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MONITORING GUIDELINES FOR THE CONDUCT OF CARCINOGEN BIOASSAYS

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
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Bethesda, Maryland 20205

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Comments and questions about the National Toxicology Program Technical Reports on Carcinogenesis Bioassays should be directed to Ms. Joan Chase, Technical Information Section, Room A-306, Landow Building, Bethesda, MD 20014 (301-496-1152).

PREFACE

The Carcinogenesis Testing Program simultaneously conducts bioassays on hundreds of chemicals through the use of extramural contracts. The Program includes a major quality assurance effort intended to ensure the accuracy of the findings from these bioassays. It extensively monitors laboratory performance, both on and off site, to maintain high standards (including good laboratory practices), state of the art laboratory methods, and conformance with contractual requirements. This document provides formal guidelines for monitoring carcinogen bioassays, particularly in small rodents.

Preparation of this document began with a discussion group on monitoring guidelines that was conducted at the National Cancer Institute on June 18-19, 1979. Comments from the group were reviewed, organized, and incorporated into a draft document that was circulated to participants, members of the National Toxicology Program, other Federal agencies, and industrial scientists skilled in monitoring laboratory performance.

Although there is some redundancy between scientific disciplines, duplication was deliberately retained for the sake of completeness within each area.

We appreciate the assistance of the following participants in the discussion group on monitoring guidelines and reviewers of the final draft of the text, and we hope that their practical ideas and experience will be useful to those who monitor carcinogen bioassays and related studies. We are especially grateful to Dr. Cipriano Cueto, Jr., for so elegantly serving as chairman.

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I. GENERAL MONITORING GUIDELINES

A. OBJECTIVE

The main objective in the monitoring of *in vivo* carcinogenesis bioassays is to ensure the quality and integrity of the bioassays and the safety of the personnel assigned to the program. Monitoring is intended to ensure that the tests are adequately conducted according to a specific protocol and that the data as reported are valid.

B. TYPES OF MONITORING

1. *Offsite* monitoring requires an assessment of the program from written reports, computerized data, and telephone conversations with the personnel assigned to the studies at the contracting laboratory. Such audits of the data may be used to follow the performance of an assigned task and to verify the adherence to protocols. They cannot be substituted for frequent onsite monitoring, and they should precede and follow onsite monitoring visits.

2. *Onsite* monitoring is essential to assess various aspects of the program that cannot be accomplished by offsite means. Regular visits to the laboratory are made to inspect facilities, discuss and review specific protocols, standard operating procedures, and the entire program with the key technical personnel, observe techniques, and inspect recordkeeping, training programs, and safety and quality assurance procedures.

C. SELECTION AND PREPARATION OF MONITORING TEAM (Onsite)

The principal NCI laboratory monitor, in conjunction with program management, will organize and specify the objective of the site visit. The success of an onsite monitoring operation is dependent on the selection of monitoring personnel who can accomplish the specified objectives of the visit and followup on identified action items. To accomplish these objectives, the monitoring team should have the following items prepared at the appropriate time:

1. A history of the laboratory, including

    a. Statement of work contained in the contract
    b. Monthly, quarterly, and annual progress reports
    c. Previous on site monitoring reports
    d. Information on the status of significant and/or unresolved action items
    e. Milestone status
    f. Financial status

2. A previsit briefing package that includes

   a. The date and time of the proposed visit
   b. Travel and accommodation arrangements
   c. The purpose of the visit
   d. The proposed agenda
   e. The names, disciplines, positions and/or responsibilities, of key laboratory personnel
   f. A map indicating location, address, and phone numbers of the laboratory
   g. A floor plan of all facilities participating in the program
   h. Company organization charts and the curricula vitae (CV's) of key personnel
   i. Program organizational charts within the company
   j. A list of the chemicals assigned to the laboratory with

      (1) An outline of basic protocols
      (2) An outline of special protocols
      (3) Milestones
      (4) Problems encountered

   k. Previous monitoring reports (for the last 12 months), including

      (1) Action items
      (2) Followup on each action item

   l. The latest monthly report from the laboratory
   m. New developments at laboratory requiring special attention
   n. New developments in program related to laboratory
   o. Financial status of each bioassay to include

      (1) Over or under runs
      (2) Data on the prompt submission of vouchers

3. Information from the laboratory's principal investigator regarding

   a. Time of visit
   b. Main objective of visit
   c. Program agenda of visit
   d. Participants

4. A schedule for a previsit conference (with NCI/NTP laboratory monitor, discipline monitors, chemical managers, program management) to:

   a. Define visit objective
   b. Identify problem areas
   c. Delineate any specific areas to be covered by each member
   d. Discuss the critical items in the briefing package
   e. Coordinate travel

5. A monitoring program agenda, including

   a. Definition of purpose(s) (discipline/program/review/problem solving)
   b. Laboratory staff presentation for status of program
   c. Laboratory inspection
   d. Record inspection
   e. One-to-one discussion between laboratory personnel and the NCI/NTP site visit team member
   f. Executive session
   g. Debriefing of laboratory on findings

6. A summary report of the site visit by the monitoring team

   This report will be drafted by the laboratory monitor from the minutes of the debriefing meeting and circulated to the site visit team for review and comments. The report will be clearly marked as a draft until it is accepted by the project officer. The report should include the following:

   a. Date of visit
   b. Laboratory visited
   c. Purpose of visit
   d. Participants (monitoring team and laboratory personnel with each discipline indicated)
   e. A general statement of overall findings (facts, not opinions), including critical action items and recommendations (See also 7. below)
   f. A statement on chemical tracking whenever this is done
   g. Requests and/or suggestions made by contractor
   h. Distribution of report:
      1) A copy of the draft report to the NCI laboratory manager, discipline monitors, and branch chiefs involved
      2) A discussion of the draft report by above-named personnel
      3) A statement of acceptance of final report by laboratory monitor (Signed by all NCI members of site visit team)
      4) A copy of the report for each monitoring team participant, central file, the principal investigator, and the contracting officer

7. Action items

   The action items necessary to correct deficiencies or increase performance level should be handled as a separate entry in the site visit report.

   a. Discussion on the resolution of previous action items
   b. Discussion of unresolved previous action items, including date of first observation, rationale for why it has not been resolved, and current correction planning
   c. New action items and who is responsible for their resolution

It is mandatory that the laboratory monitor see that all action items are corrected. Where necessary, he/she should enlist scientific disciplines or other inputs as needed.

II. INFORMATION TO BE OBTAINED AT THE PREAWARD SITE VISIT

The purpose of the preaward site visit is to evaluate the potential of a laboratory to conduct carcinogenesis and toxicology testing and to detect areas in the proposed program that need improvement. The following list of questions is designed to provide an outline that can be used to collect important information during preaward site visits on personnel, facilities, protocols, and record keeping as they relate to the bioassay program of contract laboratories. If the questions are carefully answered, the information gathered should allow the NTP laboratory monitor to assess accurately the capabilities of the laboratory and to pinpoint potential problem areas. It is essential, therefore, that all items in the list of questions be thoroughly studied.

A. CONTRACTOR'S ORGANIZATION

1. Is the program organized by the contractor adequate to ensure a quality work performance?

2. Will management adequately support the principal investigator and the program?

3. Is the principal investigator fully qualified in terms of scientific background and experience?

4. Will the appropriate support services for the program needs be efficiently provided to the principal investigator? Are they under his/her direct control?

B. ANIMAL CARE

1. Is there a veterinarian responsible for the institution's animal care program?

2. What are his/her qualifications for this responsibility?

   a. What advanced degree or training does the veterinarian have?

   b. Is the veterinarian certified? If so, by what organization?

3. Is he/she a full-time employee, part-time employee, or consultant?

4. What will be his/her participation in this program?

5. Are the animal care technicians certified by AAALAC?

   a. If so, how many are certified as:

      Assistant laboratory animal technicians?
      Laboratory animal technicians?
      Laboratory animal technologists?
      How many hold college degrees?

b. If not, is there an ongoing training program to prepare animal care personnel for animal technician certification?

6. Is the facility AAALAC accredited?

a. If so, what is the

Date of original accreditation?
Date of last site visit?

b. If not,

Has accreditation been applied for?

7. Inspect the facility on the basis of standards of: The Guide for Care and Use of Laboratory Animals, DHEW Publication No. NIH 78-23, 1978, for sale by the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402; and The Guide for Long Term Holding of Laboratory Rodents, ILAR News, Volume XIX, Number 4, 1976.

a. Does the laboratory have standard operating procedures (SOP's) for care of animals in a long-term carcinogenicity study?

b. Does the combination of facilities and management provide an environment that minimizes the entrance of pathogenic organisms into the animal cages and the exit of test substances from the cages?

c. Does the laboratory have a diagnostic laboratory?

d. What routine health screening procedures are used to minimize the introduction of pathogens into the facility?

8. Protocols and recordkeeping (See also Section E-3 a,b,c, & d)

C. CHEMISTRY

1. Personnel

a. What are the backgrounds of the responsible chemist and chemical support staff?

(1) What is their experience in analytical chemistry or in performing analyses similar to those required in the program?

(2) What experience do they have in analyzing and handling carcinogenic material?

b. What are the backgrounds of the supervisor and technicians responsible for dose preparation?

(1) What experience do they have in preparing dosage mixtures for chronic studies?

(2) What is their experience in handling chemicals and carcinogenic materials?

c. Is there a SOP for briefing technicians (i.e., a training program) on the proper handling of program chemicals?

2. Management

a. What is the relationship of the analytical chemistry department with the bioassay program?

(1) Is the analytical chemistry department providing a service to the bioassay program, or is it a part of the bioassay program?

(2) If the chemistry department is separate from the bioassay program, is it willing to meet the requirements and time deadlines specified for the bioassay program?

b. Is the responsible chemist knowledgeable of all the chemistry requirements of the bioassay program?

c. Has the responsible chemist discussed the scheduling of sample submissions (dosage mixtures, bulk chemical, corn oil) with the bioassay principal investigator, and can the schedule be met?

d. Who is responsible for preparing reports on the analyses performed for the bioassay program?

(1) Are they reviewed for accuracy?

(2) Who receives copies of these reports?

e. What quality assurance procedures are followed by the analytical chemistry laboratory?

(1) Are SOP's available for laboratory activities?

f. Has the chemist reviewed the procedures used for dosage preparation activities?

g. Does the supervisor for dosage preparation activities know all the chemical/vehicle requirements of the bioassay program?

(1) Has a training program for briefing dosage preparation technicians on all bioassay chemicals and chemical/vehicle mixtures been set up?

(2) Have emergency procedures addressing such situations as chemical exposure or spills been established?

3. Facility and Equipment

a. Analytical facility

(1) What analytical instrumentation is available for use on the bioassay program?

- What is the maintenance schedule and quality assurance procedure used for each piece of equipment?

(2) Are the analytical laboratories designed and utilized in a safe and efficient manner? (See also Health and Safety)

- Can the facility handle carcinogenic materials?

