapter 7, Tab 1, addresses this aspect of planning. While this tab indicates supporting documentation is available regarding the time estimates for evacuation, it is not found in the plan.

10.m. Basis for choice of recommended protective actions in plume EPZ.

Chapter 7, II. D. leaves choices to "judgment of responsible officials", using EPA PAGs as a starting point.

No mention is made of protection afforded by sheltering, which is a vital piece of information needed by decision-makers in deciding whether evacuation (Chapter 7, IV. A. 4.) or sheltering (Chapter 7, IV. A. 1.) is preferred. EPA report "effectiveness of Sheltering as a Protective Action Against Nuclear Accidents Involving Gaseous Releases", EPA 520/1-78-001 should be reviewed and factored into the Plan, to assist the decision makers.

11. Protective measures for ingestion pathway.

Protective actions are given in Chapter 8, IV. F. 1. b. for milk, 8. IV. F. 2. for other foods, and 8. IV. F. 3. c. 5. for water.

No procedures were found for determining contamination levels or for estimating dose consequences of uncontrolled ingestion.

Decontamination of food stuffs is covered in Section 8, IV. F. 2.

Maps of water supply intakes and treatment plants are referenced in Chapter 8, Tab 2 and will be maintained by "the State". The specific location of such maps should be indicated, along with the name or title of the person responsible for maintaining them, and telephone number. Consideration should be given to making the maps a part of the plan.

12. Registration and monitoring evacuees in relocation centers.

Chapter 9 addresses monitoring, decontamination procedures, etc. but does not provide for registration of evacuees using or passing through the reception area.

K. RADIATION EXPOSURE CONTROL

4. Decision chain to authorize emergency workers to incur exposures in excess of PAGs.

Procedural arrangements have been made on paper (e.g., Chapter 9. V. B. 1. b.), but methods for tracking over-exposed workers are not detailed. This could be corrected by elaborating the instructions on the Dosimeter Report Form (Chapter 9, Tab 6).

The Preventive PAG levels specified in Chapter 9. V. D. are consistent with EPA guidance to minimize exposures. However, the Plan does not indicate how these PAGs are to be used. Section 9. V. D. 1. indicates exposures up to 25 Rem (W.B.) or 125 Rem (thyroid) will be accumulated "as directed by LNED". This control is not, however, reflected in the Dosimeter Report Form, which implies that there are no restrictions on exposures up to those limits. This should be clarified.

Authorization to exceed emergency PAGs can be given by the chief executive officer of the affected Parish, or by the ASOEA. Neither of these officials is required to have specialized training in radiological health physics. A health physics professional should be explicitly identified somewhere in the decision chain. The professional should be a medical doctor with radiological health training, or should have advanced training dealing with radiation injury.

5.b. Means for decontamination of emergency personnel, supplies, and equipment, and waste disposals.

External contamination control is covered in Chapter 9, Tab 2.

Persons with contaminated wounds are to be referenced to nearest medical facility. (Chapter 9, Tab 4, Item 2). No other internal contamination provisions could be found. Provisions should be added for persons exposed to the plume who may be internally contaminated through the inhalation pathway and require decontamination.

L. MEDICAL AND PUBLIC HEALTH SUPPORT

1. Local and back-up material and medical services.

The State has not yet completed a listing of hospitals equipped to accept radiation accident patients. Tensas Parish Plan provides letters of agreement with several hospitals in this category. Tab 1 to Chapter 9 in the Tensas Parish Plan refers to hospital decontamination plans but no plan was included. These plans must be reviewed before an evaluation of this element can be completed.

3. Lists, locations and capacities of public, private, military hospitals.

Chapter 10 of the plan addresses medical and public health aspects of REP planning. IV. B. 2. indicates Tab 4 lists the hospitals capable of receiving and treating radioactively contaminated persons. Tab 4 is to be developed, therefore is deficient.

4. Transportation of accident victims to medical support facilities.

Chapter 10, paragraph IV. A. 1. addresses transportation of on-site personnel needing medical treatment and IV. A. 2. indicates that parish OEPs are responsible for coordinating emergency medical services and that the ambulance services can be found under Tab 2 which is to be developed and is therefore a deficiency.

M. RECOVERY AND REENTRY PLANNING AND POSTACCIDENT OPERATIONS

1. General plans and procedures for reentry and relaxation of protective measures.

Protective actions will be relaxed by the ASOEA based on LNED recommendation (Chapter 11, III. A.). An individual should be identified to make the recommendation rather than the organizational entity (LNED).

4. Method for periodic estimation of total population exposure.

This is probably implied in Section IV. J. and is a responsibility of the LNED as stated in Section VI. B. 13. e. The plan does not, however, establish a method. Population doses are likely an output of LNED's computer capabilities noted in Chapter 6, III. C. 1. a.

N. EXERCISES AND DRILLS

2. c. Medical emergency drills (licensee/local).

Inadequate. The State plan does not address medical emergency drills and the Tensas Parish Plan states this section is "not applicable".

2.d. Annual radiological monitoring drills.

Annual drills are specified in Chapter 13. IV. A. 2. However, this chapter merely recites NUREG-0654 requirements and does not add the necessary elaboration to make the Plan a working document. For example, responsible individuals should be designated within the organizations to plan and coordinate the drills, it should be stated whether the State participates in each annual drill at each facility, the extent of realism required, i.e., simulation vs. actual data collection and response actions, etc.

2. 3. Health physics drills

Chapter 13, IV. A. 3., indicates health physics drills will be conducted semi-annually. Plan does not indicate analysis of simulated elevated airborne sample (wording of sentence is of such to indicate only liquid analysis).

2.e.(1) Semi-annual health physics drills

Chapter 13. IV. A. 3., also merely recites NUREG-0654 requirements without elaboration. There is no indication how many health physicists are to be involved, whether two drills are required at each facility, who is the individual responsible, etc.

O. RADIOLOGICAL EMERGENCY RESPONSE TRAINING

1.b. Training programs for offsite response organizations including fire, police, and ambulance/rescue personnel.

Chapter 12, III. A. 1., indicates training will be provided by the facility for offsite personnel responding on site. Did not find provisions for training of those organizations who might have mutual aid pacts or agreements with the primary emergency response organizations.

P. RESPONSIBILITY FOR THE PLANNING EFFORT

1. Training of planning personnel

Chapter 12, V. B. is listed as cross reference - incorrect. Should read Basic plan, V. B. p. 20 which says the ASOEA is authorized to direct the development and implementation of emergency response plans for FNFs. It is not indicated that State Planning Personnel shall be trained but can only be assumed.

6. A listing of supporting plans

Inadequate. Some supporting plans are referred to in various parts of the State and Parish plans but there is no detailed listing of State or local supporting plans and related documents.

7. A listing by title of SOPs

Inadequate. These procedures are not in either the Parish or State plans. Both plans indicate procedures are " to be developed".

This is an important element and must be provided to complete the plan evaluation.

8. A table of contents and cross references to NUREG-0654/FEMA-REP-1, Rev. 1

B. P. page i is a table of contents for the plan. The cross reference while not numbered as a part of the plan is included (needs corrections).

Attachment B

Consolidated and Condensed Exercise Evaluation Comments Relative to the Louisiana State Plan as Submitted to the State after Exercising the Grand Gulf Facility

OBSERVATIONS NOTED IN THE GRAND GULF NUCLEAR STATION EXERCISE

I. Emergency Operations Facilities and Resources

C.1.b. - What are the procedures to be used to request Federal resources? What Federal resources were requested during the exercise and by whom? Was the Federal response adequate and timely?

Scenario did not call for exercising this element.

C.1.c. - What procedures have been established to provide available State and local resources to support the Federal response? Were these resources needed during the exercise? Were they provided in a timely manner and were they adequate?

Scenario did not call for exercising this element.

F.1.b. - What provisions have been established for communications with contiguous state/local governments within the Emergency Planning Zone? Were these provisions effectively executed during the exercise? If there were problems, indicate what they were.

Initial warning and notification was not exercised consistent with the plan. LOEP has the capability for 24-hour warning and notification as well as LNED. Question that the dedicated telephone circuitry and the commercial telephone circuitry satisfies the requirement for dissimilar initial warning and notification from the licensee to the OPR. Unable to hear the ring of the dedicated land line circuit. OEP needs to establish SOPs for utilization of dedicated land line circuitry.

Rating: 3

F.1.c. - What were the provisons for communications with the Federal emergency response organizations? Were these provisions initiated and if so, what were the results?

Scenario did not call for exercising this element. However, LOEP did not notify FEMA Region 6 at the alert EAL.

Rating: 3

F.1.d. - What provisons were established for communications between the nuclear facility and the licensee's near-site EOF, State and local EOCs, and radiological monitoring teams? Were the systems/procedures effective to the overall emergency response as observed during the exercise?

Satisfactory

Rating: 4

H.3. - Where are the State and local emergency operating centers? If readily available, how much EOC working space is there? Is this adequate? What are the provisions for EOC security? Were the provisions initiated and what were the results? What are the provisions in the plan for EOC communications? Describe the EOC internal communications system as you observed it. Is it adequate? Are there provisions in the plan for the necessary display information and who is responsible for it? As you observed the EOC displays, were they adequate? Others needed?

Limited space at the local EOC. There was demonstrated need to keep the EOC participants better informed. Adequate local visuals, but not properly displayed and message board not kept current. State displays were adequate but not properly utilized. The state plan lacks specific procedures for internal communications. Recommend LNED and LOEP coordinate and provide for adequate displays for the governor's press room.

Rating: 4

J.10.a.- What are the provisions for maps showing evacuation routes, evacuation areas, preselected radiological sampling and monitoring points, relocation centers in host areas, and shelter areas? As observed, did the decision makers use the maps and did the maps appear to be adequate?

The radiological sampling and monitoring maps, relocation maps, and shelter area maps are not in the plan. Radiological sampling and monitoring map was not available at the local EOC. Shelter area map not at the local EOC. Maps need enlarging and vectored. The state EOC had adequate maps but they were not used to any great extent.

Rating: 3

J.10.b.- What are provisons for sector maps showing population distribution around the nuclear facility? Did the decision makers use the maps and did the maps appear to be adequate?

No deficiencies noted.

Rating: 3

II. Alerting and Notification of Officials and Staff

A.1.e. - In what manner has the organization to be evaluated provided for a 24-hour per day emergency response capability, including a 24-hour per day manning of the communications system? Did the provisions, when initiated, work well? Other comments?

The plan sufficiently addressed the 24-hour per day alert capability. Was not exercised, assumed to be sufficient.

A.4. - What are the provisions for continuous (24-hour) operations for a protracted period? Who is responsible for assuring continuity of resources? As observed, were the provisions adequate? Was there an observed capability for effective shift change over an extended period of time? Comments?

Capability not exercised.

C.2.a. - The plan indicates the Louisiana Nuclear Energy Division will dispatch a technical analysis representative to the licensee's near-site EOF. Was this done during the exercise?

Satisfactory.

Rating: 4

E.1. - What are the established procedures for notification of response organizations? If observed, how effective were these procedures, when implemented? How effective is the verification system for transmission and receipt of communications between State and local organizations?

Additional practice needed at the local level but implemented satisfactorily.

Rating: 4

E.2. - What are the procedures for alerting, notifying and mobilizing emergency response personnel? Were these procedures used and how effective did they appear to be?

There was one incorrect telephone listing, otherwise satisfactory.

Rating: 4

E.3. - Were the initial emergency messages sent by the licensee handled effectively? Did they contain the following information: (1) identification of the emergency action level (2) if a release is taking place, (3) identification of potential affected population and areas, and (4) whether protective measures may be necessary?

Received erroneous information from the utility. However, did not detract from state and local capability.

Rating: 3

F.1.a. - What are the provisions for an emergency response communications network with manning on a 24-hour basis? Was this notification capability and effective ability to activate the emergency response network demonstrated during the exercise? Was there a telephone link and an alternate means for notification available and what were they?

Satisfactorily demonstrated.

Rating: 3

F.1.e. - What procedures have been established for alerting or activating emergency personnel in the organization being evaluated? Were these procedures activated and how effective were they?

Alerting notification and activation of local personnel was demonstrated satisfactorily.

Rating: 4

F.2. - What is the provision for a coordinated communications link for fixed and mobile medical support facilities? Was the method used during the exercise and did it add to the overall emergency response capability?

Emergency personnel should use channel 2 on the radio frequency for normal operational messages.. Lack of voice communications between mobile medical and fixed medical - planned to be corrected in the future.

Rating: 3

H.4. - What are the procedures for timely activation and staffing of the emergency response centers? During the exercise, were the centers activated and staffed in a timely manner?

Procedures for activation and staffing of the EOC should be included in the plan.

Rating: 3

III. Emergency Operations Management

A.1.a. - Who are the organizations identified as a part of the overall response organization? Was each organization participating in the exercise?

No comment - each organization participated.

Rating: 3

A.1.b. - How is the organization's role in the concept of operations defined? Could the observer determine from observation the organization's role and its relationship to the total effort during the exercise?

All operations controlled through EOC effectively.

Rating: 3

A.1.d. - Who is the individual,by title, who is in charge of emergency response? During the exercise, did the designated official assume charge of emergency response?

The designated official assumed the leadership role satisfactorily.

Rating: 4

A.2.a. - What are the assigned functions and responsibilities assigned to the organization being evaluated? How well did the organizations carry out these functions and responsibilities?

All functions and responsibilities were carried out effectively.

Rating: 4

A.3. - What written agreements have been made for the organizational function being evaluated? Were these agreements implemented during the exercise?

Any written agreements which had been made and consummated were done effectively.

Rating: 4

C.1.a. - Who is the person, by title, authorized to request Federal assistance? Was such request for assistance observed during the exercise?

Not exercised.

C.4. - What nuclear and other facilities, organizations, or individuals have been identified which can be relied upon to provide emergency assistance to the organization being evaluated? Did the observer note any assistance being requested from any of these identified groups and were the responses adequate?

Assistance requested from several organizations and all responded in an effective manner.

Rating: 4

D.3. - Were State and local classification and emergency action levels used during the exercise consistent with that of the utility?

State and local EALs were consistent with that of the utility.

Rating: 4

D.4. - What procedures are in place that provide for emergency actions to be taken which are consistent with the emergency actions recommended by the nuclear facility licensee, taking into account off-site conditions? Were these procedures implemented and what were the results?

All procedures were implemented effectively with favorable results.

Rating: 4

IV. Public Alerting and Notification

E.5. - What system is to be used for dissemination to the public of information received from the licensee (includes EBS)? Was activation of this system observed and what were the results?

The EBS was activated satisfactorily.

Rating: 4

E.6. - What is the administrative and physical means planned for prompt notification to the public? Is the system in place? Was it used during the exercise and was it adequate to warn all the population requiring notification?

The system is in place and was tested. Questionable whether all people can be warned by the present system. The alert notification system will be tested at a later date. A rating will be given at that time.

E.7. - Are there messages in the plan intended for the public, giving appropriate instructions with regard to specific protective actions to be taken, etc. Were these messages used during the exercise? Did the messages provide all the information needed?

Was not exercised completely.

Rating: 3

J.10.c.- How does the plan provide for notification to all segments of the transient and resident population? Is this system adequate as demonstrated during the exercise?

All systems were not tested during the exercise. The ones that were, were satisfactory - the remainder will be tested at a later date.

Rating: 3

V. Public and Media Relations

G.1. - What is the method to be used for periodic dissemination of information to the public regarding how they will be notified and what their actions should be in an emergency? Was there any evidence during the exercise to indicate that this periodic dissemination was being made?

Method for periodic dissemination has been accomplished. However, there is a need for coordination between MP&L spokesperson and media at the local level in Louisiana.

Rating: 4

G.2. - What provisions have been made for a public information program for the permanent and transient population in the 10 mile EPZ? Was evidence of this observed during the exercise?

Provisions for information to permanent and transient population has been addressed and was observed to be sufficient.

Rating: 4

G.3.a. - Who are the points of contact and physical locations for use by news media during an emergency? Were there any problems finding the contact persons or the location of the PIOs?

Recommend that LNED and LOEP discuss and settle on one news media site at the state level taking into consideration the desires of the governor. Appropriate functions of each should be worked out. Also, needed in the plan is the procedure whereby news releases would be cleared with the utility media center.

Rating: 3

G.3.a. (con't) - If there was a joint media facility, what were the good and bad points?

The Mississippi side is responsible for the emergency news media center. However, the state of Louisiana did experience some difficulty in coordinating news responses with them.

No rating.

G.4.a. - Who is the designated media spokesperson for the organization being evaluated, or if that organization does not have a spokesperson, who is to speak for it?

Reference remarks under G.3.a.

Rating: 3

G.4.b. - What arrangements have been made for a timely exchange of information among designated spokespersons? Were these arrangements used during the exercise and were they adequate?

A lack of advance arrangements for inter-media exchange of information.

Rating: 3

G.4.c. - What are the coordinated arrangements planned for dealing with rumors? Were these arrangements initiated and were they effective?

Arrangements had been made for dealing with rumors but were not put into effect during the exercise.

VI. Accident Assessment

H.7. - What are the provisions for offsite radiological monitoring equipment in the vicinity of the nuclear facility? Were these provisions adequate?

Observed and were adequate.

Rating: 3

H.12. - Has each organization established a central point (preferably associated with the licensee's near-site EOF) for the receipt and analysis of all field monitoring data and coordination of sample media? Were there any problems in getting the data/sample media to the specified central point?

The requirement has been met.

Rating: 4

I.7. - Was the capability and resources for field monitoring, as described in the plan observed to be adequate during the exercise?

Adequate, but need additional training.

Rating: 3

I.8. - What are the provisions for methods, equipment, and expertise to make rapid assessments of the actual or potential radiological hazards through liquid or gaseous release pathways? During the exercise, were adequate assessments made? (The assessment should include the magnitude and location of the release as well as the method of activation, notification means, field team compositions, transportation, communications, monitoring equipment, and estimated deployment times).

Misinterpretation of technical data was believed to be by GGNS and not a fault of NED. Activation of field deployment teams was very satisfactory.

Rating: 4

I.9. - Does the organization being evaluated have the capability to detect the measure radio-iodine concentrations in the air within the plume exposure EPZ as low as 10'4Ci/cc under field conditions? Were any such measurements made during the exercise and did they appear to be accurate?

Not observed but sufficiently addressed in the plan.

I.10. - Does the State level organization have the means for relating measured parameters to dose rates for Key isotopes and gross radioactivity measurements? Are there detailed provisions described in separate procedures? Were these procedures implemented during the exercise and what were the results?

Good capability exists.

Rating: 4

I.11. - What are the arrangements to locate and track the airborne radioactive plume? Were these arrangements carried out during the exercise and were they workable?

Arrangements were satisfactory and demonstrated during the exercise.

Rating: 4

J.10.m.- At the State level does the plan provide bases for the choice of recommended protective actions from the plume exposure pathway during emergency conditions? During the exercise were the recommended protective actions made in accordance with the plan bases?

There was some confusion on interpretation of protective actions recommended by EPA PAGs (apparently confusion by GGNS).

Rating: 3

C.3. - What radiological laboratories were named in the plan, and what are their general capabilities and expected availability? Were requests made or simulated to these laboratories during the exercise? What were the results?

Requests were simulated for laboratory assistance during the exercise but were not needed.

Rating: 4

VII. Actions to Protect the Public

J.2. - Are there contingency plans for movement of onsite individual to offsite locations? Were these plans implemented during the exercise and what were the results?

Not applicable.

J.9. - How did the State and local organizations implement protective measures based on protective action guides (PAGs) and other related criteria? Was it consistent with the plan? Was this activity adequate for the protection of the public?

Protective measures consistent with PAGs.

Rating: 4

J.10.d.- What are the provisions for protecting those persons whose mobility may be impaired, confined at home or in institutions? Were these provisions exercised and what were the results?

Provisions for protecting persons with impairments, disabilities, etc., well addressed and simulated during exercise.

Rating: 5

J.10.g.- What are the procedures for implementing relocation of the populace? Were these procedures followed during the exercise and what were the results?

A need for more information to personnel responsible for shelters.

Rating: 3

J.10.h.- Where are the planned relocation centers? Were these centers activated during the exercise and what were the results?

Relocation centers were reactivated. Demonstrated need for additional training by shelter staff.

Rating: 4

J.10.k.- What are the potential impediments to use evacuation routes identified in the plan? What are the means of dealing with these potential impediments? Did the exercise include an impediment as identified and how was it handled?

None identified in the plan. However, it was noted there is a railroad track passing through St. Joseph which could be a potential impediment. The exercise did not include any impediments.

J.10.l.- What are the time estimates for evacuation as projected in the plan? Was evacuation simulated during the exercise? If there was a partial evacuation, describe. What potential problems might be encountered as seen from the exercise?

Misdirection of traffic at roadblock 6 (simulated evacuation).

Rating: 3

J.11. - What protective measures are specified in the plan for use in the ingestion pathway, including the methods for protecting the public from consumption of contaminated food stuffs? Was there any exercise of the ingestion pathway protective measures and what were the results?

The scenario did not call for implementation of 50-mile ingested pathway zone precautionary measures.

J.12. - What are the provisions for registration and monitoring of evacuees at relocation centers? Were these functions observed during the exercise? Were they handled in an effective manner, or were there problems?

More information to registrants at relocation centers.

Rating: 4

VIII. Health, Medical, and Exposure Control Measures

J.10.e.- What are the provisions in the plan for the use of radio-protective drugs, particularly for emergency workers and institutionalized persons in the plume EPZ, including quantities, storage, and method of distribution? Was the use of such drugs simulated during the exercise? Comments?

The use of radio-protective drugs not exercised.

J.10.f.- What is the method to be used by the State Health Department in decisions to administer KI to the general population during an emergency and the predetermined conditions under which such drugs may be used by offsite emergency workers?

Not implemented.

J.10.j.- What are the provisions for control of access to evacuated areas and organizational responsibilities for such control? Were these provisions demonstrated during the exercise? Simulated? Comments?

Provisions for control of access to evacuated areas were adequate.

Rating: 4

K.3.a. - What are the provisions for a 24-hour-per-day capability to determine the doses received by emergency personnel? Were these provisions implemented during the exercise and what were the results?

Provisions were covered in the plan. However, only self-reading devices were observed.

K.3.b. - What are the provisions for frequent emergency worker dosimeter readings and how are dosage records to be kept? Were these provisions carried out during the exercise and how successfully were they carried out?

Sufficiently addressed in the plan - minimal observation during exercise.

K.4. - What is the decision chain established for authorizing emergency workers to incur exposures in excess of EPAs PAGs? Was this action a part of the exercise? Comment?

Scenario did not call for exercising this element.

K.5.a. - What are the action levels specified for determination of the need for decontamination? As used during the exercise, were the action levels adequate?

Not exercised.

K.5.b. - What means is provided for radiological decontamination of emergency personnel wounds, supplies, instruments and equipment, and waste disposal? Was decontamination required during the exercise? How effective?