(3) Is there adequate cold storage (at 5° and -20°C or lower) for bulk chemical reference standards? (See also Toxicology)

(4) How are wastes generated in the analytical facility disposed of? (See also Health and Safety)

b. Dosage preparation facility

(1) What equipment is available for use on the bioassay program?

(a) What are the number and sizes of Patterson-Kelley "V" blenders for dosed feed studies?

(b) What type of balances are to be used?

(c) What equipment will be used for preparing gavage mixtures? Skin painting mixtures?

(d) What is the maintenance schedule and quality assurance procedure used for each piece of equipment?

(2) Is the dosage preparation area designed and utilized in a safe and efficient manner? (See also Health and Safety)

(a) Can the facility handle carcinogenic materials?

(b) Is there a "breathable air" line available for use with an air-supplied respirator?

(c) Is it located inside the barrier facility?

(d) If not, how will dosage mixes be brought into the facility?

(3) Is there adequate storage (both at ambient and 5°C temperatures) for both bulk chemical and chemical/vehicle mixes and dosed diets?

(4) How are wastes generated in the dosage preparation area disposed of? (See also Health and Safety)

(5) How will dosage mix samples be taken and transferred to analytical chemistry for analysis?

(6) How will the dosage mixes be transferred from the mixing vessel to the storage container?

(7) How will the dosage mixes be stored and labeled?

- If the dosage mixes are stored, what quality assurance procedure will be used to ensure that dose levels, etc., are not mixed up during distribution for dosing?

(8) How will dosage mixes be distributed to the animal rooms?

c. Shipping and Receiving

(1) What is the SOP for receipt of bulk chemicals and transfer to the bioassay facility?

- What procedures will be used to report receipt of shipments?
- How will unacceptable shipments be handled?

d. Inhalation facilities

(1) What capabilities exist for generation of gases? Of aerosols?

(2) What instrumentation is available to monitor chamber concentrations?

(3) Is there adequate storage space for large numbers of gas cylinders or other containers of bulk chemicals?

D. HEALTH AND SAFETY

Review/Approval of Laboratory Health and Safety Plan: Plans should be reviewed as the laboratory enters the program and periodically thereafter, reflecting changes/improvements as circumstances dictate. Each laboratory's health and safety plan should contain, as a minimum, consideration of the following:

1. Administrative Aspects

    a. Clear definition of health/safety responsibilities, personnel qualifications
    b. Facility design/operation
    c. Medical surveillance
    d. Material inventory, disbursement (SOP required)
    e. Work area access control (SOP required)
    f. Work area hazard evaluation
    g. Emergency contingency plans (e.g., power failure, fire/explosions, severe weather, spills, accidents) (SOP required)
    h. Housekeeping practices (all areas) (SOP required)
    i. Waste disposal (SOP required)
    j. Recordkeeping (SOP required)
    k. Training and evaluation
    l. Compliance procedures (occupational, environmental)

2. Engineering Aspects

    a. Local and area exhaust ventilation
    b. Personnel protective devices/equipment
    c. Facility design/operation, shipping/receiving areas, bulk chemical storage, analytical chemistry, dosage preparation (e.g., access to utilities, barriers), and personal hygiene areas
    d. Waste treatment and disposal (e.g., incineration, waste water control)

3. Personnel Protection Aspects

    a. Work practices (e.g., equipment operation, proper laboratory techniques, housekeeping, personal hygiene) (SOP required)
    b. Personnel protection program (e.g., clothing respirators, emergency plans) for specific work areas (SOP required)

4. Before testing begins, a site visit should be conducted to ensure that all health and safety programs are operational.

E. PATHOLOGY

It is desirable, but not mandatory, that the evaluator be an experienced pathologist. Although some of the questions can be answered by a layman, many others require a specific knowledge of pathology and related techniques.

1. Personnel

   a. Pathologist(s)

      (1) Is there a full-time pathologist(s) at the laboratory?

      (2) How many hours per week does the pathologist devote to the study?

      (3) What advanced degree or training does the pathologist have?

      (4) How many years of postdoctoral experience does the pathologist have?

      (5) How many years of postdoctoral experience in rodent pathology does the pathologist have? Does this include tumor and aged rodent experience?

      (6) Is the pathologist certified? If so, by what organization?

      (7) How many years has the pathologist been at the laboratory?

   b. Prosectors

      (1) How many prosectors are available to work on the project?

      (2) Do any of the prosectors serve in other capacities (animal caretaker, histotechnician, etc.)? If so, specify the task and respond to the following questions:

      (a) How many weeks does each prosector spend in these activities?

      (b) How many hours per week are spent with formal lecture, formal training, on-the-job training?

      (c) How many prosectors have been at the laboratory for 1 to 3 years? More than 3 years?

      (d) How many prosectors have had less than 1 year of experience performing necropsies on laboratory rodents? 1 to 3 years? More than 3 years?

   c. Histology Technicians

      (1) How many technicians are available to work on the project?

      (2) Do any of the technicians serve in other capacities?

      (3) Is the histology supervisor certified by the American Society of Clinical Pathologists (ASCP)?

(4) How many of the technicians are certified by ASCP?

(5) Is there a training program for technicians at the laboratory? If so, how long does it last? Who supervises the training?

(6) How many technicians have less than 1 year experience? 1 to 3 years? More than 3 years?

(7) How many of the technicians have worked at the laboratory for less than 1 year? 1 to 3 years? More than 3 years?

d. Clinical Laboratory

(1) What degree does the direct supervisor of the clinical laboratory hold?

(2) Is the supervisor certified? If so, by whom?

(3) How many certified medical technologists are employed in the laboratory?

(4) How many technicians employed in the laboratory have less than 1 year experience? 1 to 3 years? More than 3 years?

2. Facilities and Equipment

a. Necropsy Room

(1) Is the necropsy room adequate?

(2) Is the room used for any other purpose? (If so, what?)

(3) How many individual work stations are there?

(4) Are the stations ventilated?

(5) Is there a vented hood for general purposes?

(6) What type of lighting is used?

(7) Is the lighting adequate for close work?

(8) Is there a balance in the necropsy room? What type is it? How frequently is it calibrated?

(9) Is there a calibration log book?

(10) How many sinks are there in the room? Are they conveniently located?

(11) Are the following pieces of equipment available in the necropsy room:

- Refrigerator?
- Cutting boards? (Specify number)
- Dissecting microscopes?
- Gross photography equipment?

(12) Are the numbers and quality of dissecting instruments adequate?

(13) Are the following types of personal equipment available for the prosectors:

- Gloves?
- Masks? (Specify type)
- Safety glasses?
- Caps?
- Smocks, lab coats, etc.?

Is the equipment properly maintained and cleaned?

b. Histology Laboratory

(1) Is there adequate laboratory space?

(2) Is there adequate bench space?

(3) Is ventilation adequate in the following areas:

- Trimming?
- Processing?
- Embedding?
- Staining?

(4) Is the lighting adequate for close work in the work areas?

(5) How many processors are present in the laboratory?

(6) What type of processors are present?

(7) How many embedding centers are present? Are they vented?

(8) What methods of embedding are used?

(9) Is staining done manually or automatically? (If automated, specify the number, type of equipment.)

(10) How many and what type of microtomes are present?

(11) Is a cryostat present? (If so, what type?)

(12) How many and what types of knife sharpeners are present?

(13) How many and what types of balances are present?

(14) Are there safety cabinets for storage of flammable chemicals?

(15) Is there a room for storage of wet tissues and blocks?

(16) Is the room used for any other purpose? (If so, specify.) How large is the room? Is access to the room controlled? Is the temperature and humidity in the room controlled?

c. Clinical Laboratory

(1) How many square feet in the laboratory are devoted to hematology?

(2) How many square feet are devoted to clinical chemistry?

(3) What types of automated equipment are used for hematological tests?

(4) What types of automated clinical chemistry analyzers are available?

3. Protocols and Recordkeeping

a. How long are animals quarantined before they are put on test?

b. On what proportion of the quarantined animals are the following types of observations made:

(1) Clinical?

(2) Gross necropsy?

(3) Microbiological?

c. Who evaluates the data obtained from quarantined animals to determine if they are suitable for testing?

d. Who makes the day-to-day clinical observations on test animals? Where are the observations recorded?

e. Necropsy

(1) To complete parts of this section, it will be necessary for a qualified pathologist to observe completed Individual Animal Data Records (IADR's) as well as complete necropsies by as many of the prosectors as time permits.

(a) Is a list of SOP's readily available in the necropsy room?

(b) Is there a necropsy log?

(c) Is a pathologist present for all scheduled necropsies (subchronic and chronic)?

(d) Is a pathologist present for all unscheduled necropsies? If not, who supervises and conducts necropsies?

(e) If an animal dies on a weekend, is it stored until the following week or necropsied immediately?

(f) How are dead animals stored?

(g) Are pathologists available on weekends?

(2) Observe one or more of the prosectors doing a complete necropsy.

(a) Is the necropsy procedure adequate? (If not, specify.)

(b) Does the procedure deviate from NCI guidelines? (If so, specify.)

(c) How long are tissues fixed? Is there sufficient fixative (there should be 3 to 5 times as much fixative as tissue)?

(d) Where are tissues stored during fixation?

(e) Are labels legible?

(f) Can tissues readily be retrieved?

(3) Ask to see certain specific IADR's.

(a) Were the forms easily located?

(b) Are the forms legible?

(c) Are the forms complete?

(d) Do descriptions of lesions include the location, size, shape, and color when appropriate?

(e) Are all gross and clinical observations accounted for by histopathologic diagnoses?

(f) Who checks IADR and CBDS tables for accuracy?

f. Histology

(1) Is a list of SOP's readily available in the histology laboratory?

(2) Is there a histology log?

(3) Are all handlers of the tissues identified?

(4) Does an individual technician perform all steps (from trimming through coverslipping) on a given animal's tissues, or do different technicians complete the separate steps?

(5) Who trims the tissues? Is a pathologist available if necessary?

(6) Are additional gross observations made during tissue trimming?

(7) How are tissues identified to insure proper labeling of blocks and slides? Are standard NCI bioassay labeling methods used?

(8) How are blocks identified?

(9) Are slides permanently labeled by etching or other non-removable mark?

(10) Are blocks sealed with paraffin after sectioning?

(11) Are stock solutions dated and initialed?

(12) Are positive controls prepared when special stains are requested?

(13) Are all slides checked against the necropsy record to insure that all submitted tissues have been sectioned?

(14) Ask to see certain specific slides, blocks, and tissues.

(a) Was the material easily located?

(b) Are the sections free from artifact?

(c) Are all tissues specified in the necropsy report accounted for on the slides?

(d) Is there a master inventory for stored slides?

(e) What procedure is required to obtain release of slides?

g. Histopathologic Evaluation

(1) What materials are made available to the pathologist along with the slides?

(2) Are the slides read in a particular sequence?