Not exercised.

L.1. - What arrangements have been made for local and backup hospital and medical services with capability for evaluation/treatment of contaminated individuals? How did the implementation of the arrangements work out? What facilities were used and how well did the medical personnel perform? Were the facilities adequate?

Question the need for medical decontamination capability. Was exercised and additional training needed.

Rating: 3

L.3. - Has the State developed a list of the location of public, private, and military hospitals and other emergency medical services facilities considered capable of providing medical support for any contaminated injured individual? Were any of these facilities called on for assistance during the exercise?

Failed to see the need for this capability for this facility and was not exercised.

L.4. - What arrangements have been made for the transportation of accident victims to medical support facilities? During the exercise, were there any accident victims? If so, how effective was the pre-arranged transportation?

Sufficiently addressed.

Rating: 3

M.4. - What method has the State outlined for periodic estimation of total population exposure? Was this in evidence during the exercise? Comments?

Vaguely implied in the plan and was not observed during exercise.

IX. Recovery and Reentry Operations

M.1. - What generally is planned to accomplish recovery and reentry functions? Was recovery and reentry exercised? Describe what took place? Are the provisions adequate?

Exercised minimally.

M.3. - What are the State procedures for informing response organizations that reentry has been initiated? Exercised? Adequate?

Exercised minimally.

X. Relevance of the Exercise Experience

N.1.a. - Did the exercise test the integrated capability of the various plans and organizations? Did it test a major portion of the basic elements of the plans? Comments?

A need for additional training and education at the local and state level. Public warning devices need attention.

Rating: 3

N.1.b. - Did the observer feel the scenario was adequate to verify the capability to respond to a radiological accident? Comments?

The scenario was limited. The ability of the state and local emergency organizations to respond to a radiological accident is adequate.

Rating: 3

Did the exercise appear to benefit the participants? Explain.

Brought out items that needed further clarification in the plan and some weak areas that could be enhanced by further training and education.

Attachment C

Consolidated and Condensed Plan Review/Evaluation Comments

Relative to Enclosures 1 and 2 of Attachment 1

(Waterford 3 Site-Specific Plans for St. Charles and St. John the Baptist Parishes)

WATERFORD III
CONSOLIDATED RAC COMMENTS

Enclosures 1 and 2 to Attachment 1

ELEMENT 0654	COMMENTS
A. ASSIGNMENT OF RESPONSIBILITY (Organization Control)	
A.1.a.	Both parish plans define responses and agencies including parish support on p. 66, encl. 1, and p. 10, encl. 2.
A.1.b.	Parish concept of operations defined p. 74, encl. 1, and pp. 236-239, encl. 2.
A.1.c.	Emergency preparedness organization charts p. 80, encl. 1, and p.241, encl. 2.
A.1.d.	Respective parish presidents have responsibility for overall safety. Emergency Preparedness Coordinators coordinate emergency operations, p.73, encl. 1, and p. 230, encl. 2.
A.1.e.	St. Charles and St. Johns parish EOCs are capable of 24 hour per day operations over extended periods of time, p. 73, encl. 1, and p. 235, encl. 2. St. Charles parish maintains communications link with state, utility, and St. Johns parish, p. 121, encl. 1.
A.2.a.	Emergency functions and responsibilities chart found on p. 79, encl. 1, and p.124, encl. 2. St. Charles and St. Johns parish accident assessment is not specifically addressed in parish plan but is carried as a state function in the state plan. USDA recommends assignment of responsibility to USDA emergency Boards for the purpose of providing coordination and assistance for damage assessment activities. The USDA SEB in LA is Mr. Willy F. Cooper. P. 79, encl.1, and p. 241,encl. 2 - primary responsibility for highway maintenance incorrectly assigned to health and medical.
A.2.b.	P. 66, encl. 1, p. 228, encl. 2 - addressed adequately.
A.3.	Indication is that parish will enter into appropriate agreements with applicable organizations, p. 217, encl. 1, and p. 375, encl. 2. Letters of agreement are not included.
A.4.	P. 73, encl. 1., and p. 235, encl. 2.
B. ONSITE EMERGENCY ORGANIZATION	
B.2.	No cross reference. Check licensee facility plan for identification of emergency coordinator.

ELEMENT 0654	COMMENTS
C. EMERGENCY RESPONSE SUPPORT AND RESOURCES	
C.1.c.	State responsibility.
C.2.a	State responsibility.
C.3.	State responsibilty.
C.4.	Support and resources noted on page 10 of Attachment 1, p. 217, encl. 1, and p. 375, encl. 2. Need letters of agreement or understanding from non-governmental organizations being relied upon to provide resources.
D. EMERGENCY CLASSIFICATION SYSTEM	
D.3.	Chapter 4 of the general plan sufficiently addresses emergency level guidelines, p. 36.
D.4.	Actions at various EALs are outlined on pp. 74-77, encl. 1, and pp. 236-239, encl. 2.
E. NOTIFICATION METHODS AND PROCEDURES	
E.1.	Notification consistent with emergency classification scheme- p. 117, encl. 1, and p. 278, encl. 2. The chapters provide the concept of operations but not procedure. It does indicate that operational hotline procedures are to be developed. USDA chair person should be notified.
E.2.	P. 119, encl. 1, and p. 280, encl. 2. Operational hotline procedures not developed.
E.3.	Attachment 1, pp. 13-18. Not cross-referenced. Not indicated state will call in accordance with revised 0654.
E.5.	EBS use and messages addressed. Pp. 130-134, encl. 1, and pp. 280-315, encl. 2.
E.6.	Time and population coverage found on p. 133, encl. 1, and p. 300, encl. 2.
E.7.	Messages to the public included, pp. 135-145, encl. 1, and pp. 302-312, encl. 2. Reference evacuation message no. 3 - question advisability of having farmers call USDA county agent due to line overload and limited personnel.
F. EMERGENCY COMMUNICATIONS	
F.1.a.	Twenty-four hour per day communications capability is assumed on p. 118, encl. 1. and p. 278, encl. 2. Operations concept described pp. 122 and 123, encl. 1, and p. 283, encl. 2.

ELEMENT 0654	COMMENTS
F.1.b.	Communications with St. Johns parish provided p. 122, encl. 1 and with St. Charles parish, p. 283, encl 2.
F.1.c.	Communications with Federal emergency response defined p. 123, encl. 1, and p. 284, encl. 2.
F.1.d.	Communications with facility and local government addressed p. 123, encl. 1, and p. 284, encl. 2.
F.1.e.	Alerting of emergency response personnel, p. 118, encl. 1, and p. 243, encl. 2. and alerting of EOC personnel, p. 81, encl. 1, and p. 243, encl. 2.
F.2.	Coordinated EMS communications link, p. 198, encl. 1, and p. 357, encl. 2.
F.3.	Periodical testing of communications system addressed, p. 124, encl. 1, and p. 285, encl. 2.
G. PUBLIC EDUCATION AND INFORMATION	
G.1.	Public education and information addressed, p. 25, attachment 1.
G.2.	Information programs for transients and residents provided for, p. 25, attachment 1 (to be developed).
G.3.a.	Designated spokesperson found on p. 25, attachment 1. Not specific in locating media center.
G.4.a.	Spokesperson designated, p. 25, attachment 1.
G.4.b.	Coordination of news releases, p. 25, attachment 1.
G.4.c.	Rumor control center to be established, p. 25, attachment 1.
G.5.	Programs for informaing news media addressed, p. 26., attachment 1.
H. EMERGENCY FACILITY AND EQUIPMENT	
H.3.	Local EOC established St. Charles parish, p. 73, encl. 1, and St. Johns parish, p. 235, encl. 2.
H.4.	Activation and staffing of St. Charles and St. Johns parish EOCs not addressed. Implementing procedures to be developed, p. 116, encl. 1, and p. 277, encl. 2.
H.7.	Equipment for monitor/survey listed, p. 221, encl. 1, and p. 378, encl. 2.; but quantity and storage location not identified. Resource management officer for suppling equipment not identified in enclosure 1 or enclosure 2.

ELEMENT 0654	COMMENTS
H.10.	Periodic calibration of instruments not addressed.
H.11.	P. 221, encl. 1, and p. 378, encl. 2. has form for resources inventory but inventory has not been tablulated on form. Kit contents, location and custodian not identified.
H.12.	Satisfactorily covered in state plan, pp. 6-12.
I. ACCIDENT ASSESSMENT	
I.7.	State assumes responsibility - see state plan, pp. 6-22.
I.8.	State assumes responsibility - see state plan, pp. 6-22.
I.9.	State responsibility.
I.10.	State responsibility.
I.11.	State responsibility.
J. PROTECTIVE RESPONSE	
J.2.	Not addressed in state and/or local plan.
J.9.	Parish plans consistent with state plan, p. 155, encl. 1, and p.320, encl. 2. Need capability for implementing protective measures (ie., respiratory protective measures, protective clothing, and drugs).
J.10.a.	Tab 5, Chapter 4, p. 177, encl. 1, and tab 5, chapter 4, p. 342, encl. 2 includes maps. However, maps and instructions to public should be more definitive than those in plan, did not find maps designating monitoring or sampling points.
J.10.b.	Pp. 20-22, attachment 1, show population by evacuation areas (16, 22 1/2° sectors) but areas are not consistent with evacuation area maps found in Tab 5. Suggest evacuation area maps be consistent with population distribution maps.
J.10.c.	Encl. 1, p. 132, and encl. 2, p. 290, indicates a combination of fixed sirens and alerting teams will accomplish alert notification.
J.10.d.	Pp. 67, 95, and 183, encl. 1., and pp. 229, 264, and 345, encl. 2. address capability for protecting handicapped or institutionalized persons.

ELEMENT 0654	COMMENTS
J.10.e.	State has responsibility for administration of radio-protective drugs and is sufficiently addressed in state plan.
J.10.f.	State plan and attachment 1, p. 45, encl. 1. address use of KI for emergency workers but not for general public (assume general public not to be considered for KI administration).
J.10.g.	Attachment 1, p. 9 generically addresses means of evacuation. Inserts to Tab 7, encl. 1., should be completed.
J.10.h.	Pp. 169-176, encl. 1. and pp. 332-339, encl. 2. addresses reception centers and shelters.
J.10.i.	P.162, encl. 1, and p. 328, encl. 2., references evacuation team study completed. Assumption made study included route capacities under emergency conditions.
J.10.j.	P. 83, encl. 1 and p. 246, encl. 2., assigns responsibility for access control to parish sheriff.
J.10.k.	P. 111, encl. 1, and p. 272, encl. 2., assigns responsibility for removal of impediments to Public Works Officer. However, other than stalled autos impediments have not been identified as required by the criteria.
J.10.l.	P. 162, encl. 1, and p. 328, encl. 2, references team studies. Assumption is made study included time estimates by sector for evacuation within PEP.
J.10.m.	Adequately covered in state plan.
J.11.	State responsibility and sufficiently covered in state plan.
J.12.	P. 61, encl. 1, and p. 327, encl. 2. indicates evacuees will be surveyed and registered but does not describe means for doing so nor lists supplies or storage locations of instruments for surveying.
K. RADIOLOGICAL EXPOSURE CONTROL	
K.3.a.	Responsibility assumed by state, chapter 9, p. 2.
K.3.b.	State assumes all responsibility for dose records, see state plan.

ELEMENT 0654	COMMENTS
K.4.	P. 47, attachment 1, p. 157, encl. 1., and p. 323, encl. 2., established decision making chain for excess exposures to emergency workers. Parish president makes authorization, should be done after consultation with trained medical doctor or health physicist. Plan should indicate ALARA principles still applicable and levels are upper limits not to be exceeded, section V.D.2.a. and b. should be separated from section V.D.2. and combined with section V.D.3. Limit of 75 Rem should be defined as absolute upper limit and reason for not giving upper limit thyroid dose explained.
K.5.a.	Chapter 5, p. 48 of attachment 1 gives decontamination levels of .1 MR/hr for general public. Assume same level would apply for decontamination of emergency workers and/or inanimate objects.
K.5.b.	State responsibility and generally covered sufficiently. No special arrangements for necessary supplies.

L. MEDICAL AND PUBLIC HEALTH SUPPORT

L.1.	P. 193, encl. 1, and p. 354, encl 2.. Plan does not indicate training has been accomplished, equipment/supplies available, or written hospital plans for handling contaminated persons.
L.3.	State responsibility. P.204, encl. 1., and p.362, encl. 2. identify medical facilities for handling contaminated persons.
L.4.	Pp. 196 & 197, encl. 1, and p. 354, encl. 2. provide for transportation and pp. 207 and 208, encl. 1, and p. 365, encl. 2. list ambulance services and vehicles available.

M. RECOVERY AND REENTRY PLANNING AND POSTACCIDENT OPERATIONS

M.1.	P. 210, encl. 1, and p. 368, encl 2., notes considerations for recovery/reentry recommendations. Meaning of ambient levels of radioactivity, section II.B.1., is not clearly defined.
M.3.	State responsibility.
M.4.	State responsibility.

N. EXERCISES AND DRILLS

N.1.a.	Attachment 1, p. 32, notes criteria.

ELEMENT 0654	COMMENTS
N.1.b.	Attachment 1, pp.32-33, meets criteria minimally, does not specifically address scenario variation annually or exercises under variable weather conditions or at varying 24-hour time intervals once every six years.
N.2.a.	Attachment 1, p. 34, addresses communications drills.
N.2.c.	Attachment 1, p. 34, addressed adequately.
N.2.d.	Attachment 1, p. 34, addressed adequately.
N.2.e.	State responsibility.
N.3.a.b.c.d.e.f.	Attachment 1, p. 35, addressed adequately.
N.4.	Attachment 1, p. 33,addressed adequately.
N.5.	Attachment 1, p. 33, addressed adequately.

O. RADIOLOGICAL EMERGENCY RESPONSE TRAINING

O.1.	Attachment 1, p. 27, addressed adequately.
O.1.b.	Attachment 1, p. 27 and 28, generally, meets criteria, does not specifically reference training of emergency personnel having mutual aid agreement with emergency organizations responding on site (is implied in para. III.A.1.).
O.4.a.b.c.d. f.g.h.j.	Attachment 1, pp. 28-29 addressed adequately.

P. RESPONSIBILITY FOR THE PLANNING EFFORT

P.1.	Attachment 1, p. 30 indicates people may attend planning course.
P.2.	Pp. 68 and 73, encl. 1, and p. 230, encl. 2. Individual identified for emergency operations. This person assumed to have responsibility for emergency planning.
P.3.	P. 68, encl. 1, and p. 230, encl. 2., indicates that the Emergency Preparedness Coordinator is responsible for planned development and maintenance.
P.4.	Attachment 1, pp. 10 and 11, indicates parish plan to updated annually.

ELEMENT 0654	COMMENTS
P.5.	Attachment 1, p. 11, addresses authority for and distribution of plans. The plan does not indicate revised pages to be dated and marked to indicate changes.
P.6.	P. 66, encl. 1, and p. 228, encl. 2, cites local authority for plans and state plan, pp. 2-4, cites state authority for plans. The state plan contains some supporting plans and their sites, but there is no detail listing.
P.7.	P. 116, encl. 1. and p. 277, encl. 2 indicate implementing procedures to be developed.
P.8.	Attachment 1, pp. i and ii, is a Table of Contents for attachment 1, pp. 63-65, and pp. 63-65, encl. 1 and pp. 225-226, encl.2 are the Table of Contents.
P.10.	Attachment 1, p. 11, indicates phone numbers will be verified each calendar quarter.

February 19, 1982 SECY-82-77

For: The Commissioners

From: William J. Dircks
Executive Director for Operations

Subject: USE OF POTASSIUM IODIDE FOR THYROID BLOCKING

Purpose: To respond to the March 26, 1981 memorandum from the Secretary of the Commission (S80-257/257A) and to inform the Commission of the progress of the staff in developing criteria and strategies for implementing the use of potassium iodide (KI) to block the thyroid gland in the event of an accidental release of radioiodine from a nuclear power plant. It also is provided to assist the Commission in deciding whether to recommend the procurement and distribution of KI for thyroid blocking use by the general public.

Discussion: In a May 4, 1981 memorandum, the EDO informed the Commissioners of the progress of the staff and other Federal Agencies regarding the use of KI. Since that time, the following actions and continued progress have occurred:

- The Food and Drug Administration (FDA) published its proposed guidance covering the projected radiation dose levels to the thyroid (10 to 20 rads) at which the use of KI blocking should be instituted; the information on establishing provisions for medical assistance to individuals who may experience side affects; and further identification of individuals whose medical condition may cause them to experience side effects. This proposed guidance was published in the Federal Register on June 5, 1981, requesting comments (46 FR 30199). A copy of this Federal Register notice is provided as Enclosure A. The comment period on the guidance expired on October 1, 1981; however, the FDA has not published its final guidance to date because of a number of adverse comments from the medical community on the low projected dose level recommended for instituting thyroid blocking. An example of these comments is the statement

Contact:
B. Grimes, IE, Ext. 24614
S. Ramos, IE, Ext. 29602

from the American Thyroid Association on this matter, a copy is provided as Enclosure B. In addition, staff studies conducted in FDA and NRC indicate that the fatal carcinogenic risk is greater from the whole body dose than from the thyroid dose using the airborne plume from various accidents in WASH-1400, "Reactor Safety Study". These studies are based on the analysis presented in NUREG/CR-1433, "Radiation Protection: An Analysis of Thyroid Blocking", and indicate that for a ratio of 20 to 1 thyroid to whole body dose, the resulting risk of cancer death is three times greater for the whole body dose. This difference in risk is a result of the low percentage of deaths for thyroid cancer as compared to a higher percentage of deaths for many other types of cancer and the fact that the risk of a carcinoma is lower per unit thyroid dose as compared to the carcinogenic risk per unit whole body dose. This makes the value of administering KI to the general public questionable. Also, it may give the general public a false belief that they are protected from the radiation effects of an airborne release when, in fact, the critical dose is to the whole body which they may feel they do not need to protect. In addition, the analysis in NUREG/CR-1433 indicates that the national distribution of KI for thyroidial blocking of the general public would be questionable from a cost/benefit standpoint even assuming the administration would be 100% effective.

- The Bureau of Radiological Health of FDA has published HHS Publication FDA 81-8158, "Background Material for the Development of the Food and Drug Administration's Recommendation on Thyroid Blocking with Potassium Iodide." This publication covers the mechanism of action, efficacy, safety and availability of KI as well as examples of potential accidents and pathways of exposure, previous recommendations for protective action, populations of concern, and problems with procurement, storage and distribution. A copy of this publication is provided as Enclosure C.

- The NRC staff is continuing its studies on the abundance and the chemical and physical forms that radioiodine might take in a release resulting from various nuclear power plant accident scenarios. As discussed in NUREG-772, "Technical Bases For Estimating Fission Product Behavior During LWR Accidents", the most probable form for the radioiodine release during a light water reactor accident may be cesium iodide, a non-volatile particulate; however, a variety of chemical forms are possible.

- The NRC staff is continuing its studies on the use of expedient measures (e.g., breathing through a bath towel or using a dust respirator) rather than thyroid blocking to protect the general public. A paper on this subject was presented by J. A. Martin, RES, at the annual Health Physics Society Meeting on June 23, 1981, titled: "On the Efficacy of Ad Hoc Respiratory Protection During A Radiological Emergency." Enclosure D is a copy of the abstract of this paper.

- The Federal Emergency Management Agency (FEMA) is continuing its in-house study of developing a system for the storage and distribution of KI to the general population within 10 miles of a nuclear power plant in the event of an accident. As a part of this study, FEMA has negotiated an agreement with the Conference of Radiation Control Program Directors (CRCPD) to have the CRCPD Task Force #12 propose a system of storage and distribution. This CRCPD group met for the first time on August 25, 1981, and submitted draft recommendations for distribution procedures to FEMA in December 1, 1981. The proposal is being reworked internally by the FEMA staff. In addition, we understand that FEMA is considering plans to purchase KI and arrange for regional stockpiles at Veterans Administration Hospitals. FEMA would then leave the decision on the distribution of this KI to State and local authorities.

- On November 4, 1981, the Tennessee Department of Public Health began implementation of the distribution of KI to all the households within a radius of five miles of the Sequoyah Nuclear Power Plant. This material was purchased for the State of Tennessee by the TVA.

- A "Workshop on Technical Factors Relating Impacts from Reactor Releases to Emergency Planning" sponsored by EPRI/NSAC was held on January 12 and 13, 1982. Most of the day on January 13 was devoted to discussions regarding the distribution and administration of KI for thyroidial blocking. Speakers from Federal and State health agencies, foreign agencies, the National Laboratories, university medical facilities and licensees gave their differing opinions. The general consensus of opinion (excluding State health authorities) was to question the validity of distributing KI for use by the general public.

It is the opinion of the staff, and of several medical advisers consulted separately, that:

1. The utility of distributing KI to the general public for thyroid blocking in case of a reactor accident is very questionable.

2. There are potential side effects from KI which some medical authorities believe warrant further investigation.

3. The decision should not be turned over to individual states without some guidance since they are in a poorer position to make such decisions than is the federal government.

In view of these conclusions, I propose to have staff work with other federal agencies and medical consultants to assist in developing a policy position on KI which could be provided to the states, subject to Commission review and consideration.

Unless otherwise directed by the Commission, staff testimony before Congressman Markey on March 5 will reflect the above conclusions and plan of action.

William J. Dircks
Executive Director
for Operations

Enclosures:
A. Federal Register Notice (46 FR 30199)
B. American Thyroid Association Statement
C. HHS Publication FDA 81-8158
D. Abstract on The Efficacy of Ad Hoc Respiratory Protection During a Radiological Emergency

DISTRIBUTION:
Commissioners
Commission Staff Offices
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Secretariat

[Docket No. 81N-0087]

Potassium Iodide as a Thyroid-Blocking Agent in a Radiation Emergency; Draft Recommendations on Use

AGENCY: Food and Drug Administration.

ACTION: Notice of availability.

SUMMARY: The Food and Drug Administration (FDA) announces the availability of draft recommendations about administering potassium iodide to the general public in a radiation emergency. The draft recommendations prepared by FDS's Bureau of Radiological Health and Bureau of Drugs are being made available for public comment to provide FDA with views to be considered for incorporation into any final recommendations that the agency may develop on this use of potassium iodide.

DATE: Comments by September 3, 1981.

ADDRESS: Written comments to the Dockets Management Branch (formerly the Hearing Clerk's office) (HFA-305), Food and Drug Administration, Rm. 4-62, 5600 Fishers Lane, Rockville, MD 20857. The draft recommendations are on display in the Dockets Management Branch, and copies may be obtained from Bernard Shleien at the address below.

FOR FURTHER INFORMATION CONTACT: Bernard Shleien, Bureau of Radiological Health (HFX-4), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, 301-443-6220, or Edwin V. Dutra, Jr., Bureau of Drugs (HFD-30), Food and Drug Administration, 5600 Fisher Lane, Rockville, MD 20857, 301-443-6490.

SUPPLEMENTARY INFORMATION: In the Federal Register of October 22, 1980 (45 FR 69904), the Federal emergency Management Agency (FEMA) outlined the responsibilities of several Federal agencies concerning emergency response planning guidance that the agencies should provide to State and local authorities. The October 22, 1980, notice updated a prior notice on the same subject that the General Services Administration (GSA) published in the Federal Register of December 24, 1975 (40 FR 59494). (GSA responsibility for emergency management was transferred by Executive Order 12148 to FEMA.)

The Department of Health and Human Services (HHS) responsibilities for emergency-response planning include assisting State and local authorities in developing plans for perventing adverse effects from exposure to radiation in the event that radioactivity is released into the environment. These plans are to include the prophylactic use of drugs that would reduce the radiation dose to specific organs from the sudden release into the environment of large quantities of radioactivity that might include several radioactive isotopes of iodine.

As one step in meeting HHS responsibilities, FDA issued a notice in the Federal Register of December 15, 1978 (43 FR 58798), announcing FDA's conclusion that potassium iodide is safe and effective for use as a thyroid-blocking agent in a radiation emergency under certain specified conditions of

use. The notice also announced, however, that potassium iodide has not been used to such an extent or for such [illegible] d of time under radiation [illegible]ncy conditions to permit the co[illegible]usion that the drug may be marketed without an approved new drug application; thus, in the interest of the public safety, the notice encouraged interested persons to submit to the agency new drug applications for potassium iodide in oral dosage forms for use as a thyroid-blocking agent. Now, potassium iodide as a thyroid-blocking agent for use in a radiation emergency is available commercially in both a tablet and a solution form (see 45 FR 11912; February 22, 1980).

FDA is now making available draft recommendations on the use of potassium iodide to provide information and guidance to State and local public health agencies and other persons responsible for formulating response plans for use in developing emergency plans and for use in the emergency response to radiation accidents.

The draft recommendations prepared by the agency's Bureau of Radiological Health and Bureau of Drugs discuss projected levels of radiation following a radiation accident that would warrant the [illegible]e of potassium iodide as a thyroid-[illegible]g agent for the general public. It [illegible]ends establishing an integrated system for disseminating information about the proper use of potassium iodide and for reporting suspected or actual occurrences of side effects from the use of the drug. The draft also recommends that authorities make the general public aware of where and how they may contact medical personnel for assistance in a radiation emergency.

One purpose of the draft recommendations is to facilitate a national consensus on the use of potassium iodide during a radiation emergency. They also may serve as provisional guidance to State and local officials and the nuclear industry, should a serious nuclear accident occur. These draft recommendations are not free of controversy. The agency specifically invites comment on the following controversial issues:

(1) Whether the benefits to be derived from protection of the thyroid gland from projected doses of 10–20 rads outweight the potential for side effects from potassium iodide administered at the recommended dose regimen, and if not, at what projected radiation dose the benefit/risk decision is favorable?

[illegible]hether the probability of a [illegible]power plant incident resulting in re[illegible]s of radiodine at levels high enough to justify the administration of potassium iodide to the public is so small that the recommenations are unnecessary?

Further, the is[illegible]uance of such guidance on potassium iodide use must be put into the entire context of radiation emergency planning. The use of potassium iodide in a radiation emergency is not a panacea, and needs to be balanced against the cost and effectiveness of other protective measures such as seeking shelter, evacuation, or respiratory protection. Persons are invited to comment in addition on these issues as well.

Interested persons may, on or before September 3, 1981, submit to the Dockets Management Branch (HFA–305), Food and Drug Administration, Rm. 4–62, 5600 Fishers Lane, Rockville, MD 20857, written comments regarding these draft recommendations. Comments received after September 3, 1981, may be considered, depending on the stage of development of any final recommendations. Four copies of any comments should be submitted, except that individuals may submit one copy. Comments should be identified with the docket number found in brackets in the heading of this document. The draft recommendations and receiving comments may be seen in the Dockets Management Branch between 9 a.m. and 4 p.m., Monday through Friday.

Dated: May 27, 1981.

Arthur Hull Hayes, Jr.,

Commissioner of Food and Drugs.

[FR Doc. 81-16586 Filed 6-4-81; 8:45 am]

BILLING CODE 4110-03-M

Enclosure B

1980 1981

THE AMERICAN THYROID ASSOCIATION

GRAVES
1796 - 1853

INCORPORATED
FOUNDED 1923

October 5, 1981

Gentlemen:

The Environmental Hazards Committee of the American Thyroid Association has reviewed the issue of the use of iodine as a blocking agent in the event of a nuclear reactor accident. A statement prepared by this Committee was presented to the Membership of the American Thyroid Association at its recent meeting in Minneapolis where it was approved unanimously.

A copy of the statement is attached for your information.

Sincerely yours,

David V. Becker, MD
Chairman, Environmental Hazards Committee

fr

Enclosure

The Nuclear Regulatory Agency
1710 H Street, N.W.
Washington, DC 20014

THE USE OF IODINE AS A THYROIDAL BLOCKING AGENT IN THE EVENT OF A REACTOR ACCIDENT

THE AMERICAN THYROID ASSOCIATION

The membership of the American Thyroid Association includes persons with special competence pertinent to the issues both of radiation hazards from radioactive iodine and the possible side effects of protecting against those hazards with stable iodine. The Association believes it has the expertise and responsibility to express an advisory opinion on these questions to various governmental and other agencies concerned with radiation protection decisions.

Induction of Thyroid Neoplasms by Radiation

At some level of exposure, radioactive iodine is tumorigenic for the human thyroid gland, and at higher levels it is ablative. It should be noted that radiation induced thyroid neoplasms are usually benign or well differentiated carcinomas with a good prognosis. The precise level of radioiodine incorporation into the thyroid that is tumorigenic is unknown because most of the available low dose (non-ablative) data derives from X-ray studies (1) and relatively little from radioiodine studies (2). There is growing evidence that a rad of ^{131}I is substantially less tumorigenic than a rad of X-ray (3, 1, 4). Estimates of the relative tumorigenic potential of ^{131}I as compared to X-ray vary from 1/5 (5, 6) to 1/50-1/70 (1). This ratio may be influenced by the relative contribution to the total radiation dose of harder (short-lived) isotopes of iodine in subjects exposed to fallout as well as by dose rate and distribution differences between these modes of irradiation. Evidence from subjects exposed to relatively large amounts of diagnostic ^{131}I in Sweden and carefully followed (7) suggested no increase in thyroid tumor incidence in populations exposed to about 100 rads (adults) or 159 rads (persons under 20 years of age). For these reasons, projected thyroidal doses from radioiodine as high as 500 rads have recently been proposed (8) as a realistic threshold for the institution of blocking countermeasures in the event of a reactor accident releasing radioiodines into the environment.

Pharmacologic Blockade of the Thyroid with Iodine

Numerous studies have considered various protective measures against environmental contamination with radioiodine. Although various other agents might be useful, all analysts come to the conclusion that thyroid blockade by iodides is the most effective

treated pregnancies although the dose and duration are probably greater than that considered here. On the other hand, fetal thyroids are quite radiosensitive so they should be protected via the mother.

c) Very High Doses - (more than 1000 mg/day) Thyroiditis - probably not worrisome in a protective program.

II. Extrathyroidal

a) Low Doses - Ioderma; rare but incidence unknown
- Periarteritis nodosa-like syndromes
- Hypocomplementemic vasculitis - rare but serious (17)
- Dermatitis herpetiformis - (18)
- Allergies - edema, nasal polyps

b) High Doses - Sialadenitis (iodide mumps). Perhaps the most common adverse reaction; occurs in parotid and submaxillary glands; easily reversed by iodide withdrawal.
- Iodide fever

c) Very High Doses - (more than 1000 mg/day) - gastric disturbance; not expected with protective doses.

If general distribution of iodine is contemplated, carefully prepared public information materials should be distributed and individuals potentially at risk for iodide side effects (and previously known iodide sensitivities) should be identified and alerted.

Conclusions and Recommendation

What are needed for the development of an appropriate strategy for proper protection against radioiodine contamination are risk-benefit (risk ratio of radioiodine hazards to stable iodine hazards) and cost-benefit evaluations, but adequate data are not now available for either the numerator or the denominator.

The American Thyroid Association recommends more vigorous attempts be made to obtain such essential data through funding by the appropriate governmental agencies such as

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Enclosure C

radiological health

Background Material for the Development of the Food and Drug Administration's Recommendations on Thyroid-Blocking with Potassium Iodide

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Food and Drug Administration

BRH PUBLICATIONS

Publications of the Bureau of Radiological Health (BRH) are available as paper copies from either the U.S. Government Printing Office (GPO) or the National Technical Information Service as indicated by the GPO or PB prefix, respectively, on the ordering number. Publications are also available in microfiche from NTIS at $3.50 per copy. To receive all BRH reports in microfiche, at $0.85 each, you may establish a deposit account with NTIS and request automatic distribution of "FDA/HFX" reports under the "Selected Research in Microfiche" program. Publications without GPO or PB number are available only from BRH, without charge.

Addresses for ordering are: Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402 ($1.00 minimum order); National Technical Information Service, Springfield, VA 22161 (outside North America, prices are double those listed); and Bureau of Radiological Health, Technical Information Staff (HFX-28), 5600 Fishers Lane, Rockville, MD 20857. All prices are subject to change.

FDA 79-8082 Some Considerations of Hazards in the Use of Lasers for Artistic Displays (GPO 017-015-00156-3, $1.10) (PB 294 513/AS, mf only).

FDA 79-8083 National Conference on Referral Criteria for X-Ray Examinations (GPO 017-012-00279-0, $3.75) (PB 296 173/AS, mf only).

FDA 79-8086 Evaluation of Commercially Available Laser Protective Eyewear (PB 80-103039, $11.00).

FDA 79-8087 X rays, Pregnancy and You...(brochure).

FDA 79-8088 X Rays: Get the Picture on Protection (brochure).

FDA 79-8088S Sepa Como Protegerse de los Rayos Equis (brochure).

FDA 79-8090 Source Book of Educational Materials for Radiation Therapy (GPO 017-015-00159-8, $4.50) (PB 299 415/AS, mf only).

FDA 79-8094 Quality Assurance for Radiographic X-Ray Units and Associated Equipment (PB 80-101 405, $11.00).

FDA 79-8097 Analysis of Retakes: Understanding, Managing, and Using an Analysis of Retakes Program for Quality Assurance (PB 80-102445, $6.50).

FDA 79-8098 Bureau of Radiological Health Index to Selected Acoustic and Related References (PB 80-120967, $27.50).

FDA 80-8024 FDA X-Ray Record Card (card).

FDA 80-8034 Report of State and Local Radiological Health Programs, Fiscal Year 1978 (PB 80-130867, $6.50).

FDA 80-8035 Regulations for the Administration and Enforcement of The Radiation Control for Health and Safety Act of 1968 (July 1980) (supersedes FDA 79-8035) (GPO 017-015-00173-3, $3.75).

FDA 80-8057 Nationwide Evaluation of X-Ray Trends: Dental X-Ray Data (brochure) (supersedes FDA 78-8057).

FDA 80-8092 Biological Bases for and Other Aspects of a Performance Standard for Laser Products (PB 80-128648, $6.50).

FDA 80-8095 Quality Assurance for Fluoroscopic X-Ray Units and Associated Equipment (PB 80-129778, $9.50).

FDA 80-8096 Quality Assurance for Conventional Tomographic X-Ray Units (PB 80-128838, $8.00).

FDA 80-8100 Implementation of a Quality Assurance Program for Ultrasound B-Scanners (PB 80-138340, $6.50).

FDA 80-8101 Ionization Chamber Smoke Detector Meeting (PB 80-128705, $8.00).

FDA 80-8102 Inexpensive Microwave Survey Instruments: An Evaluation (PB 80-114028, $5.00).

FDA 80-8103 Analysis of Some Laser Light Show Effects for Classification Purposes (PB 80-131576, $5.00).

FDA 80-8104 The Selection of Patients for X-Ray Examinations (GPO 017-012-00285-4, $3.50) (PB 80-157431, mf only).

FDA 80-8105 X Rays: So You Want To Be In Pictures? (Bookmark).

FDA 80-8106 An Evaluation of Microwave Emissions from Sensormatic Electronic Security Systems (PB 80-155385, $5.00).

Background Material for the Development of the Food and Drug Administration's Recommendations on Thyroid-Blocking with Potassium Iodide

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•

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Group Leader for Endocrine Drugs
Bureau of Drugs

March 1981

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Food and Drug Administration
Bureau of Radiological Health
Rockville, Maryland 20857

FOREWORD

The Bureau of Radiological Health develops and carries out a national program to control unnecessary human exposure to potentially hazardous ionizing and nonionizing radiations and to ensure the safe, efficacious use of such radiations. The Bureau publishes the results of its work in scientific journals and in its own technical reports.

These reports provide a mechanism for disseminating results of Bureau and contractor projects. They are distributed to Federal, State, and local governments; industry; hospitals; the medical profession; educators; researchers; libraries; professional and trade organizations; the press; and others. The reports are sold by the Government Printing Office and/or the National Technical Information Service.

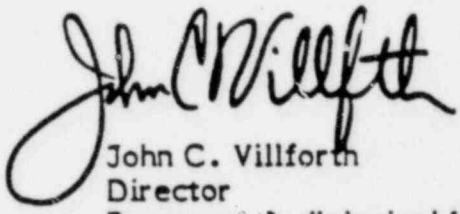
The Bureau also makes its technical reports available to the World Health Organization. Under a memorandum of agreement between WHO and the Department of Health and Human Services, three WHO Collaborating Centers have been established within the Bureau of Radiological Health, FDA:

WHO Collaborating Center for Standardization of Protection Against Nonionizing Radiations;

WHO Collaborating Center for Training and General Tasks in Radiation Medicine; and

WHO Collaborating Center for Nuclear Medicine.

Please report errors or omissions to the Bureau. Your comments and requests for further information are also encouraged.



John C. Villforth
Director
Bureau of Radiological Health

By Federal Register notice of October 22, 1980 (45 FR 69905), the Federal Emergency Management Agency (FEMA) outlined the responsibilities of several Federal agencies concerning emergency response planning guidance that the agencies should provide to State and local authorities. This updated a prior notice published in the Federal Register by the General Services Administration (GSA) on December 24, 1975 (40 FR 59494), on the same subject. GSA responsibility for emergency management was transferred by Executive Order to FEMA. The Department of Health and Human Services (HHS) is responsible for assisting State and local authorities in developing plans for preventing adverse effects from exposure to radiation in the event that radioactivity is released into the environment. These plans are to include the prophylactic use of drugs that would reduce the radiation dose to specific organs from the sudden release into the environment of large quantities of radioactivity that might include several radioactive isotopes of iodine.

By Federal Register notice of December 15, 1978 (43 FR 58798), FDA requested submissions of new drug applications for potassium iodide in oral dosage forms for use as a thyroid-blocking agent. This was the first FDA step in meeting the Departmental responsibilities as outlined by GSA. Now potassium iodide as a thyroid-blocking agent for use in a radiation emergency is available commercially in both a tablet and a solution form (see 45 FR 11912).

FDA is taking another step in meeting HHS responsibilities as outlined by FEMA by publishing proposed recommendations on the use of potassium iodide for the general public. FEMA and the Nuclear Regulatory Commission are charged with the responsibility for issuing recommendations relative to the procurement, storage and distribution of potassium iodide.

This report provides background material for the FDA proposed recommendations on thyroid-blocking with potassium iodide. Notice of availability of the proposed recommendations will appear in the Federal Register.

This background report presents: 1) the mechanism of action, efficacy, safety and availability of potassium iodide as a thyroid-blocking agent; 2) examples of actual and theoretical nuclear accidents and pathways of exposure which could require thyroid-blocking; 3) a discussion of previous recommendations relative to the use of potassium iodide as a thyroid-blocking agent as well as alternative protective actions; 4) descriptions of populations of special concern; and, 5) problems dealing with the procurement, storage and distribution of the drug.

Jerome A. Halperin, M.P.H.
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ABSTRACT

Halperin, J.A., B. Shleien, S.E. Kahana, and J.M. Bilstad. Background Material for the Development of the Food and Drug Administration's Recommendations on Thyroid-Blocking with Potassium Iodide. HHS Publication (FDA) 81-8158 (March 1981).

This report provides background material for the development of FDA's recommendations on thyroid-blocking with potassium iodide in the event of a radiation emergency.

It presents: 1) the mechanism of action, efficacy, safety and availability of potassium iodide as a thyroid-blocking agent; 2) examples of actual and theoretical nuclear accidents and pathways of exposure which could require thyroid-blocking; 3) a discussion of previous recommendations relative to the use of potassium iodide as a thyroid-blocking agent as well as alternative protective actions; 4) descriptions of populations of special concern; and, 5) problems dealing with the procurement, storage and distribution of the drug.

ADMINISTRATION'S RECOMMENDATIONS ON THYROID-BLOCKING WITH POTASSIUM IODIDE

INTRODUCTION

There is considerable debate about the appropriate role of a thyroid-blocking agent as an element of a public health response to an accidental release of radioiodines from a nuclear power plant. As one step in facilitating the availability of and providing guidance for the use of prophylactic drugs to reduce radiation doses in the event of accidental releases of radioactive materials (1), the Food and Drug Administration (FDA) published a notice in the Federal Register of December 15, 1978, entitled "Potassium Iodide as a Thyroid-Blocking Agent in a Radiation Emergency" (2). This notice requested submission of New Drug Applications (NDAs) for potassium iodide in specific oral dosing forms, announced the availability of labeling guidelines, and declared such preparations to be suitable under specific conditions for marketing as over-the-counter drug products. Prior to publication, the FDA worked closely with the Ad Hoc Committee on Thyroid Blocking of the National Council on Radiation Protection and Measurements (NCRP); the FDA notice closely followed the recommendations of NCRP Report No. 55, "Protection of the Thyroid Gland in the Event of Releases of Radioiodine" (3).

The Three Mile Island accident emphasized the need for radiation emergency planning, which should include consideration of the potential use of potassium iodide to reduce the uptake of radioiodines by the thyroid gland, thereby mitigating the possible adverse effects of such exposure. Thyroiditis may occur as an early effect but, since it has been observed only with very large doses of ^{131}I, it is unlikely to be a complication of off-site releases (3). Hypothyroidism and thyroid nodules with either benign or malignant characteristics are complications of lower dose exposure and occur later in time. The levels of radiation exposure associated with these abnormalities have recently been reviewed and planning officials are urged to familiarize themselves with the available human data (4,5). Minimizing the risk of such complications is of obvious significance to the public health.

Understanding the mechanism of action of potassium iodide is essential to its appropriate use as a radiation protection measure. To be most effective, potassium iodide would have to be administered promptly--either before, simultaneous with, or within 2 hours of the onset of exposure. Also important is an understanding of the rationale for the recommended dosage regimen and the possible side effects. Finally, guidelines for potassium iodide use, including the nature of the radiation hazard, pathways of exposure, population at risk, methods of storage and distribution, alternative or complementary protective actions and possible legal ramifications must be considered.

MECHANISM OF ACTION

Iodine in the diet reaches the circulation as iodide. The thyroid gland has an active iodide transport system which enables it to concentrate iodide so that the ratio of thyroid to plasma iodide concentrations is usually between 20-to-1 and 50-to-1. The ability to concentrate iodide is not limited to the thyroid gland but is found to a lesser degree in other organs, including the salivary glands, parts of the gastrointestinal tract, mammary glands, and placenta (6). The latter two have special significance for pregnant women and nursing infants.

Once in the thyroid, iodide is rapidly incorporated into organic molecules that are synthesized into thyroid hormones and ultimately released into the general circulation.

There is also an intra-thyroidal cycling of iodide from deiodination of hormone precursor molecules. Iodide so produced is available in the gland for re-organification or it may "leak" from the gland back into the general circulation (7). Estimates of the fractional turnover rate of incorporated iodine from the adult thyroid have varied, but it is generally considered to be about 1 percent per day; this corresponds to a biological half life of approximately 70 days (8).

Although a variety of chemical substances can block the accumulation of radioiodine in the thyroid gland, and hence its conversion to relatively long-lived organic compounds, stable iodide in the form of therapeutic doses of potassium iodide appears to be most suitable for this purpose. A number of factors were considered in choosing potassium iodide over other blocking agents such as propylthiouracil, methimazole, perchlorate, thiocyanate or iodate. These factors included the degree of blocking achieved, the rapidity of onset and duration of the blocking effect, the safety of the blocking agent, and the ease with which the drug could be made available under present FDA regulations.

The effectiveness of large doses of stable iodide in reducing the amount of radioactive iodine taken up by the thyroid gland appears to be dependent on two factors: (1) the proportion of radioactive iodine relative to the increased amount of stable iodide in circulating blood is greatly reduced (dilution effect); (2) as the levels of iodide in blood increase, there is an autoregulatory mechanism that limits the rate at which further iodide is accumulated by the gland; the precise nature of this iodide-induced effect has not been established. The suppression of uptake of radioiodine persists for as long as the intake of stable iodide is maintained at adequate levels. The administration of potassium iodide, however, would not significantly reduce the amount of radioiodine already trapped in the gland in hormonal form.

Of possible additional benefit is an acute inhibition of organification of iodine observed with excess iodide intake (Wolff-Chaikoff Effect) (9). This phenomenon is, however, usually short-lived in euthyroid individuals. Unlike the inhibitory effect on the transport system, the thyroid escapes from iodide inhibition of organification despite the maintenance of high concentrations of circulating iodide. The usual duration of this effect in humans has not been precisely defined; in rats, escape occurs after about 48 hours. It should be re-emphasized that such an escape would <u>not</u> affect the blocking of uptake of radioiodine by protective doses of potassium iodide. As far as is known, the latter can continue for long periods of time.

EFFICACY

As noted above, the usefulness of potassium iodide depends on its capacity to block entry of radioiodines; therefore, it is important to review briefly the pattern of a standard 24-hour uptake curve after ingestion of a single bolus of ^{131}I. (Of the various iodine isotopes, ^{131}I is the one of principal concern to the neighboring population. See NCRP Report No. 55 for further information regarding other isotopes and their properties and significance.) Figure 1 demonstrates that, in normal individuals retaining 10-40 percent of the administered amount of ^{131}I, most of the radioiodine is accumulated in the thyroid over a 10- to 12-hour interval; a smaller amount is accumulated over the next 12-hour period. Hence, an important factor in obtaining satisfactory blocking of peak radioiodine uptake is the temporal relation of stable iodide administration to radioiodine exposure.