(3) Are the slides read without knowledge of the group to which they belong?

(4) How does the pathologist record his/her diagnosis?

(5) Is there an in-house quality assurance (QA) program? If so,

(a) Who makes the original evaluation?

(b) Who reviews the slides for QA?

(c) What is reviewed?

F. TOXICOLOGY

1. Administration

a. Are the key people qualified for the bioassay program?

(1) Is the toxicologist

- Experienced with rodent studies?
- Experienced with long-term chronic studies?
- Experienced with carcinogens?
- Experienced with feeding, gavage, dosed water, skin painting, inhalation?
- How many hours per week does a toxicologist devote to the study?
- What advanced degree or training does the toxicologist have?
- Is the toxicologist certified? If so, by what organization?
- How many years has the toxicologist been at the laboratory?

(2) Is the clinical chemist

- Experienced in hematology?
- Experienced with automated microanalysis techniques for various markers in blood and urinalysis?
- Experienced in clinical enzymology?

(3) Are personnel available in special disciplines as required:

- Immunology?
- Pharmacokinetics?
- Biochemistry?
- Residue analysis in biological samples?

(4) Are experienced personnel available for dose preparation?

b. Is there a designated QA Unit?

(1) Are there SOP's for various toxicologic operations, i.e. gavage?

(2) Who writes and reviews SOP's?

(3) How often are the SOP's updated?

c. Is there a safety committee that reviews toxicology activities?

(1) Who serves on this committee?

(2) To whom does it report?

(3) How often does it meet?

2. Facilities and Equipment

a. Is the facility properly designed for toxicology studies?

(1) Is management willing to make changes in the facility to insure the integrity of the bioassay program?

(2) Does the facility design allow for controlled access, and are SOP's in existence as to how the facility is used to insure controlled access?

(3) Has the flow of the following been considered:

- Personnel?
- Equipment?
- Laundry?
- Bulk chemicals?
- Dose preparations?
- Contaminated material?
- Incoming animals?
- Animal carcass?
- Other waste?

b. Is general housekeeping adequate for the following areas:

- Offices?
- Halls?
- Personnel hygiene stations?
- Laboratories?
- Animal rooms?
- Storage areas?
- Dose preparation laboratories?
- Cage/washing facilities?
- Disposal/pick up sites?

c. Are there adequate storage areas for:

- Bulk chemicals (Room temperature, +5°C, -20°C)?
- Dose preparations (+5°C and room temperature)?
- General supplies?
- Feed and bedding?
- Clean cages and racks?
- Analytical tissue samples/sera (-20°C)?
- Animals for necropsy (+5°C)?
- Animal carcasses (-20°C)?

d. If the facilities are equipped for inhalation toxicology, determine the following:

(1) What type of chamber equipment is available?

(2) How many chambers are available?

(3) What size are the chambers?

(4) What types of generation procedure are available?

(5) Can the chamber concentration be monitored intermittently or continuously?

(6) Is the generation system isolated from the chamber room?

(7) Is emergency power automatic? What is handled by emergency power?

(8) Are animals housed in the chamber or removed when it is being cleaned?

(9) What techniques are used to determine particle size?

(10) Does each chamber have temperature and humidity controls?

(11) Is the pressure/vacuum in the chamber negative to the room?

3. Special Studies

a. Clinical chemistry

(1) Is the laboratory equipped to carry out clinical chemistry tests by micro methods?

(2) What type of equipment is available?

(3) Does the laboratory have experience with clinical chemistry tests on rodents?

(4) How do they bleed their mice, rats, and other animals for such studies?

(5) Do they have adequate storage for preserving samples?

(6) How many specimens do they process per day?

(7) What QA programs do they have regarding the proficiency of their laboratory?

b. Behavioral studies

(1) What procedures do they use with rodents?

(2) What equipment do they have?

(3) Are the environmental conditions conducive to producing reliable results?

(4) Where are their facilities for behavioral studies located in relationship to the bioassay animal facilities?

c. Pharmacokinetics studies

(1) What experience have they had with radioisotopes in rodent studies?

(2) Do they have the analytical capability to identify impurities? Metabolites?

(3) Have they analyzed tissues for parent compounds? For metabolites?

(4) Do they have the capabilities of collecting urine? Feces? Exhaled air?

(5) Have they computed half-lives, volumes of distribution, or clearing rates from organs?

d. Assay for metals and organic chemicals in biological samples (See also Pharmacokinetics)

(1) Do they have experienced chemical/analytical/toxicology personnel?

(2) Do they have experience in collecting necropsy tissues for analytical samples? (Mandatory)

(3) Do they have the following items available for the preparation of their samples? (Mandatory)

- Refrigerated centrifuges?
- Refrigerators?
- Freezers?
- Ultra-low freezer?
- Freeze-dryer?
- Back-up electrical system with alarms on equipment?

(4) Do they have available the following homogenization, digestion, and extraction equipment for tissues and solvents:

- Homogenizers?
- Soxhlets?
- Hoods?
- Reflux units?
- Concentrators and dryers?

(5) Do they have available the following separatory equipment?

- Separatory funnels?
- Reflux systems?
- Liquid chromatography?
- High performance liquid chromatography?
- Gas chromatography

(6) Do they have the following analytic equipment:

- Atomic absorption spectrometer (single-element analysis-lowest minimum technology)?
- Atomic fluorescence spectrometer?
- X-ray fluorescence spectrometer?
- Neutron activation analysis capability?
- Ion selective electrodes?
- X-ray emission spectrometer?
- Inductively-coupled argon plasma spectrometer (multi-element analysis with good sensitivity and specificity)?
- Gas chromatography-mass spectrometer?

(7) Do they have SOP's for the following:

- Receipt, storage, and processing of samples?
- Analytical methods selected and employed for single or multiple element analysis?
- Quality control maintenance?

4. Immunotoxicology

a. Determine the laboratory's capabilities to perform the following immunoassays:

(1) Immunosuppressive effects:

(a) Humoral effects

- Immunoglobulin quantitation by radial diffusion and specific levels in serum
- Jerne plaque assay to enumerate antibody-producing cells (T-cell dependent antigens by keyhole limpet hemocyanin or Tetanus Toxoid) (T-cell-independent antigens by Type III pneumococcal polysaccharide)

(b) Cell-mediated immunity

- Rosette assay for quantitating T-cells
- Fluorescent antibody for quantitating B-cells
- Esterase cytochemical stain for macrophages
- T- and B- mitogens or antigens for lymphocyte proliferation or blastogenesis
- I^{125} isotopic footpad swelling to measure delayed hypersensitivity response to T-cell antigens

(2) Immunopotentiating effects

(a) Transplant rejection

(b) Macrophage activation as growth inhibition or cytolysis of tumor cells

(3) Hypersensitivity, allergenicity, and immunogenicity

(a) ELISA

(b) RIA

(c) Ouchterlony gel diffusion

(d) Passive hemagglutination

(e) Passive cutaneous anaphylaxis

(f) Reverse passive Arthus's phenomenon

b. Determine if the immunology group has experience with known or suspect carcinogens.

III. OFFSITE MONITORING

A. ANIMAL CARE

1. Review SOP's for adequacy (See Standard Operating Procedure Evaluation Procedure: Animal Care, p. 25).

2. Review monthly reports.

3. Review environmental data record for adequacy.

4. Review colony data record for adequacy.

5. Review animal group data record for adequacy.

6. Review animal shipping records.

7. Review serology reports.

8. Is the veterinarian providing animal care as a full-time employee of the facility? If not, is he/she a part-time employee or consultant?

9. Is the veterinarian responsible for animal care board certified by the ACLAM?

   If not, is he/she board eligible?

10. How many hours does the animal care veterinarian devote to the bioassay program (monthly)?

11. Are the forms used for the Animal Disease Screening Program being completed as required?

12. Are serum samples from sentinel animals being prepared and submitted in accordance with instructions?

13. Are results from the testing laboratory on the animal disease monitoring program being submitted with the monthly report?

STANDARD OPERATING PROCEDURE EVALUATION PROCEDURE: ANIMAL CARE

Operation	Written SOP?	Implementation*	Comments
1) Receipt of Animals			
2) Operation and Maintenance of Rack and Cage Washer			
3) Sanitization of Racks, Feeders, Cages, and Automatic Watering Systems			
4) Testing Performance of Automatic Watering System Valves			
5) Changing Cages			
6) Changing Racks			
7) Receipt and Storage of Bedding			
8) Receipt and Storage of Feed			
9) Cleaning Areas Housing Animals			
10) Cleaning Areas Not Housing Animals			
11) Feeding Rats and Mice			
12) Handling Clean Bedding			
13) Quarantine of Animal			
14) Vermin Control and/or Prevention			
15) Changing Pellet Feeders			
16) Handling Dead or Moribund Animals			
17) Preliminary Health Check in Quarantine			
18) Final Health Check Clearing Quarantine			
19) Observing Animals for Clinical Signs			
20) Escaped Animal Incidents			
21) Unsatisfactory Animal Shipments			
22) Sentinel Animal Program			
23) Handling of Emergencies			
24) Technician Training			
25) Terminal Sacrifice of Test Animals			
26) Temperature and Humidity Recording			
27) Isolation of Animals			
28) Room Preparation			

*Is Implementation and Monitoring System Adequate?

B. CHEMISTRY

1. Offsite monitoring will require that a number of reports be reviewed on a routine basis to determine the status of the chemistry at the bioassay laboratories. These reports will include:

   a. Analytical report from the analytical contractor

   b. Experimental design (protocol) for each study

   c. Statement of work for each contract

   d. Chemistry SOP's

   e. Monthly progress reports from the bioassay laboratories, including all chemistry reports

   f. Reports submitted upon completion of the various phases of a bioassay study (e.g., acute, RD, etc.)

   g. Copies of all site visit reports

   h. Copies of all program review reports

2. Some of the parameters that need to be followed off site and that can be monitored by review of the previously mentioned reports include the following:

   a. Receipt, storage, and analysis of chemicals

      (1) Is the chemical available for shipment to the bioassay laboratory from the analytical contractor?

      (2) Is the receipt and initial analysis of the chemical reported in the bioassay laboratory's monthly report?

   b. Acute test

      (1) Did the analytical contractor provide the mixing protocol for the acute study?

      (2) Did the bioassay laboratory report any difficulty with chemical/vehicle mixing during the acute study?

   c. Repeated dose study

      (1) Did the analytical contractor provide the mixing protocol for the chronic study?

      (2) See d.(2) below.

d. 90-day study

(1) Did analytical contractor provide the mixing protocol for the chronic study?

(2) Did the bioassay laboratory perform the required chemical/vehicle analysis and report the results in monthly report?

(a) Were there any problems encountered with the mixing or analysis?

(b) If a feeding study was conducted, was the bioassay laboratory able to obtain a homogeneous mixture?