This has been investigated by performing 24-hour radioactive iodine uptakes, varying both doses of potassium iodide and time intervals between administration and exposure (10). When doses approximating 100 mg of stable iodide (equivalent to 130 mg of potassium iodide) have been given just prior to or simultaneous with an oral tracer dose of ^{131}I, a 90 percent or greater reduction in peak thyroid accumulation of ^{131}I has been observed. (For example, if the unblocked 24-hour uptake of ^{131}I were normally 20 percent of the administered dose, then the uptake would be expected to be 2 percent or less with such a prior or simultaneous administration of potassium iodide.) A substantial benefit (e.g., a

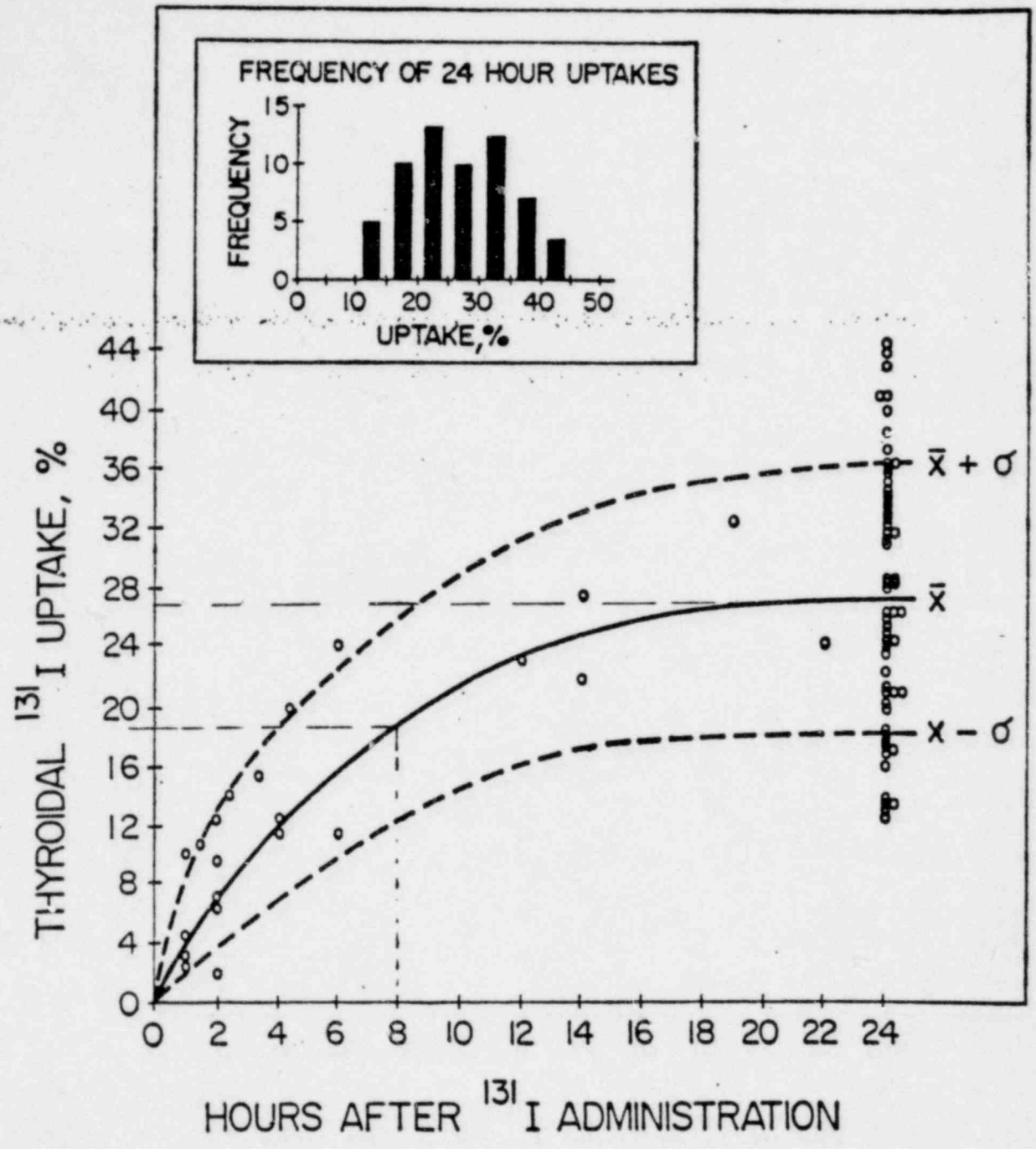

Figure 1. Thyroidal ^{131}I uptake in 62 euthyroid volunteers administered sodium iodide ^{131}I and their frequency distribution (from reference 10).

limited and little benefit can be expected after 10 to 12 hours for a single exposure. For more prolonged radioiodine exposure, potassium iodide will, of course, be useful at any time during the exposure as it will reduce further accumulation. A smaller dose, 50 mg iodide (65 mg of potassium iodide), can be used in infants under 1 year of age.

The range and mean 24-hour ^{131}I uptake in the normal adult U.S. population was reported in early 1970 publications to be significantly lower than that illustrated here, most likely reflecting the effect of an increase in dietary iodine. A more recent assessment in one metropolitan area has indicated a trend toward higher uptakes (mean 20.5 percent ± 6.1 percent) in association with a marked decrease in the iodine content of bread as a source of dietary iodine (11). It is probable that geographical variations in mean 24-hour ^{131}I uptakes exist based on regional differences in dietary iodine content from bread or other sources. Despite such variations in the numerical value of the mean 24-hour ^{131}I uptake in the normal population, the pattern of accumulation of ^{131}I is similar, so that conclusions regarding the temporal relationship of exposure to the efficacy of potassium iodide as a blocking agent are still valid. Such variations would obviously affect the calculation of the radiation dose received by the gland from any given exposure and for any degree of block.

The onset of blocking with an oral dose of 100 mg of stable iodide is readily demonstrated within 30 minutes of administration (12). The decay of the blocking effect after iodide administration is relatively slow; single daily doses of 130 mg of potassium iodide should be adequate to maintain effective blocking. Repeated daily administration is necessary not only for chronic exposure but also for a period of time after acute exposure in order to allow for renal excretion of circulating radioiodine. Renal clearance of iodide, usually in the range of 30 to 50 ml of serum/min, is closely related to glomerular filtration and is little affected by the iodide load (8,13). Most radioiodine not taken up by the thyroid gland after a single oral bolus of ^{131}I is excreted in the urine over the subsequent 48-hour period (14). Thus, the minimum duration of potassium iodide administration should be 3 days even if there is no continuing exposure. It is unlikely that administration would be required beyond 10 days in view of the availability of other countermeasures (e.g., interruption of contaminated milk supplies, evacuation). However, should extraordinary circumstances prevail and significant radioiodine contamination still be present, continued use of potassium iodide would be required.

SAFETY

The incidence of significant adverse reactions from short-term administration of potassium iodide in daily doses of 65 or 130 mg is unknown but is expected to be low.

Potassium iodide in large doses (300-1200 mg daily) and on a long-term basis has been widely used for years in the management of bronchial asthma and other pulmonary disorders. Doses of 100 mg or more have been employed for prolonged periods in children (15). Individual reports of complications of iodide administration in the medical literature for the most part do not identify the size of the patient population taking iodides from which the cases have been drawn. While under-reporting undoubtedly exists, the number of reports of adverse reactions from potassium iodide received by the FDA has been low. (The 160 adverse reactions to potassium iodide received by the Division of Drug Experience of the FDA from 1969 to 1980 were reviewed. Particular attention was paid to identifying those reported as having occurred in the group less than 10 years of age; none were noted. In 19 of these reports the age was unknown.) Furthermore, the occurrence of most side effects and toxicities appears to be proportional to the dose and duration of therapy. Known allergy to iodide would appear to be the only contraindication to its use in a radiation emergency.

The adverse reactions can be grouped into thyroid and non-thyroid effects (16). The non-thyroid side effects include skin rashes, swelling of the parotid glands ("iodide mumps"), and

burning mouth and throat, sore teeth and gums, rashes, symptoms of a head cold, gastric upset and diarrhea. Systemic hypersensitivity reactions (allergic-like) may also occur with, for example, fever, joint pains, and edema of various tissues. Discontinuing iodide, instituting supportive or specific medical therapy, and evacuation may be necessary depending upon the nature and severity of the observed adverse reactions. It would obviously be desirable to be able to identify particular members of the population who might be at greater risk of exhibiting a sensitivity reaction to potassium iodide. That iodide and iodine administration in patients rarely have been associated with sensitivity reactions was noted in a recent publication in which four patients with hypocomplementemic vasculitis associated with either chronic idiopathic urticaria or systemic lupus erythematosis were reported as exhibiting sensitivity to potassium iodide at doses of 500 mg daily on 5 of 11 days or 1000 mg as a single challenge (17). It was suggested that patients with similar clinical features might also be sensitive to the drug. The sensitivity reactions were non-fatal in outcome and varied from an exacerbation of urticaria to systemic manifestations. Patients with this uncommon disorder should preferentially be evacuated. However, if potassium iodide administration is unavoidable, such patients should be hospitalized where they could be monitored closely.

Complications of iodide administration involving the thyroid gland include hyperthyroidism, hypothyroidism and iodide goiter. Goiter may also occur in infants born to mothers who have taken large doses of potassium iodide for prolonged periods of time during pregnancy (18). However, for the recommended dosage and duration of administration, the incidence of such changes in thyroid function in the general population should be very low. Physicians should nevertheless be prepared to recognize and manage such conditions, most of which are readily reversible by cessation of potassium iodide administration.

Persons using potassium iodide as a thyroid-blocking agent in a radiation accident should be familiar with the possible side effects. Labeling guidelines which include such information in readily understandable form are available (2,19). Even though the occurrence of significant side effects is not anticipated with any degree of frequency, public awareness should result in their early recognition, prompt treatment and, hence, minimal risk.

AVAILABILITY

In the Federal Register notice of December 15, 1978 (2) the FDA requested submission of New Drug Applications (NDAs) for potassium iodide in oral form for use as a thyroid-blocking agent in a radiation emergency. The Agency waived the requirements for submission of full reports of toxicology studies in animals and clinical studies in humans stating that the requirements were met by citing the scientific literature. Potassium iodide was declared to be suitable as an over-the-counter drug if specific conditions were met: these include special labeling to accompany the immediate container, and use as a thyroid-blocking agent in a radiation emergency only upon the direction of responsible State or local public health officials. The availability of a labeling guideline for potassium iodide for use as a thyroid-blocking agent in a radiation emergency also was announced. Notice of a revision of this labeling guideline was published in the Federal Register of August 17, 1979 (19).

Dose-specific tablet and liquid forms of potassium iodide were included in the request for submission of NDAs. At the time of the Three Mile Island accident, no such applications had been received. To meet the possible emergency need, FDA arranged for the manufacture of a supply of Potassium Iodide Solution USP and delivery to Harrisburg, Pennsylvania, where the drug was stockpiled in a State-owned warehouse. None of the drug was distributed to local centers; however, Pennsylvania officials did have a plan (20,21) for distributing supplies of the drug accompanied by patient information sheets printed by FDA. This plan would have been put into effect if a decision had been made to employ the drug.

Applications for scored 130 mg tablets of potassium iodide and for Potassium Iodide Solution USP in a calibrated light-resistant dropper system delivering 21 mg of potassium iodide per drop. Public health officials and nuclear utility operators must now determine whether potassium iodide has a role in their emergency preparedness plans and, if so, must acquire adequate supplies and locate them in accordance with established plans for their distribution and use.

NUCLEAR ACCIDENTS: ACTUAL AND THEORETICAL

ACTUAL

In addition to the accident at Three Mile Island there have been four accidental releases of iodine-131 to the environment from nuclear facilities over the past 2 decades. Table 1 provides estimates of the magnitude of these releases and the resultant thyroid doses.

During the Three Mile Island accident the total amount of radioiodine released to the environment was estimated to be 13 to 17 Ci (22). There was no report of thyroid doses via the inhalation pathway; the maximum dose to an infant thyroid via milk was estimated to be 0.005 rem, far below the level at which any protective action is needed (23).

THEORETICAL

A sudden release from an operating nuclear power plant of large quantities of gaseous fission products (including several radioisotopes of iodine) could result from a loss-of-coolant accident due to a melting of the fuel cladding. The potential off-site absorbed doses to the thyroid may be estimated on the basis of a given set of reactor, meteorological and topographic conditions. These dose estimates can vary widely, depending on the assumptions chosen. Illustrations of two such calculations are presented in NCRP Report No. 55. In the "conservative" model it is presumed that the releases of radioiodine from the fuel are large and engineered safeguards are not fully operable, whereas in the "realistic"

Table 1. Accidental releases of iodine-131 to the environment from nuclear facilities

Event	Year	Magnitude of iodine-131 release (Ci)	Estimated maximum off-site dose to thyroid (rem*)
Windscale[24]	1957	20,000	16
SL-1[25,26]	1961	10 in first 16 hours; total of 80 over next 30 days	0.035
Hanford[27]	1963	60	0.03
Savannah River[28]	1964	94 in first few days; total of 153 in 26 days	1.2

*Doses for iodine-131 to the thyroid, for the purposes of this paper, are expressed in rems or rads interchangeably. The two are essentially equal for iodine-131.

The doses listed in this table have been calculated for a child's thyroid (2-5 grams in weight). The corresponding adult thyroid dose at Windscale is estimated at 9.5 rem. In no case was a thyroid-blocking agent used, although milk was dumped during the Windscale incident if the thyroid dose to children was projected to be in excess of 20 rem.

(See Appendix B of NCRP Report No. 55 for further definition of the assumptions involved, including reactor power level, primary containment leak rate, years of reactor operation, filter efficiencies and meteorological conditions.) Table 2 is a reproduction of the resultant calculated doses, in rads for the conservative model and in millirads for the realistic model. While the values are highly qualified and do not relate to any particular reactor and its site, the table is included for the purpose of illustrating the orders of magnitude that might obtain under a given set of conditions. It will also be employed later in the paper to show how such projections, when calculated for a specific accident, can be used to define the area within which the use of potassium iodide might be an appropriate protective measure.

It is the burden of the reactor operator to maintain the capacity for rapid generation of such projections as are appropriate to the particular facility, and to take into consideration varying conditions that can influence the nature, quantity and environmental distribution of an accidental release. In the event of a radiation emergency, nuclear utility operators are also responsible for the immediate communication of such information to designated public health officials.

Thyroid dose estimates for a loss-of-coolant accident without emergency core cooling and with a breach of the reactor containment were not included in NCRP 55. A recent estimate for such an unlikely accident in which very large quantities of radioiodines are released suggests that 10-60 percent of the children exposed within 200 miles downwind from the reactor could eventually develop thyroid nodules (29). The detailed assumptions upon which this projection was made were not given.

PATHWAYS OF EXPOSURE

The primary route of exposure to radioiodines, when airborne radioiodines are being released, is inhalation of contaminated air. Uptake of radioiodines over subsequent days to weeks occurs primarily via the pathway from pasture to cow to milk to human. The total human dose from the milk pathway can even exceed the inhalation dose by substantial amounts if large dairy farming areas are contaminated. However, other protective action measures are available, such as the use of uncontaminated feed and diversion or confiscation of contaminated milk, which could mitigate further exposure through the ingestion pathway. The primary radiation protection measure for airborne radioiodine is avoidance of exposure by remaining inside buildings, by use of respiratory protective equipment, or by evacuation from the area.

The use of potassium iodide in the recommended dosage regimen may be considered in addition to these measures if exposure has occurred or if there is risk of exposure. When used in conjunction with evacuation, potassium iodide should be continued until the evacuees reach a "safe" area and adequate time has elapsed for renal excretion of circulating radioiodines.

USE OF POTASSIUM IODIDE AS A THYROID-BLOCKING AGENT AND ALTERNATIVE PROTECTIVE ACTIONS

INTRODUCTION

Definitive Federal guidelines for the use of potassium iodide as a thyroid-blocking agent are not as yet available nor is it the purpose of this background document to propose them. Rather, recommendations on potassium iodide use and alternative protective actions recommended by the NCRP, the Environmental Protection Agency, and the Nuclear Regulatory Commission will be summarized (30,31). (In the Radiobiology Forum of 1970, the question of iodide prophylaxis was addressed (32). It is the authors' understanding that the British have considered potassium iodate as well as potassium iodide for this purpose. The

Distance (miles)	Time after release (hours)				
	2	8	24	96	720
	Dose*				
	Conservative estimate (rads)				
Pressurized water reactor					
0.5	110	230	280	320	350
1	50	110	120	140	150
2	21	45	51	55	58
5	6.3	14	15	16	17
10	2.6	5.6	6.2	6.6	6.7
20	1.2	2.5	2.8	2.9	3.0
30	0.8	1.6	1.8	1.9	1.9
40	0.6	1.2	1.3	1.4	1.4
50	0.4	1.0	1.0	1.1	1.1
Boiling water reactor					
0.5	210	790	1100	1500	1700
1	100	370	460	580	660
2	42	150	180	230	250
5	13	47	54	65	71
10	5.2	19	22	26	28
20	2.3	8.7	9.9	13	13
30	1.5	5.6	6.3	7.2	7.7
40	1.1	4.2	4.7	5.3	5.7
50	0.9	3.3	3.7	4.2	4.5
	Realistic estimate (millirads)				
Pressurized water reactor					
0.5	110	210	490	810	1100
1	53	100	200	300	400
2	22	41	73	110	140
5	6.7	12	20	28	36
10	2.8	5.1	8.3	12	15
20	1.2	2.3	3.6	4.9	6.1
30	0.8	1.5	2.3	3.0	3.7
40	0.6	1.1	1.7	2.2	2.6
50	0.5	0.9	1.3	1.7	2.1
Boiling water reactor					
0.5	0.21	1.1	5.1	17	29
1	0.0	0.55	1.8	5.4	9.2
2	0.04	0.22	0.66	1.9	3.2
5	0.01	0.06	0.18	0.49	0.79
10	0.005	0.03	0.07	0.19	0.30
20	0.002	0.01	0.03	0.08	0.12
30	0.001	0.008	0.02	0.05	0.07
40	0.001	0.006	0.01	0.03	0.05
50	0.001	0.005	0.008	0.02	0.04

*Contributions to the total absorbed dose in the first few days from the various radioiodine nuclides are approximately as follows: 60 percent ^{131}I; 30 percent ^{133}I; 10 percent ^{132}I, ^{134}I, and ^{135}I. The contribution at later times is almost all due to ^{131}I because of the more rapid decay of the other iodines. The absorbed dose to the whole body due to external radiation from the plume is generally less than the total absorbed thyroid dose by about one or two orders of magnitude.

will examine the practical impact of these differing views should they be adopted by responsible public health officials in a given radiation emergency.

Each State has the public health responsibility for formulating guidance and decision-making rules to define when the public would be given potassium iodide and instructed to use it. Such guidance was not available in Pennsylvania during the Three Mile Island emergency. The Department of Health, Education and Welfare recommended to the Governor of Pennsylvania that persons on Three Mile Island begin taking potassium iodide and that persons within a radius of about 10 miles of the reactor site be given bottles of the drug for potential use. The Pennsylvania Secretary of Health declined to accept that advice, stating that by the time the HEW recommendation reached the State the acute phases of the emergency had passed and prophylactic use of potassium iodide was not necessary (20).

In preparing guidance and decision-making rules, State agencies and local officials should be cognizant of their duty to warn citizens of the nature of the radiation hazard and of the potential adverse effects of potassium iodide. In those instances where the States will administer or direct the administration of the drug to its citizens, States may be subject to the same kinds of liability as exist in public immunization programs (33,34). States should assure that citizens are provided with, and are encouraged to read, the patient information leaflet before they receive a dose of the drug.

NCRP REPORT NO. 55

NCRP Report No. 55 recommends that a blocking agent be considered if initial estimates at the facility project total absorbed doses of 10-30 rads or more to the thyroid. When such doses are anticipated, the NCRP recommends the blocking agent be administered immediately to employees at the facility and to other support personnel coming to or working near the facility. For the general population residing outside the plant boundaries, if the anticipated thyroid absorbed dose is less than 10 rads, the NCRP states it may be preferable to consider instructing people to remain indoors and await further instructions. If the estimates of the total thyroid absorbed dose to the population exceed 10 rads, the NCRP recommends that use of a blocking agent be considered.

The report goes on to state that because of the substantially reduced effectiveness of the blocking agent after exposure to the radioiodines has commenced, and because reliable radiation monitoring data may not be available promptly, the decision to administer the potassium iodide should be based upon a pre-established emergency response plan.

EPA/NRC GUIDANCE

The EPA Protective Action Guides call for evacuation and controlled access as protective actions when the projected total accumulated thyroid doses are estimated at 5-25 rem for the general population. The EPA Guides call for protective actions for emergency workers at a projected thyroid dose of 125 rem. The use of potassium iodide is not specifically noted as an appropriate protective action for the general population.

A joint NRC/EPA report on the planning basis for radiological emergency response plans (NUREG0396) recommends that planning for evacuation should extend to 10 miles, based on plume exposures with the highest assumed fission product release and adverse meteorological conditions (31).

The circumstances in which potassium iodide might be considered following a nuclear facility accident will be reviewed. For illustrative purposes only, the authors assume: (1) that exposure occurs via the inhalation pathway promptly after release of radioiodines; (2) that the level of anticipated exposure in the first 24 hours after release is such that thyroid blocking is determined to be an appropriate protective measure; (3) that potassium iodide will be employed as a thyroid-blocking agent when the average projected thyroid absorbed dose to the general population is 10 rem or greater; and (4) that the assumptions for estimating total thyroid absorbed doses are the same as those for the models illustrated in NCRP Report No. 55.

Using the "realistic" and "conservative" accident values from Table 2, one finds that the use of a blocking agent would never be required for "realistic" estimate accidents, as it is highly unlikely that any person living outside the boundary of the nuclear facility would receive a total accumulated dose to the thyroid in excess of 10 rem from such an accident.

For "conservative" estimate accidents, persons living within about 2 miles of a boiling water reactor (BWR) or about 1 mile of a pressurized water reactor (PWR) boundary would be at risk of receiving considerable whole body exposure. This estimate is based on the assumption that whole body doses would generally be about one or two orders of magnitude below the thyroid dose. In a 24-hour period, and at a distance of 2 miles from a BWR, the estimated thyroid dose is 180 rads under the "conservative" assumption; the corresponding PWR dose is 120 rads at a distance of 1 mile. If one assumes a difference of one to two orders of magnitude between thyroid and whole body doses, and considers EPA's Protective Action Guides which recommend protective action at whole body doses between 1 and 5 rem, then evacuation is appropriate (30). As previously stated, when evacuation cannot be accomplished prior to exposure, there may be a need to consider the use of potassium iodide in those persons being evacuated.

Persons living beyond about 5 miles from a PWR and 20 miles from a BWR would be unlikely to receive an estimated thyroid dose in excess of 10 rem. Thus, based on the aforementioned assumptions, for those people living between 1 and 5 miles from a PWR and between 2 and 20 miles from a BWR (i.e., beyond the immediate evacuation area but within the zone where thyroid doses are estimated to be 10 rem or greater), blocking the uptake of radioiodines by the thyroid gland with potassium iodide would be an appropriate protective measure.

For accidents that result in loss of coolant and breach of containment, the dispersion of radioiodines may affect a much greater area and persons at significantly greater distances may be at risk of thyroid doses of 10 rems or greater. State radiation control agencies or nuclear utilities desiring to consider such accidents in the development of emergency response plans should refer to the safety analysis reports of the individual reactor facilities for guidance in calculating the potential release characteristics and the resultant areas and populations that could be affected.

In this example, potassium iodide for thyroid blocking is considered to be a proper response for a portion of the population involved in a nuclear emergency for whom the projected radiation dose to the thyroid is 10 rem or greater. Public health agencies must determine the "action level" at which they will advise the general public to start taking the drug. The 10-rem level is arbitrary, although it is consistent with NCRP 55. It is based upon an assumption that on a population basis the risk of potential adverse effects from a 10-rem radiation dose to the thyroid exceeds the risk of any adverse effects that might be encountered as a result of administering potassium iodide in daily doses of 65 mg to individuals under 1 year of age or 130 mg to the remainder of the population for a period of several days. As the radiation doses decrease below 10 rem, the relative risks of the potential adverse effects of the radiation and of the drug become less clear.

If public health authorities choose to evacuate people within a larger downwind distance from the facility, and if those people are moved promptly, the use of potassium iodide may not be necessary. Evacuation is a more effective method of reducing (or eliminating) thyroid exposure and may be a more desirable protective measure, especially for the population at greater risk from such exposure (i.e., infants, children, and pregnant women) and when the population density in the area is low enough to make evacuation a reasonable option.

When evacuation is neither feasible nor deemed necessary, the risk of radiation exposure can be reduced by eliminating possible sources of ^{131}I exposure via ingestion. This can be accomplished in large part by interruption of the milk pathway through diversion or confiscation of contaminated milk or milk products.

Barrier type protective measures (e.g., staying indoors) or use of respiratory protective devices should also be considered.

POPULATIONS OF SPECIAL CONCERN

Planning officials should be cognizant that certain members of the general population may be at increased risk in the event of exposure to radioiodine:

The Pregnant Woman

The pregnant woman herself is at risk in addition to the special concern for the developing fetus. Pregnancy is often accompanied by some degree of thyroid enlargement and increased ^{131}I uptake, which potentially increases the risk to the maternal thyroid (35). Consideration of possible effects on the fetus has prompted a recommendation that therapeutic doses of potassium iodide not be used as an expectorant in pregnant women (18). Pregnancy, however, is not regarded as a contraindication to the proper use of potassium iodide in relatively low doses over a short period of time as a thyroid blocking agent in the event of a nuclear accident.

The Fetus

Iodide reaches the fetal circulation via the placenta which, in some species and probably in man, appears to have an active transport system similar to that of the thyroid (6,36). The fetal thyroid is capable of trapping iodine at approximately 12 weeks of gestation; thereafter the uptake of iodine increases with gestational age. Using animal and human data, estimates of the human fetal thyroid radioiodine burden for a given acute exposure reveal that the dose to the fetal thyroid gland during the second half of gestation derived from either maternal ingestion or inhalation may exceed that of the adult (37). In addition, the immature gland may possess greater sensitivity for radiation-induced neoplasia (38,39). When prophylactic administration of potassium iodide is employed in the mother, it should be effective simultaneously in reducing both maternal and fetal thyroid exposure to radioiodine.

The fetus, together with the premature neonate, may also be more vulnerable to certain adverse effects of stable iodide, namely, the induction of goiter and/or hypothyroidism (40,41). There have been reports of goiters in the infants of asthmatic mothers who took therapeutic doses of potassium iodide for prolonged periods during pregnancy (18). As noted above, this has led to warnings concerning the use of large doses of potassium iodide as an expectorant in pregnant women. However, sizeable goiters have not been common and would not be anticipated to occur frequently with the dosage regimen recommended for use in thyroid blocking during a radiation emergency.

The Neonate

Estimating the radioiodine burden of the thyroid of the neonate is complex. First, the radioactive iodine uptake of the neonatal thyroid has been reported to be significantly elevated during the first few days of life compared to levels in the infant and adult (42). When expressed as uptake per gram of thyroid tissue, this difference is further magnified. The nursing neonate is also at risk of increased exposure via the oral route because of the concentration of radioiodine in the mammary gland and its secretion in milk. On the other hand, the biological half life of organically incorporated radioiodine may be shorter in the neonate than in the adult; this would reduce the duration of exposure of thyroid tissue to accumulated radioiodine (37,42,43) . Moreover, estimates of exposure from inhalation of radioiodine must be adjusted to reflect relative respiratory intake (44). Finally, the net estimate of thyroid exposure must be evaluated in the perspective of an immature gland with the potential for greater radiosensitivity.

Use of suitable safe milk substitutes and confinement in the protective environment of a hospital or home (or evacuation) should therefore be considered. In making a decision whether or not to use potassium iodide under these circumstances one must weigh the possible risks from its use against the hazard from exposure to radioiodine. Human experience with large doses of iodide in this age group is limited. Neonatal hyperthyroidism, where iodide has been recommended for use therapeutically in doses of 8 to 16 mg administered at 8-hour intervals, is an uncommon disorder (45). The published case reports do not provide adequate information from which firm conclusions can be drawn concerning the potential side effects of iodide in this dosing range, particularly for the thyroid side effects that might occur in a normal neonate. Recent reports of transient hypothyroidism in newborns associated with the repetitive use of iodine containing topical antimicrobial solutions suggest an underlying susceptibility to the inhibitory effect of stable iodide on thyroid hormone synthesis (46,47). However, no alteration in thyroid function was observed in neonates in another study employing such a topical preparation despite the presence of elevated plasma iodine levels; the authors nevertheless recommended caution in regard to repeated prolonged periods of application (48). These observations have prompted concern about the appropriate use of potassium iodide as a thyroid-blocking agent in the neonatal population. Although the reported changes appear to be reversible, a brief period of hypothyroidism during this critical stage of development is not necessarily without long-term effects. Given the present preliminary stage of information, no alteration in the recommended dosage regimen appears to be indicated, particularly in view of the potential higher risk for the adverse effects of radioiodine of this same age group. Meanwhile, it is reassuring that, while it is the youngest segment of the newborn population who might be most susceptible to exposure to either radioactive or stable iodine, it is this group that would likely be in hospitals where proper medical supervision would be available.

The Infant and Young Child

The infant and young child, by comparison with adults, are population groups at greater risk in regard to the possible adverse effects of exposure to radioiodines. Throughout infancy and childhood, while the radioactive iodine uptake as conventionally measured is similar to that of the adult, the uptake expressed per gram of thyroid tissue remains significantly elevated. Thus, for an equivalent exposure there is a greater concentration of radioiodine on a per unit thyroid weight basis, i.e., since the thyroid of infants and young children is smaller but picks up the same proportional amount of radioiodine, the radiation dose for a given exposure is an inverse function of thyroid size. Second, exposure of the infant and child may even exceed that of the adult because of the dietary reliance on fresh milk with resultant significant potential for ^{131}I intake via the oral route. Finally, the glands of infants and young children are still immature and therefore probably continue to possess greater sensitivity for radiation-induced neoplasia as compared to the mature glands of adults (4,5). These reasons all point to the consideration of all available countermeasures based on potential exposure to this particular age group in the population. Appropriate countermeasures include the use of uncontaminated milk or safe milk substitutes to reduce

for protection against inhaled radioiodines.

Reports of iodide-induced goiter and skin disorders (particularly in adolescents) have resulted in the adoption of a more conservative attitude by pediatricians toward the use of therapeutic doses of potassium iodide in asthma (18). However, consideration of both the risks involved in the event of a nuclear emergency, and the lower-dose shorter-term regimen, provides support for the relative safety of using potassium iodide in a radiation accident.

PROCUREMENT, STORAGE AND DISTRIBUTION

Acquiring adequate supplies of potassium iodide raises the issue of who should supply or pay for the drug. While the FDA provided the drug during the Three Mile Island emergency, it will not provide the drug in the future. The authors are unaware of plans by other Federal agencies to provide the drug. State agencies may choose to purchase supplies and stockpile them. Both of these options require expenditure of public funds. A third option would have the utilities which operate the nuclear power plants purchase the drug and provide it to State health agencies for stockpiling. The utilities could be required to purchase and provide the drug to State agencies as a condition of the Federal construction or operating licenses or any of the licenses or permits they must obtain from the State government before construction or operation may begin.

Based on the need for rapid administration of potassium iodide for full utilization of its thyroid-blocking potential, storage and distribution of potassium iodide in tablet or solution form are of particular concern. A further confounding problem is that supplies of potassium iodide tablets and solution currently approved for marketing bear 2-year expirations. Stockpiles will therefore have to be replaced, thus adding to the logistic and economic concerns. Further studies of product stability currently underway may result in approval of longer expiration dates on future lots of the products.

Presently, little definitive planning is evident for making the drug available promptly to potentially exposed persons. The NCRP suggests stockpiling supplies of potassium iodide tablets in distribution centers including fire houses, police stations, hospitals, clinics, factories, office buildings, municipal buildings, schools, physicians' and dentists' offices, pharmacies, and the nuclear facility itself. The problem, however, is not solely storage but also distribution. It is difficult to discern how the drug can be made available to persons in the "high risk" area soon enough to allow for effective thyroid blocking unless each household is provided in advance with a supply sufficient for all residents of the household. The need to consider predistribution may extend even beyond such an area, depending upon the radiation guidelines established for the use of potassium iodide.

A recent news article noted that a draft report prepared for the Council on Environmental Quality (CEQ) suggests that a supply of potassium iodide be fastened to electric meters (49), a notion originally suggested in 1973 (50). While predistribution has the obvious advantage of putting the drug into the hands of the public before the onset of ^{131}I exposure, there are equally obvious potential problems, e.g., persons may move and take the drug with them, bottles or packages may be lost or broken, and outdated supplies may not have been replaced. Fastening packages to electric meters poses other problems such as common meters in multifamily dwellings, meters on the outside of private houses exposed to the elements, multiple meters in public areas of apartment houses within reach of children, and even possible damage to the meter and electrical system by individuals trying to hurriedly secure tablets if they are stored inside the glass covers. Thus, this particular proposal would appear to present too many disadvantages to be considered seriously.

A detailed discussion of monitoring is beyond the scope of this presentation. However, it is important to emphasize that plans for monitoring the effectiveness of thyroid blocking, including long-term clinical followup for radiation damage as well as an assessment of any complications of potassium iodide administration, should be part of any planning program. NCRP Report No. 55 discusses both early and later complications of thyroid and whole body exposure. Regarding the possible side effects of potassium iodide administration, the importance of monitoring thyroid function in the neonatal period (particularly for premature newborns) has already been noted. In addition, following the emergency it would seem prudent to reassess thyroid function in patients with pre-existent thyroid disease.

SUMMARY

The use of potassium iodide as an agent to reduce the uptake of radioiodine in the event of accidental releases of radioiodines from a nuclear power plant is a public health countermeasure for which substantial planning is required. Although this drug is recognized by the Food and Drug Administration as safe and effective as a thyroid-blocking agent in a radiation emergency under specified conditions, the decision as to when and in whom it should be used remains with responsible State and local public health officials. For the most effective utilization, pre-planning on a State, local or regional level is required that considers details of: 1) anticipated absorbed radiation doses to the thyroid that would trigger the use of the drug and a specified procedure for estimating or determining these doses; 2) rapid distribution plans so that potentially affected population groups could begin taking the drug shortly before exposure begins, or as soon as possible after its onset; 3) supplies of the drug adequate for up to 10 days' administration; 4) a mechanism for informing the public of the need for, and timing of, taking the drug; and (5) alternative and/or supplementary actions.

The availability of potassium iodide provides a protective action to be considered with other safety measures in a radiation emergency. It is not the only action by which anticipated thyroid doses can be avoided, nor is it necessarily the best one. However, under certain conditions it provides an element of needed flexibility. Potassium iodide would seem to be particularly useful in the population for whom evacuation is not planned or deemed necessary but in whom the potential absorbed thyroid dose is projected to exceed that level pre-established to warrant its use. Depending upon the circumstances, it may also have a useful role in persons being evacuated.

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P/50 ON THE EFFICACY OF AD HOC RESPIRATORY PROTECTION DURING A RADIOLOGICAL EMERGENCY. James A. Martin, Jr. (USNRC, Washington, DC 20555)

Estimates were made of the potential for dose reduction afforded by application of ad hoc respiratory protection in an emergency. These estimates were based predominantly on data obtained recently by Cooper, et al (Harvard University) of the transmission of 0.4 to 6 micron particles through various household items. Protection factors (PFs) for the inhalation pathway were calculated using these data, log-normal particle size distributions and the ICRP lung model. The calculated PFs are in good agreement with data obtained from earlier experiments using human subjects. These studies demonstrate that application by the public of ad hoc shelter and respiratory protection could provide inhalation pathway protection factors (PFs) of ten or more, with shelter providing a PF of two to ten and ad hoc respiratory protection providing an additional PF of three to twenty, or so. These potential PFs are very competitive with that of potassium iodide (KI) for the thyroid, but the former would protect other organs as well and do not require a massive and expensive stockpiling and control preparedness program. One material studied, a commercial, NIOSH approved dust mask, is attractive as potentially useful for emergency workers. It is noteworthy, however, that for the high consequence accident scenarios considered in the Reactor Safety Study, the potential whole body dose at short range and in the immediate time frame would be about evenly divided between cloud gamma, ground gamma and inhalation pathways. For these accident scenarios, over the longer term the ground gamma pathway would clearly dominate, especially where effluent plumes intercept rainfall. Nevertheless, ad hoc shelter and respiratory protection could be used to reduce doses in cases where expeditious evacuation would not be feasible, e.g., over large areas remote from a site, or in cases where evacuation would not be warranted, but minimally interruptive protective action would be desirable, e.g., for smaller puff releases as from a sudden, major, accidental release from a fuel storage pool.

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Modification of Urethan-Lung Tumor Incidence by Low X-Radiation Doses, Cortisone, and Transfusion of Isogenic Lymphocytes[1]

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COLE, L. J. AND FOLEY, W. A. Modification of Urethan-Lung Tumor Incidence by Low X-Radiation Doses, Cortisone and Transfusion of Isogenic Lymphocytes. *Radiation Res.* **39**, 391–399 (1969).

Young adult male LAF_1 mice were given a single (300 R) exposure of x-rays, followed 1 day later by an intraperitoneal injection of urethan, 0.08 mg or 0.2 mg per gram body weight. Cortisone acetate was injected subcutaneously, seven injections of 2.5 mg each, on alternate days beginning 1 day after irradiation. The mice were killed 25–26 weeks after the urethan injection, and the enumeration of lung tumor incidence was made by standard procedures. The percentage of mice bearing lung tumors was highest (50%) in the group receiving 300 R plus urethan (0.08 mg/g), as compared with 12.5% in the control mice irradiated only, and 16% in those receiving urethan only. Thus, x-radiation and urethan can be additive for lung tumorigenesis. An increase in lung tumor frequency also occurred when cortisone was administered to 300 R x-irradiated mice (no urethan). Fractionated x-radiation (50 R × 6 given daily) plus urethan (0.2 mg/g) elicited a higher lung tumor frequency than observed in the several appropriate control groups. When the radioprotective compound, AET, was injected into mice just prior to an x-ray dose of 700 R followed 1 day later by a single injection of urethan (1 mg/g), the frequency and mean number of lung tumors were considerably increased

This study was supported through funds provided by the Bureau of Medicine and Surgery, S. Navy Department. Opinions and assertions herein are those of the authors and are not be construed as necessarily reflecting the official opinions of the Naval establishment.

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over that seen in the control group, receiving 700 R plus urethan, but without AET. Finally, in two separate experiments, the intravenous transfusion of 30×10^6 normal isogenic lymph node lymphocytes 2 days after x-irradiation and urethan treatment, resulted in a significant reduction in the lung tumor frequency.

The findings support the thesis that the frequency of occurrence of lung tumors in x-irradiated-urethan-treated mice is the resultant of two separate effects on the specific cells in question: (1) carcinogenic alteration in the cellular genetic material, and (2) sterilization of cell proliferative ability. Ionizing radiation can be additive in lung tumor induction if each is used at low dose levels. A component involving the immune system is involved in urethan lung tumorigenesis. Thus, transfusion of normal isogenic lymphocytes after 300 R plus urethan results in a lower lung tumor multiplicity per mouse; whereas multiple injections of cortisone, an immunosuppressive agent, resulted in increased numbers of lung tumors.

INTRODUCTION

Previous studies from this Laboratory (*1*) have shown that the occurrence of pulmonary alveolar tumors in adult (C57L × A)F_1 hybrid mice after a single injection of urethan (1 mg/g), is markedly reduced if the mice are exposed to a sublethal potentially lethal dose of x-radiation (880 rad) and restored with bone marrow. This inhibition was found to be due to a direct effect of the radiation on the lung since external lead-shielding of the lung area during exposure of the remainder of the body did not result in a lowered incidence of urethan-lung tumors (*2*). When urethan-treated mice were exposed to varying sublethal x-ray doses (total body) from 100 rad to 700 rad, the occurrence of lung tumors in these mice, killed at 30 weeks, was in general inversely related to radiation dose; but at the 100- or 300-rad doses there was no observable significant reduction in lung tumor incidence. We assume with L. H. Gray (*4*) that the interaction of radiation and urethan relative to lung tumorigenesis in mice is in part the resultant of two separate radiation dose-response curves: (a) radiation dose vs. carcinogenic induction, and (b) radiation dose vs. proliferative killing of the cells in question (*5*, *6*). This latter curve is assumed to have the usual x-radiation cell survival kinetics, with an initial shoulder followed by an exponential fall-off in survival.

Since we had observed no reduction in urethan-induced lung tumors at the 100- or 300-rad doses, we considered that at these doses the cell-sterilizing effect of the radiation was not sufficient to reduce the effective initial population of transformed, i.e., neoplastic, lung cells. However, since a carcinogenic initiation effect of x-radiation is postulated, it follows that such relatively low doses of x-rays should be additive with urethan when given together with a minimal dose of urethan. The present experiments were therefore carried out to test this hypothesis. In addition

...of x-radiation dose fractionation of cortisone and of a radiation dose modifying ...(AET) on lung-tumor incidence in urethan-injected mice, were investigated.

MATERIALS AND METHODS

The mice were male $(C57L \times A)F_1$ hybrids, 2–3 months of age at the start of the experiments. They were housed seven to ten per cage in galvanized metal cages, with access to tap water and Purina laboratory chow ad libitum.

The irradiation procedure was the same as that described previously (*1*). The x-ray source was a 250-kVp therapy unit (Westinghouse). The radiation factors were 250 kV, 15 ma, HVL 1.28 mm Cu; filtration: 0.5 mm Cu plus 1 mm Al; target-skin distance (T.S.D.) was 100 cm; dose rate, as measured in air, approximately 7 rad per minute.

Urethan was administered in aqueous solution as a single intraperitoneal injection, 1 day after radiation exposure of the mice. The minimal urethan doses chosen were 0.08 mg per gram body weight, and 0.2 mg/g. Cortisone acetate (Cortone-Merck) was given subcutaneously, seven injections of 2.5 mg each, on alternate days beginning 1 day after irradiation.

The mice were killed 25–26 weeks after injection of urethan, except as otherwise noted. The lungs and trachea were extirpated and immediately fixed *in toto* in Bouin-Hollande-Zky's acetic–alcohol–formalin for 24 hours. Counts of gross tumors on the surfaces of the fixed lungs were made. The fixed tissues were then embedded in paraffin and serially sectioned at 6 μ. Every fifteenth section was mounted and stained with hematoxylin and eosin. The tumors were identified and counted under the microscope. Thus, a microtumor smaller than 90–100 μ in diameter could have been missed, but probably was not, since most of the tumors on the sections were of this size or larger.

RESULTS

The lung-tumor data in mice receiving 300 rad of x-rays plus a minimal dose of urethan (0.08 mg/g) are summarized in Table I. The incidence of lung tumors under these conditions was highest in the mice treated with this combination of x-radiation and urethan vs. x-rays alone, or urethan alone ($P \sim 0.001$ by the chi-square test). The small dose of urethan by itself elicits only a slight increase over "background" lung-tumor incidence. Thus, x-radiation and urethan can be additive for the production of lung tumors in mice, under proper conditions of minimal dose of urethan and relatively low dose of x-rays. A similar additive effect of a carcinogen, 3-methylcholanthrene, and x-irradiation for production of rat mammary tumors has been reported by Shallabarger (*8*).

An increase in lung tumorigenesis was observed when multiple injections of cortisone were administered to 300-rad x-irradiated mice ($P < 0.01$). An apparent rise in lung-tumor incidence (not, however, statistically, significant, $P > 0.10$) was also

TABLE I

ADDITIVITY OF X-RAYS AND URETHAN; EFFECT OF CORTISONE

Treatment	No. of mice	Lung-tumor incidence	
		% with tumors	No./mouse
300 R only	32	12.5	1.3
Urethan[a] + 300 R	20	50	1.3
Urethan[a] only	19	16	1.3
300 R + cortisone[b]	19	42	1.2
Urethan[a] + cortisone[b]	16	37	1.0
None	27	7	1.0

[a] Urethan dose: 0.08 mg/g; single ip injection.

[b] Cortisone acetate—seven injections sc, 2.5 mg each, given on alternate days beginning 1 day after irradiation or urethan.

seen in nonirradiated mice which received a single injection of urethan (0.08 mg/g) 2 days prior to starting the cortisone injections.

Fractionated x-radiation plus urethan. The effect of six daily fractions of 50 rad of x-rays plus a single injection of urethan (0.2 mg/g) vs. a single x-ray dose of 300 rad plus urethan, was next compared. The data, summarized in Table II suggest that the fractionated x-rays yield an over two-fold increase in tumor multiplicity per mouse. Three conclusions can be drawn from these results: (1) Fractionated x-radiation and urethan (at the dose schedule employed) are additive for the production of lung tumors; (2) at the equivalent single dose of x-rays (300 rad) plus the administration of 0.2 mg/g of urethan there is no additivity for the yield of lung tumors; and (3) radiation fractionation alone, under these conditions does not increase the lung-tumor incidence over background. Since it is known that x-radiation dose fractionation permits cellular recovery, as regards proliferative capacity (cf. Elkind, *10*), the observed increased lung tumor incidence following fractionated radiation plus urethan is consistent with the theoretical formulation of the interaction between these two agents, postulated above.