(3) Was a bulk chemical analysis required during this study and, if so, was it done and reported in the monthly report?

e. Dose setting and reevaluation of experimental design

(1) Was the bioassay laboratory able to perform the required analyses during the 90-day study, and were the results acceptable?

f. Chronic study

(1) Did the analytical contractor provide the mixing protocol for the chronic study?

(2) Did the bioassay laboratory perform the required analyses and report the results in the monthly report?

(a) Were there any problems encountered with the mixing or analyses of chemical/vehicle mixtures?

(b) Were there any problems with bulk chemical reanalyses?

(c) Did the referee analyses give acceptable results?

(d) Was corn oil analysis required and, if so, what were the results?

(3) Were there any site visits made where chemistry action items were generated?

g. End of study

- Are all chemistry data generated during the bioassay study available for the technical report?

C. HEALTH AND SAFETY MONITORING

The offsite monitoring and evaluation of the contractor's health and safety performance rely heavily on the data and information provided in monthly progress reports and on any other indirect contact with laboratory personnel (including specific site visits or telecommunications). Particular items useful as indices of a laboratory's health and safety position include:

1. Accident reports (resulting in employee exposure to or an environmental release of biotest material)

2. Modifications to SOP's, experimental protocols

3. Modifications to facility

4. Worker hazard inventory

5. Work area or personnel monitoring activities and results

6. Laboratory monitor site visit perspectives

D. PATHOLOGY

1. Preinitiation period

   During this period, the laboratory monitor should ensure that the laboratory is prepared to handle the aspects of the bioassay related to pathology before any chemicals are put on test. Important areas to consider and sources of information follow:

   a. Adequacy of staff

      (1) Review site visit reports for pertinent information.

      (2) Request CV's on all professional and support staff.

   b. Adequacy of facilities

      (1) Review site visit reports for pertinent information.

      (2) Request up-to-date floor plans and list of equipment.

   c. Adequacy of protocols

      (1) Review site visit reports for pertinent information.

      (2) Request copies of all SOP's related to pathology.

   It is particularly important to ensure that the laboratory has complied with all action items from recent site visit reports.

2. Acute test for dose range finding and repeated dose test

During these phases of testing, involvement of the pathology section is minimal. The laboratory monitor should ensure, however, that NCI pathologists are aware of any clinical findings in the acute tests that might require alterations in the pathology protocols for the chronic study.

3. Subchronic test

During and following the subchronic study, all animals are to be necropsied. Monthly progress reports should be reviewed for any problems. In addition, the monitor should ensure that necropsy reports are submitted within 14 days of the death of animals during the study and that these reports are being reviewed. NCI pathologists should be made aware of any abnormal findings during the study. NCI pathologists should also receive a pathology report at the end of the subchronic study.

4. Chronic Study

As in the subchronic tests, the laboratory monitor should review monthly progress reports and see that necropsy reports of animals dying while on test are being sent from the laboratory within 14 days of the death and that these reports are being reviewed by NCI pathologists. Every 1 to 2 months, the necropsy reports should be compiled and reviewed for evidence of:

a. Excessive deaths

b. Incomplete pathologic evaluation due to autolysis

c. Deaths due to infections or parasites

d. Deaths due to dosing

Before the end of the chronic test, it would be prudent for the monitor to recheck all recent records to ensure that proper staff, facilities, and protocols are being maintained. If any problems are suspected or anticipated, a visit to the laboratory by an NCI pathologist should be scheduled before or in the early stages of the terminal necropsies.

5. Study Completion and Reporting

It is important during this phase to ensure that all deadlines are being met:

a. Slides should be prepared and sent to the pathologist promptly.

b. The review of slides by pathologist should be completed promptly.

c. Slides and diagnoses should be submitted for QA.

d. The Pathology Working Group (PWG) review of the QA report and narrative should be completed.

e. Revisions should be made in narrative.

The laboratory monitor should review the comments in the QA report and those of the PWG to ensure that adequate pathology evaluations are being performed at the laboratory.

E. TOXICOLOGY

1. Monitoring is accomplished by referring to the following documents:

   a. Contract and modifications

   b. SOP's

   c. Protocols and protocol revisions

   d. Schedule projections

   e. Milestone status

   f. CV's of key personnel

   g. Monthly, quarterly, and annual progress reports

   h. Acute, repeated dose, and subchronic reports and dose approval documents

   i. Chemical analysis reports from analytical laboratories

   j. Referee analysis reports

   k. Individual Animal Data Records (IADR's)

   l. Sentinel animal sera reports

   m. Accident reports

2. The following information can be obtained from the reports:

   a. Progress of the bioassay

   b. Personnel turnover

   c. Problems related to the receipt of chemicals

d. Unusual loss of animals due to possible disease

e. An understanding of protocols and modifications

f. Early suggested toxicity of compound

g. A lack of adequate handling of moribund animals (is autolysis too frequent?)

h. Deviation from expected clinical chemistry results possibly indicating poor QA

IV. ONSITE MONITORING

A. ANIMAL CARE

1. Strains of animals

   - Are any strains of animals being used in the bioassay program other than the B6C3F1 hybrid mouse and the Fischer 344 rat?

   - Are animals from different sources housed in the same facility? Will the disease status of the animal affect bioassay results?

2. Shipment/Receipt of Animals

   a. Have any problems been encountered with animal shipments?

      (1) Are shipments on time? If not, explain.

      (2) Do shipments contain the proper numbers of animals, If not, explain.

      (3) Are the animals healthy? If not, explain.

   b. Are the procedures for reporting unsatisfactory animal shipments understood?

   c. Are receipt records adequate?

3. Quarantine Facilities

   - Are quarantine facilities available?

   Inspect animal quarantine area and examine quarantine records and procedures. If possible, discuss procedures with the animal technicians assigned to this area.

   a. Are quarantine animals receiving the same type of feed they will receive when tested?

   b. Are quarantine animals using the same water system they will use when tested?

   c. How many animals are being killed from each shipment for parasite and disease examination before animals are placed on acute, repeated dose, subchronic, and chronic tests?

   d. Who performs these disease examinations?

   e. Are technicians familiar with clinical signs?

   f. What are the quarantine procedures? How are they recorded?

g. How often does a veterinarian examine the animals? How is the examination recorded?

h. How are sick animals reported? How are records kept?

i. Are animals inspected twice daily? 7 days per week? What is the weekend/holiday schedule?

j. Are there crossovers between the clean and dirty sides of the facility? If so, how are these crossovers minimized?

k. How is the dirty/clean concept enforced?

l. Is more than one chemical being tested in the same room? If so, what are these other chemicals?

4. Inspection of Animal Rooms

Inspect animal room(s) proposed for the studies and examine records to ensure that room(s) is properly prepared. Inspect records, noting temperature and humidity controls and monitoring procedures for the animal rooms, and review emergency power systems and alerting mechanisms.

a. Are drains in animal rooms plugged?

b. How many air changes are there per hour in the animal rooms?

c. Are quantitative measurements and recording of air flow being performed as required? What action is taken when measurements are outside these limits?

d. What is the light cycle? How is it checked?

e. How is room temperature monitored and recorded?

f. What were the normal animal room temperatures? The high and low temperatures?

g. What was the normal animal room humidity? The high and low humidity?

h. What temperature limits are allowed, and what happens when the temperature is outside these limits?

i. How often are the animal rooms being wet mopped?

j. How often are the rooms cleaned? How are they cleaned? How are records kept?

k. What cleaning compounds are used? Who manufactures them?

l. What is the source of emergency power?

(1) How frequently is it tested?

(2) Is it tested under load?

(3) Is a log kept?

(4) What areas does emergency power handle?

(5) Is it of the automatic changeover type?

m. Is the emergency notification procedure adequate?

n. How frequently are the cages, filter sheets, feeders, and racks being changed?

o. Do racks, cages, filters, and water systems meet BOA requirements?

5. Sanitation

a. How are cages cleaned?

b. How often are racks washed? How?

c. What chemicals are used to wash equipment?

d. What temperature does the cage washer reach? How is it monitored? Are records kept? Is water recycled?

e. What kind of bedding is used?

f. Rat cages should receive 510 grams of bedding and mice cages 140 grams; is this amount being dispensed?

g. Is there any evidence of vermin in the animal rooms cage wash area, storage areas, bioassay support facilities, or outside environment?

h. The sanitization report describes the methods being used for sanitization of equipment used in the bioassay program, including cages, racks, feeders, watering devices, rooms, walls, halls, and building. Are the previous mentioned practices being followed?

i. Have Baygon® and/or traps been used? If so, are detailed records available for review? Was the proposed system approved prior to use by NTP?

j. Does one cycle of the rinse water reach 180°F? If not, what was the highest temperature?

k. Is the clean storage area adequate?

6. Water

a. What is the source of the water for the animals? (Well, city supply, etc.)?

b. What treatments are given the water by the laboratory? (Acidification, chlorination, filtration, deionization, distillation, etc.)?

c. How are these treatments monitored? Are records available?

d. Are water bottles washed before refilling? How?

e. How often are water bottles and stoppers washed?

f. How are sipper tubes cleaned?

g. How are automatic waterers checked to make sure they are functioning properly?

h. Does the water provided for animal use meet the USEPA drinking water standards?

i. What is the date of the last water analyses report?

7. Feed

Inspect food storage area and note food source, lot number, and manufacturing date. Check feed rotation and review feed receipt procedures.

a. Is the storage area for feed and bedding clean?

b. What brand of feed is used? Who is the supplier?

c. How is food examined on arrival, and what are the reasons for rejection of feed?

d. What is the oldest date of manufacture in current inventory? (Food should be used within 90 days of manufacture)

e. How is the food stored? Is it refrigerated? Examine storage area for insects, rodent feces, clutter, etc. Are first-in/first-out procedures followed?)

f. How is food stored after the bag is opened?

g. What type of records are kept on feed? (Examine them.)

h. How are feeders loaded, and where is loading done?

i. Are feeders emptied and washed before refilling?

j. What type of feed container is used?

k. Does the facility have the ability to autoclave feed and/or bedding for bioassay program animals? (Not a requirement)

l. Are the following reference materials available?

(1) The Guide for the Care and Use of Laboratory Animals, DHEW Publication No. (NIH) 78-23, Revised 1978

(2) A Guide to Infectious Disease of Mice and Rats, NAS, 1971 Publication ISBN 0-309-01914-1

B. CHEMISTRY

1. Receipt, storage, and analysis of bulk chemicals

a. Receipt

(1) Was the chemical received in good condition?

(2) Was it properly packaged and labeled?

(3) Was a return receipt requested?

(4) Was the project officer notified of receipt?

(5) Were the chemical reference standards for bulk chemical analyses pulled and stored at -20°C?

b. Storage

(1) Was the material transferred directly from receipt to storage, or was it repackaged?

(2) If repackaged, why and how was the repackaging done?

(3) Do storage conditions meet those recommended by the analytical contractor?

(4) If multiple batches are received over the course of a study, are they used on a first-in/first-out distribution system?