Effect of a radiation dose-modifying compound. It was of interest to ascertain whether a chemical radioprotective agent, thought to bring about a reduction in tissue radiation dose (cf. *11*), would thereby modify the lung-tumor incidence in urethan-treated, x-irradiated mice. Since our previous studies showed an inverse relationship between x-radiation dose and lung-tumor yield (urethan dose = 0.2 mg/g) it could be anticipated that the administration of a radiation dose reducing compound would elicit an increase in lung-tumor yield. Mice were given a single intraperitoneal injection (320 mg/kg) of AET (aminoethylisothiouronium bromide) 15 minutes prior to their exposure to 700 rad of x-rays. Urethan (1 mg/g) was injected 1 day later. Another group of mice receedv 700 rad followed a few h

TABLE II

INTERACTION OF FRACTIONATED X-RAYS AND URETHAN IN LUNG TUMORIGENESIS

Treatment	*Lung-tumor incidence*			
	No./no. mice(%)	*Total no.*	*No./ mouse*[a]	*Index*[b]
50 R × 6 (daily)	6/21 (29)	8	1.3	0.38
50 R × 6 (daily) + urethan (0.2 mg/g)	14/21 (67)	32	2.3	1.53
300 R + urethan (0.2 mg/g)	11/20 (55)	14	1.3	0.72
300 R only	6/20 (30)	6	1.0	0.30
Urethan only (0.2 mg/g)	10/20 (50)	13	1.3	0.65

[a] Per tumor-bearing mouse.

[b] Index = (% of mice with lung tumors) × (number of tumors per mouse).

later by an intravenous injection of 5×10^6 isogenic bone marrow cells, and a day later by urethan (1 mg/g). The marrow cells were administered in order to permit greater survival of these irradiated mice. The resulting incidence of lung tumors at 26 weeks is given in Table III. In the AET-treated group, the percentage of mice with lung tumors, as well as the mean number of tumors per tumor-bearing mouse, was increased over that seen in the group receiving 700 rad plus urethan, but without AET ($P < 0.05$). Our interpretation of these observations is that AET pretreatment reduced the radiation dose to the lung such that fewer cells were sterilized, i.e., proliferative killing; therefore, a larger population of viable alveolar cells were available to express the carcinogenic or co-carcinogenic effect of the administered urethan.

Immunological factors in urethan-lung tumorigenesis. There now exists a considerable body of experimental evidence suggesting that immunological factors (analogous to or resembling the homograft reaction) are involved in carcinogenesis (*cf. 12, 13*). On this view, a deficiency in the immunological capacity or in the immunological recognition of neoantigens would increase the probability of the emergence of neoplasms bearing new, i.e., "foreign" tissue antigens. Since both ionizing radiation and urethan are known to inhibit the homograft response in mice (*14*), it seems plausible that this effect may be implicated (at least, in part) in lung tumorigenesis. The experimental question was therefore posed whether the transfusion of normal isogenic lymphocytes into mice which had previously received x-radiation plus urethan would result in a decreased lung-tumor incidence. The results of preliminary experiments (Table IV) indicate that this is the case. In one experiment, the mice received 300 rad plus urethan (1 mg/g) followed on day 2 by a single iv injection of 30×10^6

TABLE III

MODIFICATION OF URETHAN-INDUCED LUNG TUMORIGENESIS BY AET IN X-IRRADIATED MICE

Treatment	No. of mice	Lung-tumor incidence	
		% with tumors	No. tumors
AET + 700 R + urethan[a]	11	82	2.4
700 R + isogenic marrow + urethan[a]	30	57	1.8
AET + 900 R (no urethan)	10	0	—
900 R only	10	—[b]	—
700 R[c]	13	8	1.0
700 R + urethan[a, c]	17	59	1.8

[a] Urethan dose = 1 mg/g body weight.
[b] All died within 2 weeks following irradiation.
[c] Data previously published (Ref. 3).

TABLE IV

EFFECT OF POSTIRRADIATION TRANSFUSION OF NORMAL SYNGENEIC LYMPHOCYTES

Treatment	No. of mice	Lung-tumor incidence		
		No. with tumors	(%)	No. tumors
300 R + urethan (1 mg/g)	25[a]	25	(100)	6.2
300 R + urethan (1 mg/g) + 30×10^6 lymphocytes	18[a]	17	(94)	3.2
150 R + urethan (0.2 mg/g)	19[b]	12	(63)	1.2
150 R + urethan (0.2 mg/g) + 30×10^6 lymphocytes	18[b]	5	(29)	2.2
300 R + urethan (0.2 mg/g)	20[b]	15	(75)	1.4
300 R + urethan (0.2 mg/g) + 30×10^6 lymphocytes	19[b]	14	(74)	2.2

[a] Killed at 6 months.
[b] Killed at 11 months.

lymph node lymphocytes plus peripheral blood leukocytes from normal adult ma... It is evident (Table IV) that the number of lung tumors per tumor-bearing ma... was reduced by a factor of 2 in this group, relative to that in the control mice wh... received no injection of lymphocytes ($P < 0.001$). In the second experiment, gro... of mice were exposed to an x-ray dose of either 150 or 300 R followed by an injec... of urethan at the level of 0.2 mg/g; some of the mice then received a single intra... nous transfusion of 30×10^6 lymph node cells plus peripheral leukocytes from norm...

adult syngeneic donors. These mice were killed 11 months later and examined for lung tumors. In the group receiving 150 R plus urethan followed by syngeneic lymphocytic cells, a rather striking reduction in the incidence of pulmonary tumors was observed, relative to the appropriate controls ($P < 0.02$), although the mean number of tumors per mouse was not diminished. Since it is well known that such injections of lymphocytes are able to adoptively restore immunological reactivity in x-irradiated mice (*15*), we tentatively conclude from the present results that the reduction in lung-tumor frequency is related to a restoration, or partial restoration, of the immune system by the transfused lymphocytes. Further experiments are required under conditions where this effect could be "optimized"—particularly in mice killed at 1 year or more after irradiation and urethan injection.

DISCUSSION

These findings support the thesis that the occurrence of lung tumors in x-irradiated urethan-injected mice is the resultant of two distinct effects on the specific lung cells in question, each effect exhibiting a characteristic dose-response relationship: (1) an initial alteration in the cellular genome—presumably a physicochemical change in the DNA—which constitutes the carcinogenic induction at the earliest cellular level; and (2) sterilization of the capacity of the lung cells in question to proliferate. Within this theoretical context, our experimental data show that ionizing radiation and urethan can be additive for lung-tumor production, when both agents are applied at low dose levels, such that carcinogenic induction is predominant. This implies that relatively low levels of ionizing radiation may be hazardous for lung tumorigenesis, particularly in conjunction with other carcinogenic or co-carcinogenic influences in the environment.

The injection of a chemical radioprotection compound, AET, prior to x-radiation exposure (700 rad) and urethan administration, results in an increased lung-tumor incidence. On the basis of the above theoretical considerations, this is referable to a radiation dose reduction by the AET, resulting in the survival of a relatively greater number of carcinogenically altered lung cells, able to proliferate, and thus giving rise subsequently to gross lung tumors. An analogous, apparently paradoxical increase in lymphoma incidence in x-irradiated mice receiving prior injection of a chemical radioprotective compound was noted previously by Mewissen (*16*). The fact that the final incidence of lung adenomas in urethan-treated mice can be thus modified by experimental conditions which influence lung-cell proliferation, serves to emphasize the multi-stage aspect of carcinogenesis.

Finally, a component involving the immune system, i.e., the recognition of "foreign" tissue antigens is evidently involved in urethan-lung tumorigenesis in mice. Thus, the transfusion of normal isogenic lymphocytes after 300 rad plus urethan, a procedure known to adoptively restore immunological response in x-irradiated animals, results in a lower lung-tumor multiplicity per mouse. By contrast, multiple

injections of cortisone, a well-known immunosuppressive agent, resulted in increased numbers of lung tumors. The observations on the effects of cortisone are of interest also relative to the report by Berkheiser (*9*), who found alveolar cellular proliferation associated with cortisone therapy—both in human autopsy material and in experimental studies on rabbits. The present findings may therefore have practical implications for tumor control. In current studies, the effect of bone marrow cell transfusions on the incidence of radiation and urethan-induced lung tumors is being investigated, as well as other means of reducing lung tumorigenesis in mice under risk.

Kaye and Trainin (*17*) investigated factors influencing urethan induction of lung adenomas in young adult SWR mice. A single dose of 1 mg urethan per gram body weight resulted in 100% lung-tumor incidence. Half this dose produced a 76% incidence. The number of lung adenomas per mouse increased 60-fold, linearly with dose in the range 0.25–1.0 mg urethan per gram. Their attempts to modify the incidence of such tumors by injections of pyrimidines (thymidine, thymine, and orotic acid) as antagonists to urethan, were negative. However, these workers observed that spontaneous lung-tumor formation in A-strain mice were significantly reduced when the mice were fed thymine in their drinking water.

In conclusion, it appears that the modification of lung tumorigenesis—at least in mice—is potentially feasible by a variety of means particularly under minimal dose conditions of carcinogen or co-carcinogen, and limited time factors.

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A QUANTITATIVE STUDY OF THE LEUKEMOGENIC ACTION OF WHOLE-BODY X-IRRADIATION AND URETHANE

II. In Newborn C57BL Mice

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ABSTRACT

Newborn and suckling C57BL mice exposed to a single or repeated doses of 50 R whole-body X-irradiation failed to develop leukemia later in life; those exposed to four weekly doses of 150 R did develop leukemia, but with an incidence no higher than that obtained in adults subjected to the same treatment. Newborn mice did not, therefore, seem to be hypersensitive to radiation leukemogenesis as they are to urethane leukemogenesis. The responses of newborn and suckling mice to urethane injections were as follows: One i.p. injection of urethane (1 mg/g body weight) administered within 20 hr of birth failed to induce leukemia, but two weekly injections were effective, and three even more so, though additional weekly injections failed to raise the incidence further. With three weekly injections the incidence of leukemia was highest when treatment was started within the first four days; the incidence fell off when the first injection was given seven or 14 days after birth; it reached zero when the first injection was given 21 days after birth. The results are discussed in relation to the known rates of catabolism of urethane in newborn and adult mice.

INTRODUCTION

In the preceding communication (1), whole-body X-irradiation and repeated injections of urethane were tested for leukemogenesis at different dose levels, administered separately or in sequence, in adult C57BL mice. The object was to determine the optimal conditions for leukemogenesis by the two-stage technique, with X-irradiation as initiator and urethane as promoter. The results showed that by restricting the initiating stimulus to a single irradiation, background leukemogenesis (i.e. by the radiation alone) was almost eliminated, and that urethane alone was also virtually non-leukemogenic under the conditions of the experiments. When a single irradiation was followed by repeated urethane treatment, leukemogenesis occurred, with a rising incidence depending on the dose of radiation given. The efficacy of urethane as promoter reached a maximum after about five weekly injections. It was found that younger mice (28 to 33 days old) responded more effectively to the two-stage process

leukemogenesis than somewhat older mice (37 to 44 days old at the time of irradiation). Urethane itself is, however, known to be leukemogenic when administered from birth (2–4). This raised a number of questions that could have a bearing on the two-stage mechanism of leukemogenesis: 1) Are newborn mice also hypersensitive to X-irradiation alone? 2) What are the limitations of neonatal urethane leukemogenesis in terms of dosage, i.e. with respect to the number of subsequent injections of the substance? 3) At what age after birth does the "newborn" pattern of response to urethane change to the "adult" pattern?

These were the problems submitted to quantitative study in the present investigation.

MATERIALS AND METHODS

The experimental procedures and materials were essentially the same as those described in the preceding communication (1), except for the following modifications necessitated by the fact that newborn and suckling mice were used instead of adults.

X-ray treatment. The newborn and suckling C57BL/6 mice were allowed to move freely in petri dishes with perforated covers during the irradiation. Each litter was irradiated separately and returned to the mother immediately afterwards.

Urethane injections. Urethane, dissolved in distilled water, was administered by i.p. injection as a 5% solution in the case of newborn mice and as a 10% solution in the case of suckling mice, the dose per injection being 1 mg of urethane/g body weight.

Since the urethane treatment caused a delay in the development of the young mice, they were weaned, and separated according to sex, at 50 to 60 days of age instead of at 21 to 25 days of age. Approximately equal numbers of males and females were used for each experimental group.

As in the preceding investigation (1), each experiment was terminated 14 months after the first treatment. The examination of the animals, and of their tissues after death, and the classification of the leukemias were the same as in the previous communication (1).

RESULTS

Experiment I. This experiment was designed to test the leukemogenic action of one, two and four exposures to 50 R whole-body X-irradiation, the first exposure performed within 20 hr of birth (Groups I, II and IV) and subsequent ones at weekly intervals thereafter. Additional groups were given a) a single irradiation of 100 R, administered one week after birth (Group III, as control for Group II); b) a single irradiation of 200 R, administered three weeks after birth (Group VI, as control for Group IV); c) a single irradiation of 50 R, administered three weeks after birth (Group V, as control for Group I); d) two irradiations of 50 R, administered three and four weeks after birth (Group VII, as control for Groups II and III); and e) four weekly irradiations of 150 R, starting within 20 hr of birth (Group VIII, as control for standard irradiation for leukemogenesis in adult mice, Group IX).

The results (Table 1) were virtually negative, except for Group VIII, which yielded a fairly high incidence (56%) of leukemia, though no higher (and, if anything, lower) than that obtained with similar treatment in adult mice (69% in Group IX).

Thus, in contrast to their response to urethane, newborn mice were not found to be hypersensitive to the leukemogenic action of whole-body X-irradiation.

Experiment II. This experiment was designed to determine the minimal amount (i.e. the smallest number of weekly injections of 1 mg/g body weight) of urethane for effective leukemogenesis in newborn mice when treatment was begun within 20 hr of birth.

The results (Table 2) show a) failure to induce leukemia with a single injection of urethane; b) a significant incidence (20%) of leukemia after two weekly injections and a still higher incidence (43%) after three weekly injections, and c) no further rise in incidence with additional weekly injections.

TABLE 1. *Leukemia incidence in C57BL/6 mice irradiated at birth or during suckling period*

Group	Irradiation dose — Age: < 20 hr	1 week	2 weeks	3 weeks	4 weeks	Incidence of leukemia/effective total[a]	Average latent period (weeks)
I	50 R	—	—	—	—	1/52 = 2%	39
II	50 R	50 R	—	—	—	0/62 = 0%	—
III	—	100 R	—	—	—	0/45 = 0%	—
IV	50 R	50 R	50 R	50 R	—	3/77 = 4%	33
V	—	—	—	50 R	—	0/53 = 0%	—
VI	—	—	—	200 R	—	1/53 = 2%	32
VII	—	—	—	50 R	50 R	1/30 = 3%	39
VIII	150 R	150 R	150 R	150 R	—	9/16 = 56%	24
IX[b]	in adults, 150 R × 4					95/137 = 69%	28

[a] Effective total = number of survivors at the time of appearance of the first leukemia.
[b] Results from a previous experiment (13).

TABLE 2. *Effect of different doses of urethane on newborn C57BL/6 mice*

No. of urethane injections	Thymic lymphosarcoma — Thymic only	Generalized	Incidence of leukemia/effective total[a]	Average latent period (weeks)
× 1	0	0	0/23 = 0%	—
× 2	3	3	6/31 = 20%	25
× 3	2	8	10/23 = 43%	24
× 4	5	35	40/105 = 38%	23
× 5	3	35	38/97 = 39%	26
× 6	4	33	37/80 = 46%	26
× 10[b]	6	13	19/54 = 35%	26

[a] Effective total = number of survivors at the time of appearance of the first leukemia.
[b] Results from a previous experiment (14).

TABLE 3. *Leukemia incidence in C57BL/6 mice receiving three weekly injections of urethane started at different times after birth*

Age at first urethane injection	Lymphosarcoma — Thymic only	Generalized	Incidence of leukemia/effective total[a]	Average latent period (weeks)
< 20 hr[b]	2	8	10/23[b] = 43%	24
1 day	0	10	10/18 = 36%	30
2 days	3	9	12/29 = 39%	29
3 days	2	5	7/17 = 41%	29
7 days	1	4	5/29 = 17%	28
14 days	0	3	3/28 = 10%	44
21 days	0	0	0/34 = 0%	—

[a] Effective total = number of survivors at the time of appearance of the first leukemia.
[b] Results from Experiment II (see Table 2).

The results indicate, in short, that three weekly i.p. injections of urethane started soon after birth are optimal for leukemogenic action.

Experiment III. The purpose of this experiment was to determine at what age after birth the newborn pattern of hypersensitivity to urethane leukemogenesis changed to the adult pattern of virtual nonresponsiveness. Based on the results of Experiment II (see above), three weekly i.p. injections of urethane (1 mg/g body weight per injection) were administered to all the mice in the present experiment, the treatment being started at different ages in the different groups, namely, within 20 hr of birth and 1, 2, 3, 7, 14 and 21 days after birth.

The results (Table 3) show a somewhat similar incidence of leukemia in the first four groups, a falling off in the next two groups (when treatment was started seven and 14 days, respectively, after birth), and no leukemia in the last group (when treatment was started 21 days after birth).

DISCUSSION

The results of Experiment I may be summarized as follows: Newborn and suckling C57BL mice do not respond to the leukemogenic action of whole-body X-irradiation when given in single or multiple doses of 50 R, but their response to multiple doses of 150 R is positive, though no greater than that observed when the same treatment is given to adult mice. The conclusion to be drawn from these results is that newborn mice are not hypersensitive to the leukemogenic action of X-irradiation. It should be noted, however, that under somewhat different conditions Rudali and Reverdy (5) obtained a somewhat higher incidence of leukemia in newborn than in adult C57BL or (C57BL×AkR)F_1 mice.

In the case of urethane, however, the difference in response between newborn and adult mice is very striking, the former being highly responsive (2–4) and the latter nonresponsive (6, 7). A surprising feature of neonatal hypersensitivity to urethane leukemogenesis, shown in Experiment II, is that a single injection is ineffectual; that two or three weekly injections started soon after birth are leukemogenic; while additional weekly injections thereafter fail to raise the incidence further.

The fact that injections beyond the third week are ineffectual would suggest that the animals are, by then, in the "adult" state of nonresponsiveness. This is confirmed by the results of Experiment III, in which delay in starting urethane treatment after the first few days leads to a falling off in the incidence of leukemia, and after 21 days to complete failure of leukemogenesis. These results are explicable on the basis of earlier metabolic studies—that newborn mice are less capable of catabolizing urethane than adult mice (8, 9) and that the capacity to metabolize the compound increases with age, until the age of 20 days, when the "adult" pattern is reached (10).

Responsiveness of newborn mice to urethane leukemogenesis appears, therefore, to be related to persistence of unchanged urethane in the body, and conversely, failure of adult mice to respond is attributed to the rapid catabolism of the compound, rather than to a biological loss of sensitivity. This might account for a reported difference in response of adult mice to urethane according to whether it is administered by weekly injection, when leukemogenesis does not occur (6, 7), or administered continuously in the drinking water, when leukemogenesis does occur (11, 12).

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A QUANTITATIVE STUDY OF THE LEUKEMOGENIC ACTION OF WHOLE-BODY X-IRRADIATION AND URETHANE

I. In Adult C57BL Mice

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ABSTRACT

Leukemia induction in C57BL mice by the two-stage technique—using whole-body X-irradiation as initiator and urethane injections as promoter—has been reexamined under varying conditions of dosage and length of treatment. By limiting the radiation to a single exposure, background leukemogenesis was reduced almost to that of the spontaneous incidence. The effectiveness of X-irradiation (single exposure) for initiating action was manifested as a progressive rise in leukemogenesis with increasing dose, when adequate urethane was given after it; while that of urethane, for promoting action, expressed itself as optimal activity reached after five weekly injections. Of the two age groups tested, the younger mice (28 to 32 days old) were more responsive than the older (37 to 44 days).

INTRODUCTION

Urethane, which is itself almost devoid of leukemogenic action in adult C57BL mice (1, 2), is capable of augmenting the leukemogenic action of whole-body X-irradiation when the two treatments are given concurrently (1, 2) or when the urethane is administered after the irradiation, but not when the sequence is reversed (2, 3). These results are indicative of a two-stage process, with whole-body X-irradiation as initiator and urethane as promoter (3, 4).

A complication arose with the discovery that when urethane treatment was begun soon after birth, it was itself found to be leukemogenic (5, 6). Furthermore, radiation leukemogenesis proved to be a much more complicated process than skin carcinogenesis, involving a latent virus (7) and requiring a) its release from its hidden site (8), b) the activation of the thymus as target organ (9, 10), c) depression of the animal's immune response (11, 12), and d) a more specific depression of a control mechanism residing in the bone marrow and spleen (13), involving a protein factor—RLP (14). Thus, while the two-stage process in radiation leukemogenesis may seem similar to that of skin carcinogenesis, the underlying mechanisms in the two cases may not be identical.

Nevertheless, a two-stage technique, whatever its underlying mechanism, has important uses as an analytical tool (15), and the more refined the technique, the more valuable it is likely to be for the purpose.

One of the aims in perfecting the technique was to reduce to a minimum the background leukemogenic action of the two separate components; another was to determine the minimal amount of treatment needed for optimal leukemogenic effect.

The two-stage technique for leukemogenesis used in the original study (2) involved five applications of 90 R whole body X-irradiation followed by five injections of 20 mg urethane. (In subsequent experiments other combinations have also been used.) The present communication deals with a more systematic study of the effect, in adult C57BL mice, of varying the dose of radiation (single application) and the number (i.e. length of action) of urethane injections, the two forms of treatment being administered separately or in sequence. A further communication (16) deals with the effect of varying the dose and number of X-ray applications and urethane injections administered separately to newborn C57BL mice.

MATERIALS AND METHODS

Mice. Male and female C57BL/6 mice of several known age groups were used, derived from a line originally obtained from the Jackson Memorial Laboratory, Bar Harbor, Maine, and inbred locally for many generations. The animals were kept in stainless steel cages, 10 per cage, and housed in an air-conditioned room at 21 to 25 C. They were maintained on a standard diet of Purina Laboratory Chow pellets, occasionally supplemented with barley and sunflower seeds, and tap water ad lib.

X-ray treatment. The radiation was performed with a 250 kv Maximar machine, and given at the rate of 50 rpm, with 1.5 mm Cu and 1.0 mm Al filters. Doses ranged from 50 to 400 R per treatment, according to the design of the experiment (see below).

Urethane injections. Urethane, obtained from British Drug Houses Ltd., was administered i.p. as a 10% solution in distilled water. The injections were made on the basis of body weight (1 mg urethane/g body weight per injection).

Animals that died before the appearance of the first leukemia in the experiment, or which were decomposed at the time of death, were excluded from the experimental results. The mice were examined daily and kept under observation for 14 months, when the experiments were terminated. Individual animals which appeared moribund during the course of the experiments were killed to avoid, as far as possible, cannibalism or decomposition.

Leukemia was diagnosed by periodic palpation of the lymph nodes and thymus, and subsequently confirmed by histological examination of all organs that appeared abnormal. The leukemias—lymphatic in type—were classified according to localization as "pure thymic," "thymic involving other organs as well," and "disseminated without thymic involvement." (Other types are specified in the tables.)

RESULTS

Experiment I. This experiment was designed to test the two-stage process of radiation leukemogenesis under more exacting conditions than previously (2), using single applications of radiation at two dose levels, followed by repeated injections of urethane, and including all the necessary controls.

The mice, 6 to 7 weeks of age, were irradiated with either 150 (Group II) or 300 R (Group V) followed, one week later, by the first of 10 weekly injections of urethane (1 mg/g body weight per injection). Another two groups were first given 10 weekly injections of urethane, and one week after the last injection were irradiated with 150 (Group III) or 300 R (Group VI). Control groups consisted of irradiation alone of 150 (Group I) or 300 R (Group IV) and 10 injections of urethane alone (Group VII).

It was found (Table 1) that the incidence of leukemia was 0 and 8%, respectively, with 150 and 300 R alone; 7 and 11%, respectively, with urethane followed by 150 and 300 R; but 18 and 23%, respectively, with 150 and

TABLE 1. *Incidence of leukemia in adult C57BL/6 mice irradiated and treated with urethane*

Group	Treatment		No. of mice used	Lymphosarcoma			Incidence of leukemia/effective total[a]	Average latent period (weeks)
	First	Second		Thymic only	Generalized	Non-thymic		
I	150 R	—	82	0	0	0	0/78 = 0%	—
II	150 R	Urethane	66	0	9	2	11/60 = 18%	33
III	Urethane	150 R	67	2	2	0	4/53 = 7%	41
IV	300 R	—	74	1	4	0	5/63 = 8%	29
V	300 R	Urethane	70	1	10	0	11/47 = 23%	31
VI	Urethane	300 R	74	0	7	0	7/61 = 11%	35
VII	Urethane	—	54	0	3	0	3/47 = 6%	39

[a] Effective total = number of survivors at the time of appearance of the first leukemia.

TABLE 2. *Incidence of leukemia in adult C57BL/6 mice irradiated once with different doses of X-rays, followed by 10 injections of urethane*

Group	Urethane (×10)	Age at beginning of treatment (days)	No. of mice used	Lymphosarcoma			Incidence of leukemia/effective total[a]	Average latent period (weeks)
				Thymic only	Generalized	Non-thymic		
50 R	+	37 to 44	95	1	4[b]	1[b]	6/84 = 7%	37
100 R	+	"	112	0	2	2[b]	4/79 = 5%	44
200 R	+	"	97	3	6	1	10/83 = 12%	30
50 R	—	"	101	0	0	0	0/99 = 0%	—
100 R	—	"	99	0	0	1	1/98 = 1%	56
200 R	—	"	97	0	2	0	2/95 = 2%	31
50 R	+	28 to 32	40	2	4	0	6/32 = 19%	35
100 R	+	"	40	2	2	0	4/36 = 11%	38
200 R	+	"	38	1	4	1	6/28 = 21%	34
400 R	+	"	29	3	5	0	8/23 = 35%	26
50 R	—	"	39	0	0	0	0/37 = 0%	—
100 R	—	"	40	0	0	0	0/32 = 0%	—
200 R	—	"	40	0	0	0	0/34 = 0%	—
400 R	—	"	40	0	0	0	0/21 = 0%	—

[a] Effective total = number of survivors at the time of appearance of the first leukemia.
[b] Including stem cell leukemia.

00 R followed by urethane; while the incidence with urethane alone was 6%. (The spontaneous incidence of leukemia in our C57BL mice is less than 1%.) The range in average latent period (29 to 41 weeks) did not seem very pronounced, taking into account the fact that the first indication of the development of the disease, judged by gross inspection, could not be estimated very precisely.

Experiment II. This experiment was a repetition of the previous one, but using a wider range of doses of radiation, tested on mice of two different ages: 28 to 32 days and 37 to 44 days at the start of the experiment. (The reversal effect, i.e. of urethane followed by radiation, was not tested in this experiment.)

The results (Table 2) were essentially the same as in Experiment I, with very low incidences or absence of leukemia with radiation

TABLE 3. *Incidence of leukemia in adult C57BL/6 mice irradiated with 400 R, followed by different numbers of injections of urethane*

X-ray (400 R)	No. of urethane injections	No. of mice used	Lymphosarcoma			Incidence of leukemia, effective total[a]	Average latent period (weeks)
			Thymic only	Generalized	Non-thymic		
+	1	73	0	3	0	3/61 = 5%	41
+	3	73	0	12	0	12/56 = 21%	26
+	5	50	0	9	0	9/19 = 47%	27
+	10	50	0	8	0	8/25 = 32%	26
+	15	52	4	8	0	12/32 = 37%	29
—	3	74	0	0	0	0/68 = 0%	—
—	5	52	0	2	0	2/39 = 5%	29
—	10	51	0	0	0	0/50 = 0%	—
—	15	48	1	3	0	4/47 = 8%	37
+	—	74	0	2	0	2/60 = 3%	32

[a] Effective total = number of survivors at the time of appearance of the first leukemia.

alone, over the range of 50 to 400 R, and a rising incidence of leukemia with increasing dose of radiation when followed by urethane treatment (10 weekly injections). There was, however, an overall difference in response according to the age of the mice at the start of the experiment, the values being somewhat higher in the younger age group.

Experiment III. In this experiment on mice ranging in age from 33 to 37 days, the dose of radiation was kept constant (400 R) but the number of urethane injections was varied from 1 to 15.

Once again, the background leukemogenesis with urethane alone or with radiation alone was minimal (Table 3). In the groups receiving radiation followed by urethane treatment, the incidence of leukemia rose with the number of urethane injections, reaching a plateau after about five injections.

DISCUSSION

The concept of a two-stage leukemogenic process, analogous to that operating in skin carcinogenesis, was based on an early experiment in which adult C57BL mice were found to respond differently to the double action of whole-body X-irradiation and urethane injections, according to which treatment came first (2). The mice receiving five applications of 90 R followed by five injections of urethane showed about double the incidence of leukemia as those receiving the two treatments in reverse. The disturbing element in this experiment was the high background leukemogenesis with X-irradiation alone, although the urethane control group did not produce leukemia.

In an attempt to overcome this complication of background leukemogenesis on the part of the radiation, single doses of whole-body X-irradiation, at different dose levels, were tested alone or in conjunction with 10 weekly injections of urethane before or after the irradiation, instead of five irradiations and five injections of urethane, as was the case in the earlier experiment (2). Under these new conditions (Experiments I and II) the background leukemogenesis with radiation alone was reduced almost to the level of the spontaneous incidence of the disease, while in the case of combined radiation and urethane action, higher incidences were once again observed with urethane following the radiation treatment than when the sequence was reversed. Furthermore, the leukemia in-

cidence rose progressively with increasing dose of radiation (followed by standard urethane treatment), though a direct proportionality could not be accurately established.

The effect of varying the number (i.e. length of action) of urethane injections, after a standard dose of 400 R whole-body X-irradiation, was determined in Experiment III, from which it transpired that, beyond five weekly injections, there was virtually no further increase in incidence of induced leukemia.

In the search for optimal conditions for leukemogenesis by the two-stage technique, a considerable measure of success was thus achieved, both in reducing the background leukemogenesis on the part of either of the two components and in narrowing down the amount of treatment required for effective leukemogenesis. The best conditions observed were a) the use of a single application of X-irradiation at as high a dose level as the animal can tolerate, b) five weekly injections of urethane at a dose level of 1 mg/g body weight per injection, and c) the use of young animals, but not so young as to render them sensitive to urethane leukemogenesis per se (see 5, 6).

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Enhancement of X-ray Transformation by 12-O-Tetradecanoyl-phorbol-13-acetate in a Cloned Line of C3H Mouse Embryo Cells[1]

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ABSTRACT

A phorbol ester promoting agent, 12-O-tetradecanoyl-phorbol-13-acetate, enhances X-ray transformation *in vitro* in a two-stage fashion similar to that shown previously for ultraviolet radiation and chemical carcinogens. In studies with a mouse embryo-derived cell line (C3H/10T½ clone 8), there were clear interactive effects between X-radiation and 12-O-tetradecanoylphorbol-13-acetate. These were particularly marked when a minimally transforming X-ray dose (50 or 100 rads) was followed by 12-O-tetradecanoylphorbol-13-acetate treatment beginning either immediately after the radiation exposure or 48 to 96 hr later.

INTRODUCTION

In several tissues exposure to a low dose of a carcinogen can lead to enhanced tumor formation if there is a subsequent exposure to specific noncarcinogenic agents (2, 3, 5, 6). In such experiments, in which carcinogenesis is enhanced, the carcinogen has been referred to as the initiator and the subsequent agent is called the promoter (5, 6). The study of "2-stage" carcinogenesis began with mouse skin, in which a low dose of an initiating agent, which was insufficient by itself to cause tumors, produced tumors when followed by repeated applications of croton oil, a promoting agent (2, 3, 5, 6). Such mouse skin experiments have most often used chemical carcinogens as initiators, but there is also evidence that radiation initiates skin cancer. UV can induce skin cancer in rats and mice by itself at high doses (4, 7, 9–11, 24). A single dose of UV, not oncogenic by itself, also resulted in tumor formation when followed by croton oil applications (20). Ionizing radiation followed by croton oil treatments has been less effective in the induction of skin cancer than UV radiation. Shubik *et al.* (26) reported that, while 800 reps of β-radiation to mouse skin caused no tumors, subsequent croton oil application did result in a low yield of tumors. Using graded electron doses and croton oil on rat skin, Albert and Burns (1) showed that, following a radiation dose (3000 rads) that induced tumors, repeated croton oil applications increased the tumor incidence 2-fold; however, at lower doses of radiation, croton oil treatment had no effect. The skin tumors induced by UV and ionizing radiation differed markedly in their histopathological characteristics, perhaps because the oncogenic targets for the 2 agents may be different (7).

The effects of croton oil or its constituents on cells have been reported for several tissue culture systems (8, 14, 17, 18, 27, 28, 31). Subsequent exposure to a promoting agent enhanced malignant transformation *in vitro* resulting from exposure to chemical carcinogens (14, 17) and UV radiation (18), but the effects of promoting agents following ionizing radiation on *in vitro* transformation have not yet been reported. The present study was undertaken to determine whether a promoting agent could enhance X-ray transformation *in vitro*, as has been shown previously for UV radiation (18). The promoting agent used was TPA,[3] the most active promoter in croton oil (12), and the cell system used was a cloned line of C3H mouse fibroblasts developed by Reznikoff *et al.* (22, 23). This cell line is highly susceptible to postconfluence inhibition of cell division (22, 23), undergoes 2-stage carcinogenesis with TPA as the promoting agent (17, 18), and is transformed by X-ray treatment alone (29, 30).

MATERIALS AND METHODS

Experiments reported here utilized C3H/10T½ clone 8 cells (22, 23) between passages 7 and 14. We passaged the cells according to a modified 10T½ technique (subculturing confluent 60-mm Petri dishes every 7 days at a density of 0.5×10^5 cells) (30). When the cells are handled in this manner, no spontaneous transformation is observed in untreated control cultures. The cultural conditions and detailed methodology for these experiments have been described previously (30).

Irradiation was carried out at room temperature with a 100-kV constant potential Philips industrial X-ray generator operating at 10 ma and yielding a dose rate to the cells of 78 rads/min. Experimental dishes were irradiated 24 hr after being seeded with cell suspensions. Stock solutions of TPA (Consolidated Midland Co., Brewster, N. Y.) were made in spectrograde acetone (Aldrich Chemical Co., Inc., Milwaukee, Wis.) and were kept in amber bottles at −20°. TPA (0.1 μg/ml) was added to the medium so that the final concentration of acetone was 0.5%; this concentration of TPA was shown previously to be nontoxic and did not transform 10T½ cells (17, 18). After the initial addition of TPA to cultures, it was added to the medium of TPA-treated plates every time the medium was changed. Therefore, in all experiments described in this report, TPA was present for the entire 6-week expression period.

In the initial experiment, 4 radiation doses (25, 50, 100,

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[3] The abbreviation used is: TPA, 12-O-tetradecanoylphorbol-13-acetate.

and 400 rads) were used at 2 cell densities: the routine 300 to 400 viable cells/plate (30), as well as twice this number of viable cells. For the 50-rad point, an additional group was added with approximately 6 times the usual number, or 2400 viable cells. The 3 treatment groups at each dose of radiation were: (*a*) radiation alone, (*b*) radiation immediately followed by TPA treatment, and (*c*) radiation followed by TPA treatment beginning 96 hr later. Control cultures included groups treated with 0.5% acetone or TPA only. In additional experiments cultures were irradiated with 100 rads followed by TPA beginning either immediately after treatment or 48 or 96 hr later.

The morphology of colonies scored as transformants has been previously described (22, 30). Radiation transformation experiments such as those reported here usually result in, at most, 1 transformed focus/plate (*i.e.*, no colony seeding), thus allowing the expression of results as transformation frequencies, or transformants/surviving cell based on plating efficiency. We currently score types II and III foci separately. Previous results indicate that type III cells are tumorigenic in 80 to 100% of inoculated mice, while type II cells are tumorigenic in 60 to 75% of inoculated mice (22, 30).

RESULTS

The initial experiment utilizing 300 to 400 viable cells/plate showed a clear interaction between radiation and TPA, which was particularly marked when exposure to minimally transforming X-ray doses (50 or 100 rads) was followed by TPA treatment beginning immediately after the radiation exposure or 96 hr later. There was no great difference nor any general trend in transformation frequencies resulting from TPA addition immediately after radiation or at 96 hr later in any of the irradiated groups with subsequent TPA treatment. These results were confirmed in additional experiments with the use of 100 rads and subsequent TPA treatment beginning immediately after the radiation exposure or 48 or 96 hr later, as shown in Table 1. While 100 rads alone led to a nondetectable transformation frequency ($<3.3 \times 10^{-4}$), previous experiments have shown that 100 rads produce a transformation frequency of about 7.5×10^{-4} (30). X-irradiation (100 rads) with subsequent TPA treatment resulted in a transformation frequency of about 1.4 ± 0.1 (S.E.) $\times 10^{-3}$ (average of Groups 3 to 5 in Table 1), a 19-fold enhancement in transformation over 100 rads alone. As is shown in Table 1, the greatest proportion of transformed foci were of type II morphology following exposure to radiation and subsequent TPA treatment.

Table 1

Transformation frequencies for X-radiation and TPA with 300 to 400 viable cells/plate

Treatment	Experiment No.	Transformation frequency/surviving cell ($\times 10^{-4}$) Type III foci	Types II and III foci
1. 100 rads	1	<3.3	<3.3
	2	<1.9	<1.9
2. TPA only	1	<1.7	<1.7
	2	<4.0	<4.0
3. 100 rads + TPA immediately following radiation	1	6.0	12.0
	2	2.3	14.0
4. 100 rads + TPA 48 hr after radiation	3	1.0	11.0
	4	2.0	15.0
5. 100 rads + TPA 96 hr after radiation	1	2.3	16.0
	2	<3.3	15.0

X-ray transformation is greatly influenced by the number of cells plated: the transformation frequency dropped markedly as the initial cell inoculum was increased above 400 cells (30). Experiments were therefore designed that employed high cell densities (800 and 2400 viable cells/dish) with X-ray and TPA treatment to determine whether TPA had an effect on X-ray transformation in high-density cultures. The results of these experiments are tabulated in Table 2. In such high-cell-density cultures, TPA enhanced X-ray transformation at 400 rads and resulted in detectable transformation frequencies at the lower doses, where the transformation frequencies for radiation treatment alone were below the level of detection.

The data on transformation frequency for radiation exposure at different doses with and without subsequent TPA treatment at 400 and 800 viable cells/100-mm dish are shown in Chart 1 for a comparison with the data previously obtained for 600 rads alone (30). Since there was no difference in transformation frequencies (types II and III foci) with time of addition of TPA to the medium, the data for X-ray exposure with TPA treatment include experiments in which TPA was added immediately after the radiation exposure or 48 or 96 hr later. For both 400 or 800 viable cells/dish, increasing transformation frequencies resulted from increasing the dose of radiation for all X-ray exposures with subsequent TPA treatment. At 400 rads TPA treatment resulted in transformation frequencies higher than those usually observed for doses as large as 1500 rads, since a plateau was observed for transformants/surviving cell at doses greater than 600 rads (30).

As is shown in Chart 1, 400 rads yielded a measurable transformation frequency in these experiments; the enhancement by subsequent TPA treatment was 3-fold at 400 viable cells and 10-fold at 800 viable cells. The largest enhancement in transformation with TPA was with X-ray doses on the rapidly rising portion of the dose-response curve. For the radiation treatment alone at 25, 50, and 100 rads, transformation frequencies were below the level of detection at all 3 cell densities studied in these experiments. Data previously obtained (30) for 50 and 100 rads at 400 viable cells are indicated in Chart 1 so that the magnitude of enhancement of radiation transformation by TPA treatment can be easily seen. At 50 rads TPA treatment resulted in a 26-fold increase in transformation over radiation alone, and at 100 rads it resulted in a 19-fold increase.

DISCUSSION

It is clear from the experiments reported here that TPA can promote X-ray transformation *in vitro*, as has been shown previously for UV (18) and chemical carcinogens (17). TPA worked most effectively in enhancing X-ray trans-

Table 2

Effect of TPA on X-ray transformation with the use of high cell densities (800 to 2400 viable cells/plate)

	Transformation frequency/surviving cell (× 10^{-4})[a]									
	25 rads		50 rads				100 rads		400 rads	
	≈800 viable cells		≈800 viable cells		≈2400 viable cells		≈800 viable cells		≈800 viable cells	
Treatment	Type III foci	Types II + III foci	Type III foci	Types II + III foci	Type III foci	Types II + III foci	Type III foci	Types II + III foci	Type III foci	Types II + III foci
1. X-irradiation alone	<1.2	<1.2	<1.4	<1.4	<0.46	<0.40	<1.7	<1.7	1.4	1.4
2. TPA added immediately after X-irradiation	<0.71	2.1	0.76	2.3	<0.36	0.72	1.0	4.0	4.0	17.0
3. TPA added 96 hr after X-irradiation	<1.1	<1.1	2.2	8.7	<0.28	0.56	<1.1	8.0	3.8	9.4

[a] These results are from the experiment labeled 1 in Table 1.

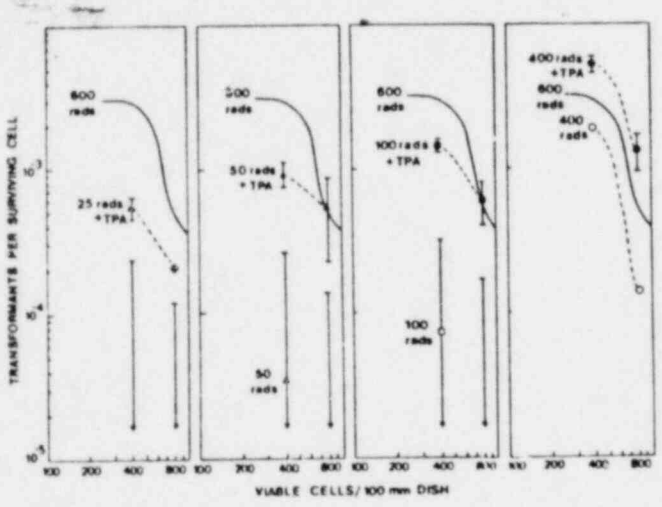

Chart 1. Transformants (types II and III foci) per surviving cell *versus* number of viable cells per 100-mm dish for 50 (△), 100 (□), and 400 (○) rads with and without TPA treatment. *Open symbols*, radiation treatment alone; *closed symbols*, radiation treatment followed by TPA. Increasing the radiation dose with TPA treatment led to an increase in total number of transformants per surviving cell. The *600 rad line* is drawn from previously published data (30) and shows the decrease in transformation frequency that normally results from an increase in the initial cell inoculum. At each radiation dose accompanied by TPA treatment, there was also a marked decrease in the transformation frequency, with 800 viable cells compared to 400 viable cells, which is similar to that observed for radiation alone. *Data points* for radiation plus TPA are the means ± S.E. from 2 experiments (adding TPA immediately or 96 hr later), except for the *100 rads-400 viable cells point*, which represents the mean ± S.E. from the 6 experiments utilizing radiation and TPA shown in Table 1. For the radiation-alone groups utilizing 25, 50, and 100 rads, the transformation frequencies were below the level of detection in these experiments (as indicated by the *top* of the *vertical arrows* at *bottom* of *graph*); the actual transformation frequencies reported previously for 50 and 100 rads (30) are also indicated. Compared with these transformation frequencies, there is a 26-fold increase with TPA treatment at 50 rads and a 19-fold increase with TPA at 100 rads over radiation alone at 400 viable cells. The transformation frequency increase for 400 rads followed by TPA compared to X-irradiation alone is less pronounced: there is a 3-fold increase at 400 viable cells and a 10-fold increase with 300 viable cells.

formation at doses of radiation that yielded very low levels of transformation by themselves. Similarly, TPA promotion was most effective when used with otherwise subeffective doses of UV (18) or chemical carcinogen (17) initiators.

There are, however, differences in the TPA effect on X-radiation-, UV-, and chemical carcinogen-induced transformation *in vitro*. TPA is considerably less efficient at enhancing X-radiation transformation than it is for UV- or chemical carcinogen-induced transformation. Although TPA most effectively enhanced transformation at low doses of X-radiation, increased transformation frequencies were also observed with X-radiation doses capable of inducing relatively high transformation frequencies (Chart 1). At doses of carcinogenic chemicals that induced high levels of transformation in 10T½ cells, the addition of TPA caused no further increase in transformation (17). Mondal *et al.* (17) found that immediate addition of TPA to the medium of 10T½ cells after chemical carcinogen treatment caused a significant inhibition of transformation over that observed for the chemical carcinogen alone or for later additions of TPA to the medium. We observed no significant differences in transformation frequencies when TPA was added immediately after X-radiation or at 48 or 96 hr following the radiation exposure, nor was there any such difference observed when TPA was added just after or at 48 or 96 hr after UV irradiation (18).