(5) Is special handling required and, if so, is the bulk broken down into working batches for easier handling?

(6) Are chemical reference standards stored at -20°C?

c. Distribution

(1) Is material signed for when transferred for mixing operations?

(2) Is there an inventory system to keep track of amounts being used so that, if necessary, more can be ordered and received before current batch runs out? Has consideration been given to storing a 90-day supply for emergency situations?

(3) Is material transferred from storage to mixing operations safely?

(4) When material is returned, is it sealed and stored properly?

(5) Are special handling conditions being followed by all personnel?

d. Analysis

(1) Is the chemist provided with background information on chemicals, including the analytical contractor's analysis report?

(2) Is the bulk material analyzed on a regular basis as outlined in the BOA? Are results consistent?

(3) Is the analysis protocol provided by the analytical contractor being followed?

(a) If not, why?

(b) If so, what approved modifications, if any, were necessary?

(4) How are the results reported to the principal investigator? to NCI?

(5) What QA procedures are used by the analytical lab for

- Calibration of instruments,
- Running standard curves,
- Use of internal standards,
- Recordkeeping

2. Acute Test

a. Are the mixing protocols provided by the analytical contractor adequate?

b. Are there specific written mixing instructions available to the individual preparing the diet mix?

c. Is onsite monitoring for chemistry (other than that listed in (a) above) being conducted?

3. Repeated-Dose Study

a. Are the mixing protocols provided by the analytical contractor adequate?

b. See 4.b. listed below.

4. 90-Day Subchronic Study

a. Bulk Chemical Analysis

(1) Is the chemist provided with background information on chemicals, including the analytical contractor's analysis report?

(2) Is the bulk material analyzed on a regular basis as outlined in the BOA?

(3) Is the analysis protocol provided by the analytical contractor being followed? If not, why? If so, what approved modifications, if any, were necessary?

(4) How are the results reported to the principal investigator? To NCI?

(5) What QA procedures are used by the analytical laboratory for

- Calibration of instruments
- Running standard curves
- Use of internal standards
- Record keeping

b. Chemical/Vehicle Analysis

(1) Mixing operation

(a) Is the mixing technician briefed on the safe handling and toxicity of the materials being used?

(b) Is protective clothing and respiratory protective equipment worn when dosage mixtures are prepared?

(c) Was a mixing protocol provided by the analytical contractor and, if so, is it being used?

(d) Are the vehicles (feed, corn oil, acetone, etc.) properly stored and handled?

(e) Do the mixing operations yield an acceptable dosage mixture (i.e., homogeneous feed mixes, clear corn oil gavage solutions, etc.)?

(f) How are samples taken and transferred to chemistry?

- Are samples of vehicles, taken from the same lot used in the mixing operation, sent as blank samples?

(g) How is the dosage mixture transferred from the mixing vessel to the storage container?

(h) Are the dosage mixtures properly stored and labeled?

(i) If dosage mixtures are stored, what QA is there to ensure dose levels, etc., are not mixed up during distribution for dosing?

(j) How are dose levels prepared?

- Are the formulation instruction sheets written, detailed, and complete?

- Are the different levels weighed and mixed separately, or is the high dose mixed and then diluted for subsequent lower doses?

(k) What is the QA on the instruments (i.e., balances, pipets, etc.) used in the mixing process?

(l) Corn oil should be stored below 5°C, must be food grade, and must be analyzed monthly for peroxide level. Are these requirements followed?

(m) How are excess dosage mixtures and waste handled?

(n) In feeding studies how are feeders filled and "topped off"?

(2) Chemical/Vehicle Analysis

(a) Are dosage mixtures analyzed promptly?

(b) Are dosage mixtures analyzed by the method provided by the analytical contractor? If not, why? If so, what modifications, if any, were necessary?

(c) What is the time frame between mixing and analysis? Do they occur before the animals are dosed?

(d) What is the QA on the analysis instruments used for dosage analysis?

(e) How are the data monitored? How is the principal investigator informed of results if results are out of tolerance? What steps are taken to notify the proper personnel?

(f) How are the data recorded, verified, and maintained? Are results within specified limits?

5. Chronic Study

a. Bulk Chemical

(1) Storage

(a) Was the material transferred directly from receipt to storage, or was it repackaged?

(b) If repackaged, why and how was the repackaging done?

(c) Do storage conditions meet those recommended by the analytical contractor?

(d) If multiple batches are received over the course of a study, are they used on a first-in/first-out distribution system?

(2) Distribution

(a) Is material signed for when transferred for mixing operations?

(b) Is there an inventory system to keep track of the amounts being used so that, if necessary, more can be ordered and received before the current batch runs out?

(c) Is material transferred from storage mixing operations safely?

(d) When material is returned, is it sealed and stored properly?

(3) Analysis

(a) Is the chemist provided with background information on chemicals, including the analytical contractor's analysis report?

(b) Is the bulk material analyzed on a regular basis as outlined in the BOA?

(c) Is the analysis protocol provided by the analytical contractor being followed? If not, why? If so, what approved modifications, if any, were necessary?

(d) How are the results reported to the principal investigator? To NCI?

(e) What QA procedures are used by the analytical laboratory for

- Calibration of instruments
- Running standard curves
- Use of internal standards
- Record keeping

b. Chemical/Vehicle

(1) Mixing operation

(a) Is the mixing technician briefed on the safe handling and toxicity of the materials being used?

(b) Is protective clothing and respiratory protective equipment worn when dosage mixtures are prepared?

(c) Was a mixing protocol provided by the analytical contractor and, if so, is it being used?

(d) Are the vehicles (feed, corn oil, acetone, etc.) properly stored and handled?

(e) Do the mixing operations yield an acceptable dosage mixture (i.e., homogeneous feed mixes, clear corn oil gavage solutions, etc.)?

(f) How are samples taken and transferred to chemistry for analysis? Are samples of the vehicle, taken from the same lot used in mixing operation, sent as blank samples?

(g) How is the dosage mixture transferred from the mixing vessel to the storage container?

(h) Are the dosage mixtures properly stored and labeled?

(i) If dosage mixtures are stored, what QA is there to ensure dose levels, etc. are not mixed up during distribution for dosing?

(j) How are dose levels prepared?

- Are the formulation instruction sheets written in detail?

- Are the different levels weighed and mixed separately, or is the high dose mixed and then diluted for subsequent lower doses?

(k) What is the QA on the instruments (i.e., balances, pipets, etc.) used in the mixing process?

(l) Use of corn oil:

- Storage should be below 5°C.
- Corn oil must be food grade.
- Monthly analysis for peroxide level.

(m) How are excess dosage mixtures and waste handled?

(n) How are feeders filled and "topped off"?

(o) Are referee samples properly packaged for shipment to the analytical contractor?

(2) Chemical/Vehicle Analysis

(a) Are dosage mixtures analyzed promptly?

(b) Are dosage mixtures analyzed by the method provided by the analytical contractor? If not, why? If so, what modifications, if any, were necessary?

(c) What is the time frame between mixing and analysis? Do they occur before the animals are dosed?

(d) What is the QA on the analytical instruments used for dosage analysis?

(e) How are the data monitored? How is the principal investigator informed of results if results are out of tolerance? What steps are taken to notify the proper personnel?

(f) How are the data recorded, verified, and maintained?

(g) Were referee samples sent to the analytical contractor for analysis and, if so, what were the results?

6. End of Study

a. Was the final bulk chemical reanalysis performed?

a. Are all chemistry data generated during the bioassay study available for the technical report?

b. Was all surplus test material returned to the analytical contractor?

C. HEALTH AND SAFETY

Each laboratory participating in the Carcinogenesis Testing Program (CGT) is required to develop and implement its own health and safety plan, approved by CGT, prior to the initiation of any research involving program-related chemicals. Since universally applicable health and safety procedures for all labs are not feasible, each lab must formulate its own program that follows certain general guidelines suggested by CGT. In assessing the adequacy of any participating laboratory's health and safety program, it is necessary to monitor both the design of the program and its implementation.

Design and implementation require that different monitoring approaches be utilized, depending on the monitoring schedule or personnel involved. For example, the design of a laboratory's health and safety program should be reviewed by individuals qualified to evaluate the performance characteristics of the engineering controls selected for use by the particular laboratory, and this review should be conducted before the entrance into any contractual obligations (or at least before the initiation of any research involving suspect carcinogens). On the other hand, evidence that portions of the health and safety program are being implemented can often be established by careful review of maintenance records by informed observers. The following sections describe various criteria used to evaluate or monitor the health and safety programs at participating laboratories before they begin work on routinely conducted program review site visits, or as the need arises. Those items that can be monitored off site (via periodic laboratory reports or laboratory monitor onsite visits) are also specified.

The following sections reflect monitoring needs as particular chemicals go on test, and they can be used to validate the effective implementation of the health and safety plans previously reviewed. The degree of monitoring is dependent upon the individual conducting the effort, the experimental design, the phase of the study, and the characteristics of the biotest material. Most of the monitoring effort is expected to occur in the early stages of the study (e.g., during acute, repeated dose, and subchronic phases) because the laboratory tends to use higher concentrations of the material and may be somewhat unfamiliar with its physical/chemical properties. The following sections outline monitoring objectives according to the phase of the study

and the area within the laboratory under review. Special studies may have additional requirements (e.g., radiolabeled pharmacokinetic tests) and are addressed on an as needed basis. The routine monitoring (of a nontechnical nature) of the laboratory's health and safety program by the laboratory monitor is covered elsewhere in this document.

1. Initiation of Study (Before receipt of test chemical)

   a. Has the laboratory received a copy of the "Safety and Toxicity" package?

      Has the package been distributed internally? To whom? At a minimum, who should receive plans? Are special considerations/ safety measures
      required (e.g., for explosive, reactive materials)?

   b. Are all precautions in place or operational before the receipt of the chemicals?

   c. Does the laboratory test incompatible materials (hence the need for segregated storage conditions)?

   d. Are there special handling procedures as a result of special toxicological studies? Are they in place?

   e. Have waste handling, storage, and disposal considerations been addressed?

   f. Is the protective equipment slated for the appropriate use of the test compound (e.g., permeability of gloves, clothing)?

2. Receipt, Storage, and Analysis of Chemical

   a. Was the compound received in good condition? (See also Chemistry.) If not, who was notified? Why/how was it damaged?

   b. Was any repackaging required? Why? (See also Chemistry.)

   c. Are loading dock conditions (e.g., temperature, ventilation) adequate for short-term storage of chemicals?

   d. What are qualifications of loading dock personnel?

   e. Is the material properly labeled?

   f. How are after-hour deliveries handled?

   g. Is the handling/transportation route from the loading dock to bulk storage direct and well planned?

   h. How are working quantities of test chemical disbursed? (See also Chemistry.) Who disperses them? Where?

i. Are biotest materials stored/labeled properly? (Consult contract, safety, and toxicity package.)

j. Do any special studies require additional material (e.g., radiolabels, analytical standards)? Are they handled properly (e.g., according to NRC 10CFR 19-30)?

k. Are technicians adequately informed of handling requirements?

l. Is the analytical laboratory properly equipped? (See also Chemistry.) Does it have:

(1) Local and area exhaust ventilation. (Define air flow parameters.)

(2) Flammable solvent storage (and dating)

(3) Fire and Safety equipment

(a) Emergency shower/eye-wash stations

(b) Compressed gas/liquified gas storage

(c) Waste chemical storage/disposal

(d) Radioisotope handling/storage (as needed)

m. Are analytical procedures conducted properly (e.g., extractions, GC analysis, local exhaust ventilation)?

n. What personnel protective equipment is available? Is it in use?

o. Describe general laboratory housekeeping conditions? Is there any evidence of food or smoking in lab?

3. Dosage Preparation and Storage

a. Are dosed vehicles (feed, water, corn oil) prepared properly?

b. Do the SOP's address health and safety concerns?

c. Are the "Safety and Toxicity" precautions being followed?

(1) Are proper clothing and proper fitting respirators worn?

(2) Are cleanup procedures followed?

(3) Do they appear effective? Is there any monitoring?

(4) Do technicians know how to handle spills?

d. What exhaust ventilation is available? What are the air flow parameters?

e. Is biotest material transferred under controlled conditions? Describe them.

f. Does the route of administration cause any unusual problems?

(1) How are the test atmospheres prepared for inhalation studies?

(2) Are the techniques in agreement with safety and toxicity package guidelines?

(3) What occurs during the chamber "shakedown" phase?

g. Is the dosage preparation provided by an analytical chemist or laboratory technician?

h. Is the diet preparation area suitably segregated from the rest of the facility?

i. Are storage conditions adequate? (Environmental conditions, labeling, segregation, alarms) (See also Toxicology.)

j. Are spill cleanup procedures available?

k. How are dosages transferred to animal rooms? Are containers bagged to prevent the spread of contamination? Are feeders, bottles, etc. filled in the diet preparation area or in animal rooms?

l. How are logs and records kept, stored, and transferred? (See also Toxicology.)

4. Dosage Administration in Feed

a. Is the transfer of dosed feed minimizing aerosol generation?

b. Has dosed feeding been monitored?

c. What are the room air flow parameters? Are they adequate?

d. Is personnel protective equipment appropriate? (See Safety and Toxicity Package and Health and Safety Plan)

e. How is waste/excess dosed feed handled?

f. Do room surfaces appear contaminated?

g. How are animal logs and records kept and stored? (See also Toxicology.)

5. Gavage/Skin Painting

   a. Have animal room atmospheres been monitored for biotest material? What were the results?

   b. What protective garments are worn?

      - Do they comply with the Health and Safety Plan specifications?
      - Are they compatible with biotest material?

   c. What are the air flow characteristics? How often is air flow checked? Are potential vapors controlled?

   d. Are hoods available? Are they in use?

   e. Have any dosing accidents occurred? What kind? What remedial action was taken?

   f. How effective are clean-up procedures?

6. Dosed Water

   a. What are the water bottle transfer techniques? (See also Toxicology.)

   b. How is excess dosed water handled/disposed of?

   c. What are the spill cleanup procedures?

7. Inhalation

   a. How are test atmospheres generated? (See also Toxicology and Chemistry.) How does the system shut off in the event of a malfunction? Who is notified?

   b. Where are stock chemicals/cylinders stored? How?

   c. How are chamber rooms protected?

   d. What type of barrier system is used? (See also Toxicology and Animal Care.)

   e. How are chamber leaks tested? What documentation is available?

   f. How are explosive mixtures handled?

(1) What are the laboratory rules regarding such mixtures?

(2) How are test atmospheres exhausted?

(3) What checks are made to ensure total removal?

g. How are animals handled after exposure?

h. How is chamber exhaust treated to remove or control the test material?

i. What are the chamber room's air flow parameters?

j. How is equipment serviced?

k. What protective equipment is worn? Is it appropriate?

l. What kinds of equipment maintenance/service logs are used?

8. Animal Care

a. What are the animal handling procedures? (See also Animal Care)

b. How are spills handled in animal rooms?

c. How are animal bites reported?

d. How are animal logs handled?

(1) Do they pass outside of the barrier?

(2) How are they decontaminated?

(3) Who cleans up the animal rooms?

(4) How is clean up accomplished?

9. Necropsy

a. What protective clothing and equipment are required?

b. What are personnel wearing?

c. What local and area exhaust ventilation is used?

(1) Are the flow rates adequate?

(2) How effective is local hood design?

(3) How frequently are hoods checked?

(4) Are any odors detected? What kind?

(5) Has any air monitoring been conducted? What were the results?

d. Where are necropsy tissues/jars stored? (See also Pathology.) Do they require surface decontamination?

e. How are logs and IADR's handled and how do they flow through the lab? (Include procedures for decontamination, if any.)

10. Histology

a. What protective clothing/equipment is required? What are personnel wearing?

b. What are the handling procedures for solvents?

c. Is there evidence of food or tobacco use in the area?

d. What local area exhaust ventilation is used? Are hood designs effective? How frequently is exhaust ventilation checked? Are any odors detected? What kind?

e. Has any air monitoring been conducted? What were the results?

(1) Is there any need to decontaminate container/surfaces?

(2) Are waste solvents properly containerized?

(3) Where are they stored?

(4) How are they disposed of?

f. How are excess tissues handled/disposed of? (See also Pathology.)

g. Do disposal procedures comply with appropriate environmental statutes?

h. Do maintenance staff clean up the area? Are they properly informed of hazards?

11. Sanitation/Maintenance

a. Animal Areas

(1) Are maintenance procedures described in the Health and Safety plan being properly implemented?

(a) Are all personnel familiar with them?

(b) Are there separate sanitation crews in animal areas?

(2) How are animal rooms/support corridors cleaned? (See also Animal Care.)

(a) What cleaning techniques are used?

(b) How frequently are these areas cleaned?

(c) Are the cleaning methods and frequency adequate?

(3) What cleaning agents are being used? (See also Animal Care.)

(a) Have they been approved? By whom?

(b) Are pest controls being used? What kind?

(4) How are rooms decontaminated after a study termination? (See also Animal Care.) How is cleanliness validated?

(5) Is exhaust air filtered at the vent? How is the filter changed? By whom?

(6) How frequently is air flow monitored (qualitatively and quantitatively)?

(7) How is improper air flow rectified?

(8) Do members of the maintenance staff comply with the personnel protection requirements of the animal care staff?

(a) How are tools decontaminated?

(b) Are maintenance staff accompanied by program staff?

b. Cage Emptying/Washing Areas

(1) Is adequate exhaust ventilation provided?

(a) When and how is it checked?

(b) How are wastes handled?

(c) Are all cage wastes appropriately segregated (e.g., those for landfilling, those for incineration)?

(2) What personnel protective equipment is in use?

(3) What is the general appearance of the cleaning areas?

(4) Are certain personnel dedicated to cage cleaning operations?

(5) How are wastes transported to the incinerator or loading dock? Are wastes specially marked? How?

c. Facility (general)

(1) Where are maintenance logs located? Who updates them? Are they in compliance with SOP's?

(2) How are air filters replaced? By whom?

(3) What is the administrative structure of the engineering/maintenance staff? What is its relationship with the bioassay staff?

(4) Are maintenance personnel familiar with the needs of the bioassay program (e.g., air flow, contaminated filter replacement, etc.)? How are contaminated work areas, waste water drains, and air ducts cleaned up?

12. Decommissioning (specific bioassay termination)

a. How is the surplus chemical handled/shipped?

b. Is all material accounted for?

D. PATHOLOGY

The list of questions presented in Section II E of this report (Preaward Site Visit:Pathology) is useful for collecting information for pathology site visits.

At the pathology site visit, the emphasis may be different than that in the preaward visit. Areas that are known to be strong may not require the same close scrutiny as areas that have changed or have shown weaknesses. At the site visit, it is important to detect changes in personnel, facilities, and protocols, assess them for adequacy, and conduct in depth reviews of known problem areas. These sections in the list of questions should be stressed accordingly.

Following the site visit, the data collected should be compiled and interpreted by the pathologist. A report evaluating the laboratory's capabilities and indicating existing or potential problems should then be drafted and given to the NCI laboratory monitor.

E. TOXICOLOGY

1. Personnel

a. Are key people qualified for the bioassay program?

(1) Toxicologist?

(2) Clinical chemist?

(3) Special disciplines as required?

b. Has there been any loss of staff? Are new personnel qualified?

c. Are there training programs for the following personnel or activities?

(1) Technicians?

(2) Group leaders?

(3) Data evaluators?

(4) Techniques:

(a) Gavage?

(b) Necropsy?

(c) Skin painting (when applicable)?

(d) Inhalation equipment (when applicable)?

(e) Clinical observations?

(f) Clinical chemistry?

(g) Dose preparation?

d. Are there records to verify training programs for the following?

(1) Verification of proficiency - speed, accuracy?

(2) Periodic refresher training?

(3) Who does training? Qualifications?

2. Management

a. Principal Investigator

(1) Is the principal investigator knowledgeable in all respects about the status of the following:

(a) Protocols?

(b) Contract & modifications?

(c) Chemicals?

(d) Recordkeeping?

(e) Reporting?

(f) Scheduling?

(g) Cost incurred?

(2) Who is the designated backup for the principal investigator?

(3) Is the master schedule for bioassay studies up to date?

(4) Are reports verified for completeness and accuracy? (By whom?)

(5) Is there an adequate staff to conduct the bioassay studies?

b. Quality Assurance (QA)

(1) Is there an individual responsible for QA? To whom does he/she report?

(2) Are there SOP's for all bioassay functions?

(a) Who writes them?

(b) Who reviews them?

(c) How often are they updated?

(d) Are they available in the laboratory?

(e) Are copies available for the laboratory monitor/ discipline monitor?

(f) Are they in operation prior to the receipt of chemicals and animals?

(g) Are they kept in limited access areas?

c. Have emergency procedures been established for the following:

(1) Electrical failure?

(2) Heating/cooling failure?

(3) Airflow disruption?

(4) Chemical spill?

(5) Shortage of personnel?

(6) Food or water rejection by animals?

(7) Has an up-to-date emergency notification procedure been posted inside and outside barrier?

3. Facility - Equipment

a. Are there problem areas? If so, how are they handled?

(1) Crossovers?

(2) Filter changes?

(3) Dose preparation?

b. Have there been any changes in assigned facilities?

(1) Why?

(2) Are the new facilities adequate for the present bioassay needs? For future needs for assigned chemicals?

c. Is there controlled flow of the following for the bioassay program: (Do standard operating procedures exist to cover these items?)

(1) Access to area(s)?

(2) People on program?

(3) Equipment - for repair, preventative and emergency maintenance?

(4) Laundry?

(5) Incoming animals?

(6) Dose preparations?