There are fundamental differences in the mechanism of action of X-rays, UV, and chemical carcinogens, which may account for the differences observed in the TPA enhancement of transformation *in vitro* induced by these agents. Ionizing and UV radiation can be differentiated by the classes of DNA damage produced by each and by the nature of the DNA repair processes that they induce (16). Many chemical carcinogens can be similarly differentiated. Regan and Setlow (21) recently showed that several specific chemical carcinogens could be separated into "X-ray-like" or "UV-like" groups depending on the type of DNA repair that they induce in human cells. The fact that promotion occurred when TPA was added as long as 96 hr after irradiation, however, suggests that promotion did not result from an effect of TPA on DNA repair processes; X-ray-induced DNA repair is usually completed within 1 to 4 hr of irradiation (16). Chemical carcinogenesis and types of DNA damage induced by chemical carcinogens have been reviewed elsewhere (13, 21, 25).

If only type III colonies are scored, TPA enhancement of X-radiation transformation is not as pronounced as that seen for UV radiation (18) or chemical carcinogen (17) transformation in 10T½ cells. This is also the case for promotion of radiation carcinogenesis *in vivo*: ionizing radiation (1, 26) with subsequent croton oil application was not as effective an inducer of mouse skin tumors as were UV (20) or chemical carcinogens (2, 3, 5, 6). However, when both types II and III colonies are scored as transformants, TPA does have a clear-cut enhancing effect on X-ray-induced transformation *in vitro* (Chart 1). By our classification 25 to 40% of type II foci are not tumorigenic in inoculated mice (30). The fact that radiation with subsequent TPA treatment produces mostly type II colonies is consistent with the action of promoting agents *in vivo*. Two-stage carcinogenesis in mouse skin involving a subeffective dose of a carcinogen with subsequent treatment by a promoting agent results primarily in benign papillomas (2, 3).

Thus, the data presented here indicate that promoting agents can increase the levels of X-ray-induced transformation *in vitro* just as they enhance carcinogenesis *in vivo* (2, 3, 5, 6). Promoting agents *in vivo* also shorten the latent period for cancer development (2, 3, 5, 6). There is evidence that TPA affects transformation *in vitro* similarly by reducing the time required for the expression of transformation. One of us (S.M.) has observed that transformed foci are visible at 4 weeks rather than at the usual 6 weeks; however, the scoring of experiments reported here was not done until the sixth week.

A possible mechanism for the early observation of transformed foci may be that TPA reduces the number of postirradiation cell divisions required for the expression of transformation. As shown in Chart 1, TPA increased the transformation frequencies over those observed for radiation exposure alone at both 400 and 800 viable cells/100-mm dish. Normally, we would expect a marked decrease in transformation frequencies at high cell densities (over 400 viable cells), an observation attributed to the fact that, on the average, cells must go through about 13 cell divisions to both fix and express the damage resulting in transformation (15, 30). Dense cultures, such as those in these experiments, will prohibit this number of cell divisions from occurring (15, 30). Even with 2400 viable cells/plate at the 50-rad dose level, the higher density cultures showed higher transformation frequencies with radiation and subsequent TPA treatment than they showed with radiation alone. These results suggest to us that TPA may reduce the number of cell divisions required for radiation transformation.

Promoting agents can increase the saturation density of some cell cultures (8, 27, 31). If TPA were causing an increase in saturation density in our experiments, a probable explanation for the increased transformation frequencies observed for radiation with subsequent TPA treatment over those observed for radiation alone in high-density cultures would be that the TPA-treated cells could go through more divisions than would normally occur, thus allowing full expression of the transformed phenotype. It has been reported recently that TPA can increase the saturation density of 10T½ cells 2- to 3-fold when the cells are grown in a rich medium (*i.e.*, Dulbecco's) (31). However, TPA has no effect on saturation density when Eagle's basal medium is used (19), as was done in our experiments.

An alternative explanation for our results is suggested by previous experiments with 3T3 cells, a mouse cell line similar to 10T½ cells. Sivak and Van Duuren (28) plated mixtures of normal and SV40-transformed 3T3 cells and showed that increasing the ratio of normal to transformed 3T3 cells caused increasing growth inhibition of the transformed cells. This inhibition was reversed by the addition of phorbol esters (28). Thus, at high cell densities the growth of transformed cells induced by X-rays might also be suppressed, and TPA might reverse the suppression.

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Apparent Rat Strain-related Sensitivity to Phorbol Promotion of Mammary Carcinogenesis[1]

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ABSTRACT

It has been reported that twice-weekly i.p. injections of 4 mg phorbol for 10 weeks, after a single feeding of 6 mg dimethylbenz(a)anthracene (DMBA) in female Wistar rats, led to a significant augmentation of mammary adenocarcinoma incidence and of lymphatic leukemia incidence as compared to 6 mg DMBA alone. In an experiment reported here, in female Sprague-Dawley rats, using the same doses of DMBA and phorbol and the same injection schedule, phorbol given after DMBA did not augment mammary adenocarcinoma incidence or lymphatic leukemia incidence as compared to DMBA given alone. It thus appears that there is a strain-related sensitivity between Wistar and Sprague-Dawley rats with regard to the promoting activity of phorbol when phorbol treatment follows DMBA treatment, and mammary adenocarcinoma incidence and lymphatic leukemia incidence are studied. Further, in Sprague-Dawley rats, phorbol did not promote mammary fibroadenoma incidence in DMBA-treated rats, mammary adenocarcinoma incidence in procarbazine-treated rats, and mammary adenocarcinoma incidence or mammary fibroadenoma incidence in X-ray-treated rats. DMBA and procarbazine, with or without phorbol, tended to induce more mammary neoplasms in the anterior (thoracic) than in the posterior (abdominal) mammary glands. X-irradiation tended to induce mammary neoplasms in approximately equal numbers in the anterior and posterior mammary glands. It was suggested that regional differences in chemically induced mammary carcinogenesis were due to a difference in the transport and delivery of the chemical carcinogens to the regions rather than a difference in the amount of mammary gland tissue in the regions. An analysis of the numbers of Sprague-Dawley rats that developed either no mammary neoplasms, or only mammary adenocarcinomas, or only mammary fibroadenomas, or both mammary adenocarcinomas and mammary fibroadenomas in response to DMBA, procarbazine, and X-ray, suggested that the development of a mammary adenocarcinoma or the development of a mammary fibroadenoma are independent processes.

INTRODUCTION

Mammary carcinogenesis in the rat is relatively easy to induce by several different chemical carcinogens and by various types of ionizing radiation (7). The rat mammary carcinogenic response to either chemical or physical carcinogenic agents may be enhanced by various hormonal treatments (12) or special diets (4). However, in the sense of the initiation-promotion hypothesis of carcinogenesis, there were no reports of promotion of chemical or radiation mammary carcinogenesis in the rat, in which the promoting agent was a true, nonhormonal, promoting agent, until the publication of Armuth and Berenblum (2). These investigators reported the promotion of mammary carcinogenesis after DMBA[3] in female Wistar rats by the i.p. injection of phorbol (phorbol is the unesterified parent alcohol of the cocarcinogenically active 12-*O*-tetradecanoylphorbol-13-acetate). Because of the great theoretical importance of the finding of Armuth and Berenblum, it was decided to see if the promotion of DMBA-induced mammary carcinogenesis, as observed by them, could be extended to another strain of rat, Sprague-Dawley, and extended in this strain of rat to the induction of mammary fibroadenomas and to an additional chemical carcinogen, procarbazine (5), and to ionizing radiation.

While the current experiment was in progress, Torgersen (10) reported that DMBA administered to female Sprague-Dawley rats induced more mammary adenocarcinomas in the anterior (thoracic) than in the posterior (abdominal) mammary glands. Since the protocol of the current experiment allowed a regional, anatomical analysis of the distribution of mammary neoplasia within individual rats, the various effects of phorbol, DMBA, procarbazine, and X-irradiation on the regional, anatomical distribution of mammary adenocarcinomas and mammary fibroadenomas were examined.

During the course of the experiment, it became apparent that of the rats that developed mammary neoplasia, some rats developed only mammary adenocarcinomas, some developed only mammary fibroadenomas, and some rats developed both mammary adenocarcinomas and fibroadenomas. These results were analyzed in an attempt to determine if the development of the 2 histologically different types of mammary neoplasms were an independent process or if the development of one type of mammary neoplasm was correlated with the development of the other type of mammary neoplasm in the same rat.

Since Armuth and Berenblum (2) also reported that phorbol promoted the leukemogenic action of DMBA, the thymus, liver, spleen, and lymph nodes of all rats were examined grossly, and leukocyte counts were performed on rats that received DMBA, DMBA and phorbol, phorbol, and nontreated controls.

MATERIALS AND METHODS

The protocol of Armuth and Berenblum (2) was followed generally except that female rats of the Sprague-Dawley strain were used in the current experiment rather than those of the Wistar strain. The Sprague-Dawley rats were purchased from Taconic Farms, Germantown, N. Y., and delivered to this laboratory on their 22nd day of age. They were maintained on

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[3] The abbreviation used is: DMBA, 7,12-dimethylbenz(a)anthracene.

commercial rat chow and water *ad libitum* in metal cages with corncob bedding, 5 rats/cage, under conditions of fluorescent light from 8 a.m. to 8 p.m. at 22 ± 2°. Each rat was given a numbered ear tag so that individual records could be kept for each rat. The experiment was started when the rats were 60 days of age with the administration of DMBA, procarbazine, or X-radiation. The first phorbol injection was given 1 week later. Beginning 1 week later, each rat was palpated weekly for the presence of mammary tumors. When mammary tumors were found, they were recorded as to location, using the nipples as reference points, counting in days after the 60th day of age. Mammary neoplasms were removed under ether anesthesia at a size of approximately 2 cm, and all mammary neoplasms were sectioned, stained, and given a classification of either mammary adenocarcinoma or mammary fibroadenoma according to criteria consistent with those of Young and Hallowes (13). If a second mammary neoplasm, of the same pathological classification, was found at the site of a previously removed neoplasm, it was not recorded as a second neoplasm unless a 10-week period had elapsed between removal of the first neoplasm and detection of the subsequent neoplasm. All rats were killed 304 days after starting the experiment and examined for gross pathology, including a visual examination of lymph nodes, thymus, liver, and spleen. Leukocyte counts were done on rats that received DMBA, DMBA plus phorbol, phorbol, and control, nontreated rats.

DMBA, 3 mg in 1 ml of sesame oil, was given by stomach tube at the rate of 3 mg/100 g of body weight (to the nearest 0.1 ml and 10 g of body weight) to rats with an average weight of 199 g.

Procarbazine (procarbazine hydrochloride, a gift from Roche Laboratories, Division of Hoffman-LaRoche Inc., Nutley, N. J.), 16.6 mg/ml of water, was given by stomach tube at the rate of 16.6 mg/100 g of body weight (to the nearest 0.1 ml and 10 g of body weight) to rats with an average weight of 200 g.

Total-body X-irradiation, either 100 or 300 R, was delivered at a rate of approximately 35 R/min, 250 kVp, 30 ma, 0.5 mm Cu, 1.0 mm Al, and a target-skin distance of 100 cm; and dosimetry was done with a 100 R Victoreen chamber on the 60th day of age.

Phorbol was purchased from the same source as used by Armuth and Berenblum (Dr. Theodor Schuchardt GmbH and Co., Munich, Germany). Each phorbol-injected rat received a total dose of 80 mg of phorbol i.p. beginning 1 week after carcinogen application and continuing for 10 weeks of twice-per-week injections. Each individual phorbol injection contained 4 mg of phorbol in 0.5 ml of phosphosaline buffer (0.01 M phosphate, pH 7.6, plus 0.15 M NaCl). The phorbol solution was mixed using a 1-min pulse of low-energy ultrasonication immediately before use. After the phorbol injections were completed, a sample of the phorbol used in the present experiment was examined with UV spectra and thin-layer chromatography, and the phorbol used in the present experiment was shown to be authentic phorbol.[*]

As a control for the phorbol injections, phosophosaline buffer was given in the same volume and on the same schedule as the phorbol injections, and these groups were designated phosphosaline treated.

Differences between incidence of rats with mammary neoplasia were evaluated for statistical significance by the χ^2 test (6), and the mean time of appearance of mammary neoplasia was evaluated by the t test (8). The independence of the appearance of mammary fibroadenomas and mammary adenocarcinomas was evaluated by the χ^2 test for independence (3).

RESULTS

The survival rate of all groups was excellent and was not different between groups (Table 1). For this reason, no corrections for intercurrent mortality were made.

Of the 236 carcinogen-treated rats, 92 developed no mammary neoplasms, 58 developed only mammary adenocarcinomas, 47 developed only mammary fibroadenomas, and 39 developed both mammary adenocarcinomas and fibroadenomas. Analysis of these data, as well as the data for and within each carcinogen-treated group, suggested that the development of mammary adenocarcinomas and mammary fibroadenomas appeared to be independent processes. Thus, we have chosen to analyze the following 3 measures of mammary carcinogenesis: rats with mammary neoplasia; rats with mammary adenocarcinomas with or without mammary fibroadenomas; and rats with fibroadenomas with or without mammary adenocarcinomas.

Phorbol treatment, after DMBA, procarbazine, or X-radiation, did not increase the mean number of mammary neoplasms, mammary adenocarcinomas, or mammary fibroadenomas per rat above the values for DMBA, procarbazine, or X-radiation, respectively (Table 1). No further analysis of the mean number of mammary neoplasms per rat was done.

DMBA treatment, with and without phosphosaline injection, was followed by an increased incidence of rats with mammary neoplasia, mammary adenocarcinomas, and mammary fibroadenomas as compared to nontreated controls (Table 1). Phorbol treatment after DMBA treatment did not modify any of these 3 measures of mammary neoplasia incidence, or the mean time of appearance of the neoplasms.

Procarbazine treatment, with or without phosphosaline injection, was followed by an increased incidence of rats with mammary neoplasia and mammary adenocarcinomas as compared to nontreated controls (Table 1). Phorbol treatment after procarbazine did not modify any measure of mammary neoplasia incidence or the mean time of appearance of the neoplasms.

X-irradiation, at a dose of 300 R with and without phosphosaline injection, was followed by increased incidences of rats with mammary neoplasia, mammary adenocarcinomas, and mammary fibroadenomas as compared to nontreated controls (Table 1). Phorbol treatment after 300 R did not modify any measure of mammary neoplasia incidence or the mean time of appearance of the neoplasms. X-irradiation, at a dose of 100 R without phosphosaline injection, increased the incidence of rats with mammary neoplasia as compared to nontreated controls (Table 1). Phorbol treatment after 100 R did not modify any measure of mammary neoplasia incidence or mean time of appearance of the neoplasms.

Phorbol, by itself, had no influence on any measure of mammary neoplasia incidence, and phosphosaline injections were also without effect (Table 1).

Mammary neoplasia tended to occur more often in the anterior half than the posterior half of the rats given DMBA or

[*] The authors wish to thank Dr. Walter Troll for these examinations.

Table 1

Survival rate and incidence of mammary neoplasia

Treatment	No. of rats			Rats with mammary neoplasia		Rats with mammary adenocarcinomas		Rats with mammary fibrocarcinomas		All mammary neoplasms		All mammary fibroadenomas		All mammary adenocarcinomas	
	Start	End	Days of study	Total	%	Total	%	Total	%	Total	Per rat	Total	Per rat	Total	Per rat
DMBA	20	16	292 ± 46[a]	15[b]	75	10[b]	50	12[b]	60	43	2.2	26	1.3	17	0.8
DMBA + phosphosaline	19	17	285 ± 60	15[c]	79	10[c]	53	12[c]	63	46	2.4	32	1.7	14	0.7
DMBA + phorbol	20	19	292 ± 53	15[d]	75	10[d]	50	10[d]	50	36	1.8	20	1.0	16	0.8
Procarbazine	18	18	304	12[b]	67	11[b]	61	3	17	23	1.3	3	0.2	20	1.1
Procarbazine + phosphosaline	20	16	278 ± 62	15[c]	75	12[c]	60	5	25	30	1.5	6	0.3	24	1.2
Procarbazine + phorbol	20	18	301 ± 11	10[d]	50	8[d]	40	3	15	14	0.7	3	0.2	11	0.6
300 R	19	19	304	18[b]	95	11[b]	58	13[b]	68	40	2.1	23	1.2	17	0.9
300 R + phosphosaline	20	19	303 ± 2	13[c]	65	8[c]	40	11[c]	55	31	1.6	18	1.0	13	0.6
300 R + phorbol	20	18	291 ± 54	14[d]	70	10[d]	50	7[e]	35	29	1.4	13	0.6	17	0.8
100 R	20	19	303 ± 6	8[f]	40	4	20	4	20	9	0.4	4	0.2	5	0.2
100 R + phosphosaline	20	19	303 ± 6	5	25	1	5	4	20	6	0.3	5	0.2	1	0.1
100 R + phorbol	20	20	304	4	20	2	10	2	10	5	0.2	3	0.2	2	0.1
None	14	14	304	0		0		0							
Phosphosaline	20	20	304	0		0		0							
Phorbol	20	20	304	1	5	0		1	5	1	0.1	1	0.1		

[a] Mean ± S.D.
[b] Different from no treatment; χ^2, $p < 0.01$.
[c] Different from phosphosaline treatment: χ^2, $p < 0.01$.
[d] Different from phorbol treatment: χ^2, $p < 0.01$.
[e] Different from phorbol treatment: χ^2, $p < 0.05$.
[f] Different from no treatment; χ^2, $p < 0.05$.

procarbazine, but occurred randomly in the inrradiated rats. The actual anterior-posterior number of mammary neoplasms was: DMBA: anterior, 81; posterior, 44. Procarbazine: anterior, 42; posterior, 25. X-ray: anterior, 63; posterior, 57.

No indication of leukemia, as judged by the enlargement of the lymph nodes, thymus, spleen, and liver, was found in any animal. The leukocyte count was not different among groups that received DMBA, DMBA and phorbol, phorbol, or no treatment.

DISCUSSION

It seems clear that the promoting effect of phorbol on DMBA-induced mammary adenocarcinoma formation, as observed by Armuth and Berenblum (2) in female Wistar rats, was not found in female Sprague-Dawley rats in the present experiment. Also, the promoting effect of phorbol on DMBA-induced leukemia reported by Armuth and Berenblum in Wistar rats was not found in the present experiment with Sprague-Dawley rats. The longer follow-up period in the present experiment was adequate to disclose that there was no promoting effect of phorbol on mammary fibroadenoma formation in Sprague-Dawley rats. The experimental protocol of the 2 experiments was almost the same with the obvious exception of rat strain difference. The most probable, and the most obvious, explanation for the positive promoting effect of phorbol on DMBA-induced mammary adenocarcinoma formation in Wistar rats, reported by Armuth and Berenblum (2), and the lack of promoting effect on the same response in Sprague-Dawley rats, noted in the current experiment, has to do with inherent differences in the 2 strains themselves. Support for the strain difference explanation has been provided by Armuth (1), since he reported that phorbol induced leukemia in only 1 of 7 strains of mice, accelerated the appearance of reticulum cell sarcomas in another of the 7 strains, and, combined with thymectomy, induced leukemia in yet another strain. Other aspects of mammary carcinogenesis (7) and leukemogenesis (9) in the rat are known to exhibit strain-related differences; therefore, it should not be surprising if promotion also exhibits strain-related sensitivity. The biological and biochemical mechanisms that would allow phorbol to exhibit promoting activity in Wistar rats but not in Sprague-Dawley rats are poorly understood but might involve whether or not a particular strain has the capacity to esterify phorbol, thus transforming the weakly active phorbol to the strongly active esterified phorbol.

The failure of phorbol to promote procarbazine-induced or X-ray-induced mammary carcinogenesis in Sprague-Dawley rats cannot be taken as proof that phorbol has no promoting activity for these 2 agents because of the lack of a "positive control" (11). The promoting activity of phorbol on DMBA-induced mammary carcinogenesis was demonstrated (2) in Wistar rats. In the absence of demonstrated phorbol-promoting activity on DMBA-induced mammary carcinogenesis in Sprague-Dawley rats, it is not clear whether the lack of phorbol-promoting activity in regard to procarbazine and X-irradiation is due to an intrinsic lack of promoting activity of phorbol itself or due to the incapability of Sprague-Dawley rats to exhibit the phenomenon of promotion with this agent.

In the present experiment, the analysis of mammary carcinogenesis has been divided into 3 separate analyses; mammary adenocarcinoma formation; mammary fibroadenoma formation; and mammary neoplasia formation of either type. This was done in part because the 2 types of mammary neoplasms display different latent periods as well as different histological characteristics (13) and because a statistical test for independence suggested that the 2 types of mammary neoplasms oc-

curred independently. Presumably, this means, in the Sprague-Dawley rats at least, that there are some rats which are at risk in response to DMBA, procarbazine, and x-Irradiation for the development of mammary adenocarcinomas and some other rats which are at risk for the development of mammary fibroadenomas, and some other rats which are, presumably by chance, at risk for developing both types of mammmary neoplasms. It would seem profitable to profile and compare, in an endocrinological and an immunological sense, the rats that develop only mammary adenocarcinomas to the rats that develop only mammary fibroadenomas in order to gain additional insight of the factors that modify the mammary carcinogenic response to chemical carcinogens and to radiation.

It has been reported (10) that more mammary neoplasms appear in the anterior than in the posterior mammary glands of Sprague-Dawley rats following DMBA administered by the i.v. route. The general tendency in the present experiment was to find about twice as many mammary adenocarcinomas and mammary fibroadenomas in the anterior than in the posterior mammary glands following DMBA given p.o. Procarbazine also had the tendency to produce more mammary adenocarcinomas in the anterior than in the posterior mammary glands but too few mammary fibroadenomas for analysis. In contrast, X-irradiation tended to produce approximately equal numbers of both types of mammary neoplasms in anterior and posterior mammary glands. It seems reasonable to assume that the magnitude of a carcinogenic response depends directly upon both the size of the carcinogenic stimulus and the amount of tissue undergoing interaction with the carcinogenic stimulus. Since total-body irradiation with X-rays must deliver the same amount of carcinogenic stimulus to both the anterior and the posterior mammary glands and since the carcinogenic response of the anterior and posterior mammary glands to X-irradiation was approximately equal, it is possible to conclude that the relative amount of mammary tissue in the anterior and posterior portions of the rats must be approximately equal. If the equality of the carcinogenic response in the anterior and posterior mammary glands in response to X-irradiation can be taken to indicate an equal amount of mammary tissue in both regions, then the larger response to DMBA and to procarbazine of the anterior mammary glands must be taken to mean that there was a larger carcinogenic interaction with the chemical carcinogens in the anterior than in the posterior mammary glands. This conclusion implies that a larger carcinogenic stimulus from DMBA p.o. and procarbazine p.o. was delivered to the anterior than to the posterior mammary glands, perhaps because of different clearance rates or other vascular differences of the 2 areas. It would appear relatively easy to compare the distribution of chemical carcinogens to the anterior than to the posterior mammary glands by use of isotope-labeled carcinogens to verify the suggestion that regional differences in carcinogenesis in response to chemical carcinogens are due to a difference in delivery of the carcinogens to the regions rather than a difference in amounts of mammary tissue in the regions.

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