(7) Contaminated material?

(8) Animal carcasses?

(9) Other waste?

d. Is general housekeeping adequate for the following bioassay areas:

(1) Halls?

(2) Change rooms?

(3) Laboratories?

(4) Animal rooms?

(5) Wash rooms?

(6) Storage area?

(7) Dose preparation?

(8) Disposal/pickup site(s)?

e. Maintenance for the bioassay program. Are there SOP's and record verification for following maintenance activities?

(1) Decontamination procedures for lab equipment, etc.?

(2) Schedules for maintenance of equipment, filters, alarm systems, and emergency power?

(3) Flow of maintenance personnel?

(4) Temperature check records?

(5) Humidity check records?

4. The following items are to be monitored for each chemical assigned to a laboratory:

a. Has the principal investigator received and understood the following protocols for each chemical:

(1) Safety and toxicity?

(2) Chemical analysis?

(3) Dose preparation?

(4) Storage?

(5) Animal studies?

(6) Special studies?

(7) Revisions of various protocols?

b. Are the records adequate for each chemical assigned to the laboratory? (See Chemical Site Visit section.) Do they include:

(1) Log-in receipt?

(2) Inventory system for bulk?

(3) Access controlled storage for bulk?

(4) Bulk storage, as specified?

(5) Storage of analytical samples?

(6) Dose storage?

(7) Confirmation of chemical identity?

(8) Referee samples and reports?

(9) Procedure for transfer of chemical, doses, etc., from the preparation room to storage, analytical laboratory, and inoculation center?

c. Depending on the stage of each bioassay at the time of the on-site visit, it is necessary to determine if the principal investigator has submitted and received approval for doses to be used in the following phases of the bioassay:

(1) Acute?

(2) 14-day?

(3) 90-day?

(4) Chronic?

(5) Special study?

d. Dose preparation: dosed feed

(1) Who is responsible for calculations?

(2) Are calculations recorded for each dose preparation?

(3) Who supervises dose preparation?

(4) Are written mixing procedures and dose levels available for each chemical?

(5) Are dose levels weighed and mixed separately?

(6) Have records been verified for:

(a) Lot numbers of bulk chemical, feed, corn oil, etc.?

(b) Who is technically responsible (who signs record book)?

(c) Calculations?

(d) Date of dose preparations?

(e) Dates doses used?

(f) Analytical samples taken, labeling, storage?

(7) How are mixers cleaned?

(8) Are there labels on dosed feed containers?

(9) Are balances calibrated? How frequently?

(10) Are chemical and dose specific containers used for storage of dosed preparations?

(11) Are doses analyzed before use?

(12) Is there an up-to-date schedule for dosed feed preparation?

e. Dose preparation: gavage/skin painting

(1) Who is responsible for calculations?

(2) Are calculations recorded for each dose?

(3) Who supervises?

(4) Are the dose levels for each chemical available in the laboratory?

(5) Are SOP's available in the laboratory for each chemical preparation?

(6) Is each dose prepared separately or by diluting the highest concentration?

(7) Are records kept for the vehicle (corn oil, etc.), the supplier, and the lot number? How old is the supply, the chemical analysis, and the record of storage conditions?

(8) Is an SOP available for each chemical for cleanup of equipment?

(9) How are doses transferred from the preparation room to storage and to the animal room?

(10) Is the type of container adequate? Is the cap liner or stopper soluble in the chemical?

(11) Are any of the chemicals light sensitive?

(12) Are the storage conditions of the gavage/skin painting preparations those prescribed by the chemical analysis contractor? Is the storage method safe? Is there limited access?

(13) What is the length of storage for dose preparations?

(14) Are color-coded labels used? Is the key posted?

f. Dose preparation: water

(1) Who is responsible for calculations?

(2) Are calculations recorded for each dose?

(3) Who supervises?

(4) Are written mixing procedure and dose levels available in the preparation room?

(5) Is water analysis -- pH adjustment, hardness, source, and additional treatment information -- available and a part of permanent records?

(6) Are there dedicated water bottles, rubber stoppers, and sipper tubes per chemical? Per dose?

(7) Does the dose chemical deposit on bottles or discolor bottles (glass or plastic)?

(8) Are storage conditions adequate? How long is the chemical stored?

(9) Was analysis done before the chemical was used?

(10) Has the schedule for preparation been verified? Has it been updated?

(11) How is disposal of excess chemicals performed?

g. Dose preparation: inhalation

(1) What is the generation procedure?

(2) Is the concentration determined before animals are exposed? How?

(3) What is the schedule for a chamber concentration monitoring of each chemical? Is monitoring intermittent or continuous?

(4) Is there layering (nonhomogeneity) of material in the chamber?

(5) Is the generation system isolated from the chamber room?

(6) For gas exposure, are all cylinders secured at all times?

(7) For aerosols, is particle sizing performed? How frequently?

(8) Is emergency power automatic?

(9) Have records for calculations and analysis, new lots, etc., been verified?

(10) Are there SOP's for clean up of generator vessels?

5. Animals (See Animal Care Site Visit section)

a. Is there a log-in procedure for receipt of animals? Does it specify:

(1) Number/sex/strain/species?

(2) Birthdates? What is the range?

(3) Condition of animals on receipt?

(4) Source? (supplier and location, since some supplies have several locations)

b. Is there a quarantine period?

(1) How long is it?

(2) Where are animals quarantined?

(3) What observations are made?

(4) Does a doctor of veterinary medicine certify the fitness of animals for test?

c. Has randomization of animals been verified?

(1) When?

(2) What procedure was used? (Assignment by weight, random numbers)

(3) How are animals assigned to groups?

(4) Are cages/racks rotated? How? How often?

d. How are animals identified?

e. Animal care: Are there special problems related to specific chemicals?

f. How are animals observed?

(1) Have twice daily observations been verified?

(2) What times of the day during the week are observations made?

(3) What are the weekend/holiday schedules for observations?

(4) How often is weighing done; what time of day are animals weighed; what experience do personnel have? Is weighing combined with some other test? If so, what?

6. How are clinical signs observed:

a. Who makes observations?

b. Do technicians understand the terminology?

c. Who verified the uniformity of terminology?

d. Does the veterinarian spot check the accuracy of records? How often?

e. Who determines food consumption? How? How often?

f. Who determines water consumption? How? How often?

g. How are unscheduled deaths handled?

(1) Is there a log for unscheduled deaths? For necropsies?

(2) How are carcasses stored until necropsy?

(3) What is the time period from death to necropsy?

(4) How is disposal of carcasses handled?

h. How are sentinel animal serum samples handled:

(1) Are sera prepared and submitted according to instructions?

(2) Are sera reports submitted with monthly reports?

7. Bioassay Toxicology Monitoring

a. General

(1) Are protocols being followed?

(2) Does the principal investigator have the modified protocols (when such exist)?

(3) Are protocols available in the laboratory for responsible technicians?

(4) Are animals properly identified?

(5) How are clinical signs observed:

(a) When are animals observed? Is observation done in conjunction with other functions?

(b) Who makes clinical observations?

(c) Who supervises clinical observation?

(d) Are findings spot checked for verification by a veterinarian? How often?

(e) Do the staff making clinical observations understand terminology?

(f) Are clinical signs recorded?

(g) Are records inspected for verification.

(6) Who palpates animals for tumors? What training does the person have?

(a) How frequently are animals palpated?

(b) Are observations recorded and verified?

(7) Are animals weighed individually or by cage?

(a) How frequently are animals weighed?

(b) Is calibration of the balance a standard procedure?

(8) Are cages/racks rotated?

(9) What is the condition of filters?

(10) Are daily records kept of the number of dead animals?

(11) Does the number of moribund animals killed seem reasonable?

(12) Are unscheduled necropsies performed in less than 24 hours?

(13) How frequently are animals observed on weekends? (Check sign-in book.)

b. Dosed feed

(1) Is food consumption dose related?

(2) Are dedicated feeders used for each chemical?

(3) How are samples selected for analysis? Who makes the selection?

(4) How is excess food disposed of?

(5) Is the mixing schedule posted?

(6) What are the topping-off procedures for dosed feed?

(7) What are the dosed feed storage conditions and length of storage time?

(8) Is the same lot of feed used for controls and dosed feed preparation?

c. Dosed water

(1) Are water bottles replaced instead of refilled?

(2) Is water consumption dose related?

(3) What are the storage conditions (temperature, light, etc.) for dosed water?

(4) What is the condition of bottles, sippers, and stoppers?

(5) Are the bottles labeled as to chemical, dose, and date of preparation?

(6) How is disposal of extra dosed water carried out?

(7) When bottles are washed, are those for controls washed first? Is the wash water discarded when bottles for other chemicals are to be cleaned?

(8) Are there dedicated bottles, sipper tubes, and stoppers?

d. Gavage

(1) What are the training procedures for personnel?

(2) What procedure is used by the laboratory?

(3) What syringe size is used relative to dose size?

(4) Are Luer-Lok syringes used? What canular size is used?

(5) Are animals fed or fasted? (For fasted animals, 4 hours for mice, 12 hours for rats is recommended)

(6) What is the inoculation sequence for control/test doses?

(7) Is the individual or group weight used as a basis for dose size?

(8) What time of day is dosing done?

(9) What vehicle/solvent is used? What is the lot number and source? Is this information accurately recorded?

(10) What is the temperature of the doses? (Are they room temperature or iced?)

(11) Is chemical stability related to exposure to light?

(12) Are doses homogeneous?

(13) Is a hood used for gavaging? Is proper ventilation provided?

(14) Is volume recorded? (Is it same for all tests?)

(15) Is the accuracy of delivery determined?

(16) What is the schedule for changing needles or canulars?

(17) Are records of accidental inoculation and deaths per technician kept as an indication of performance?

(18) Are necropsies of all unscheduled deaths performed to determine the cause?

(19) Are different concentrations of chemical kept in different size bottles? Separate bottles for mice and rats - are bottles color coded?

e. Skin Painting

(1) Are animals housed as groups or individuals?

(2) What are the clipping procedures? How is clipping done? How frequently?

(3) What is site of application?

(4) How are descriptions of observations made?

(a) Are observations recorded on individual animal diagrams?

(b) How are lesions measured?

(c) How is regression recorded?

(5) What is the frequency for recording lesions?

(6) What is the present potential for irritation?

(7) Is the accuracy of the dose checked periodically?

(8) What is the dosing procedure -- glass rod or rubber tip for application, use of automatic syringes, stirring devices?

(9) What is the volume administered?

(10) What is the vehicle?

(11) What is the suitability of the chemical for skin painting?

(12) Is there build-up of material? What is the procedure for cleaning of skin?

(13) Are the techniques of all technicians comparable?

(14) Is skin painting being done with the skin as the organ site, or is it used for systemic exposure?

(15) Are systemic effects visible? Are they documented?

(16) Is application carried out under a hood?

f. Inhalation

(1) Chamber operation

(a) What is the type of exposure (gas, aerosol, vapor, particulates)?

(b) If gas, are gas cylinders properly secured at all times?

(c) If aerosol or particulates, is particle sizing performed to insure that a majority of particles are in the respirable size range? How frequently are samples taken and measured?

(d) What types of contaminants (chemicals and particulates) are added by the generating system?

(e) Are the generating systems checked for leaks on a regular basis?

(f) Are generating systems "isolated" from the chamber room?

(g) Can temperature and humidity be individually monitored, controlled, and recorded for each chamber? Is monitoring continuous or intermittent?

(h) Are chambers maintained at a negative pressure with respect to the chamber room during all exposures?

(i) Is monitoring continuous for each chamber?

(j) Are chambers tested for layering (nonhomogeneity) with each test material?

(k) Is chamber cleaning done daily after the exposure period, and is cleaning confirmed by sampling before opening chambers?

(l) How are concentrations in chambers determined? How often?

(m) Is the sampling system automatic? Are there manual backup sampling systems?

(n) When are calibrations of analytical instruments made to determine chamber concentrations?

(o) Do personnel wear full protective equipment while working in chamber rooms during the exposure period?

(p) What precautions are taken with highly flammable or explosive materials?

(q) Are there alarm systems? What kind? How often are they checked?

(r) Is there automatic emergency power to the chambers? Is it adequate to continue the exposure operation? Is it tested regularly?

(s) How often is equipment serviced? By whom?

(t) How long is the up-and-down time per day? What is the equilibration time per chemical?

(2) Animal care and observation

(a) Is the chamber volume adequate for maximum loading for the study?

(b) Is food removed from the chamber during the exposure period?

(c) Are animals caged individually?

(d) Is automatic watering used? If so, is it checked daily for proper functioning?

(e) Are cages rotated within the chamber? How often?

(f) Are catch pans used during exposure? If so, were measurements made showing that uniform concentrations can be maintained?

(g) Are animals housed in the chambers between exposure periods? If so, are chambers open or sealed with respect to chamber rooms?

(h) Are morbidity/mortality checks done twice daily during exposure and nonexposure periods?

(i) Where are animals housed during the chamber cleaning period?

8. Special Studies Monitoring

a. Clinical Chemistry Monitoring

(1) Personnel

(a) What are the qualifications of the clinical chemistry supervisor?

(b) What is his/her training and experience in clinical chemistry?

(c) How do the personnel maintain their proficiency? (Training and observation)

(d) Are safety precautions taken in handling the tissues of dosed animals?

(2) Animal handling

(a) What is the method of bleeding?

(b) What volume of blood is taken?

(c) Is the physiological state of the animal at the time of bleeding recorded?

(d) Have records been verified?

(3) Specimen handling

(a) Are the specimens properly identified and labeled?

(b) What is the serum or plasma separation method?

(c) What are the storage conditions for specimens?

(d) How long are specimens stored before they are tested?

(e) What are the preparation procedures for specimen analysis?

(4) Testing and analysis

(a) What is the scheduling of testing and analysis?

(b) Is instrumentation adequate? Has the service record been verified? Is the preventive maintenance scheduled?

(c) What is the method of analysis, the accuracy of the determination, and the sensitivity of the test? How precise is the method?

(d) What are the SOP's for preparing and storing reagents? Has labeling and dating on reagents been verified?

(e) How many samples are analyzed at a time?

(f) How are the data captured?

(g) Is any statistical analysis carried out?

(h) In regard to diagnostic evaluation, is there any correlation of clinical chemistry results with clinical signs and histopathologic findings? Is the correlation with historical values checked?

(i) What is the procedure for tracking and retesting values that are outside set limits?

(5) Are quality control measures adequate? Have the following items been verified:

(a) Proficiency of testing record (CAP, AAB)?

(b) Program standards analysis?

(c) Standard specimen (in and above normal range). Interbatch? Intrabatch? Day-to-day?

(d) Acceptability of data related to historical standards?

(6) Are records for the following adequate:

(a) Specimen/animal identification?

(b) Method of analysis?

(c) Identification and dating of entries?

(d) Location of records?

(e) Accessibility to involved personnel?

b. Behavioral Monitoring

(1) Examine the environmental conditions for the following:

(a) Are studies carried out in a quiet room separate from animal quarters?

(b) Is there white noise to mask external noises?

(c) Are tests done in the same place, at the same time of day, and by the same person?

(2) Equipment

(a) What is the cleaning schedule (between usage for motor active screens and mazes)?

(b) Are strain gauges and electronic gear calibrated with each session?

(3) Are tests run blind?

(4) Is there a standard sequence of tests employed (preferably the least invasive to the most stressful)?

(5) When food and water consumption are determined, have spillage of food and leaking of water bottles been examined?

(6) Since monitoring of activity measurements is highly dependent on environmental factors,

(a) Are individual animals in isolated cubicles?

(b) Is each chamber cleaned before each animal is monitored?

(c) Are tests made between 10 a.m. and 2 p.m. to avoid diurnal cycle?

(d) Is the actual time of the test recorded?

(e) Are the locations of printout counters and digital counters to test chambers adequate to avoid noise interference?

(f) Are there lightened or darkened conditions during the test?

(g) Are calibrations of the chamber made to determine shifts in sensitivity of activity monitor?

(h) What is the time between removal of an animal from its home cage to placement in activity chamber? (Acclimation time should not be counted.)

(i) What is the ambient temperature and humidity? ($72^{o}\pm5^{o}F$ and $50\%\pm20\%$)

(7) In testing for the presence or absence of autonomic signs, the appearance of normal or depressed motor/pain reflexes, or the visual placement, is very subjective. Monitor the following items:

(a) Are tests carried out in an area free of distractions (change in orders, sounds, light)?

(b) Are tests carried out on a blind basis?

(c) Are personnel familiar with literature regarding test conditions?

(8) In regard to forelimb grip and hindlimb extensor responses,

(a) Has precalibration of the stress gauge been carried out?

(b) Are three readings made under defined criteria?

(c) Are new, revised techniques used?

(9) In regard to hindlimb response (smooth movement, not muscle jerk, for limb grip strength), are animals habituated to sitting on the T-bar attached to the meter? When an air puff is used to elicit a response, is a short blast of air applied to rump?

(10) For startle responses,

(a) Are these tests carried out in a quiet area?

(b) Are animals positioned over the center of the transducer and are they motionless at time of stimulus?

(c) Are the startle tests isolated from waiting animals to avoid variable responses?

(d) Is the source of sound calibrated frequently?

(11) For measuring tremor,

(a) Which of the several techniques that distinguish small motor movements (fine motor fasciculations) from gross motor movements are being used?

(b) Can the technique separate tremor from respiration?

(c) Is the apparatus calibrated before it is used?

(d) Is the environment quiet?

(e) Are tests taken over a short period of time (10 to 15 seconds)?

(12) For measuring rectal temperature,

(a) Do instruments have a digital readout?

(b) Is the probe inserted exactly the same distance each time?
(Calibrate by placing a marker on the probe.)

(13) During tests,

(a) Are tests carried out in a controlled environment?

(b) Is the shuttlebox contained inside a sound and light-attenuated outer chamber?

(c) Are grids cleaned after each trial to remove urine and feces which short out the shocking device?

c. Pharmacokinetics Monitoring

(1) Regarding purity of the test compound,

(a) Is the compound 98 percent or more pure?

(b) Are there toxic impurities? (Impurities must not have any toxicity or inducing capacity greater, on a molar basis, than that of parent compound)

(2) For verifying the accuracy of dose measurements,

(a) Is the actual dose determined?

(b) Is the effect of the size of the dose on absorption determined?

(c) Is the effect of the number of doses on absorption determined?

(d) Is the effect of the injection site determined? (No more than 10 percent of dose should be in the injection site at the time that the tissues are assayed).

(e) Is the amount of material at the injection site determined?

(3) Are facilities, equipment, and expertise adequate for measuring the concentration of the compound administered?

(a) With radioactive compounds, the capability to separate parent compound from metabolites must be available. Does such capability exist?

(b) Is the laboratory measuring relative amounts at 3 to 4 selection time points?

(4) Has the relative importance of excretory routes been taken into account? Are the following being determined:

(a) Urine?

(b) Feces?

(c) Exhaled air?

(d) Nature of excreted compound?

(e) Relative amounts of parent compound and metabolites?

(f) Is the parent compound in feces? Are parent compounds and metabolites in the bile?

(5) Do personnel have analytical chemical capability and expertise in the following:

(a) Isolation and identification of metabolites?

(b) Concentration of metabolites in selected tissues at selected time points?

(c) Determination of the relative amount of metabolites excreted?

(6) Have the following data in reports been verified:

(a) Small animal data in triplicate test?

(b) Individual animal data?

(c) Mean for three animals and standard deviation of mean?

(d) Percent recovery of material?

(e) Computed half-lives, volume of distribution, and clearance rate/organ?

(7) Have other special areas received adequate monitoring?

V. A CHECKLIST FOR THE LABORATORY MONITOR

A. ADMINISTRATION

1. Have there been any changes in professional staff? (Obtain CV's of new personnel.)

2. Is the master schedule for bioassay studies up to date?

3. Is the principal investigator knowledgeable about the status of the following:

   (a) Contract and modifications?

   (b) Chemicals?

   (c) Recordkeeping?

   (d) Reporting?

   (e) Scheduling?

   (f) Costs incurred?

   (g) Any problems (by discipline)?

4. Has the principal investigator received and understood the following protocols for each chemical:

   (a) Safety and toxicity?

   (b) Chemical analysis?

   (c) Dose preparation?

   (d) Storage?

   (e) Animal studies?

   (f) Special studies?

   (g) Revisions of various protocols?

5. Has the laboratory received all "Safety and Toxicity" packages before the receipt of individual chemicals?

6. Are protocols available to appropriate staff?

7. Are all precautions (as necessary) in place before receipt of the chemical (e.g., handling, storage or disposal requirements)?

8. How many types of accidents have been reported? What remedial action was taken?

9. Have any personnel developed medical problems?

10. Is the contract statement of work understood by key personnel in each scientific discipline?

B. SHIPPING, RECEIVING, AND STORAGE

1. Have any problems been encountered with animal shipments?

2. Are animal receipt records adequate?

3. Are there problems with the feed supply? (Record oldest feed in the current inventory.)

4. Were chemicals received in good condition? If not, how were they handled? Was damage reported?

5. Were chemicals properly packaged and labeled?

6. Were chemical reference standards for bulk chemical analysis pulled and stored at -20° C?

7. Are all chemicals stored according to prescribed conditions (i.e., according to the Safety and Toxicity package and the contract)?

8. Is the individual chemical disbursement log up-to-date? Where are records maintained?

9. Are labeling requirements being met (including the use of any radiolabeled material)?

10. Do technicians appear well informed about standard operating procedures and specific requirements?

11. What is the general appearance of the analytical areas?

12. How effective does the exhaust ventilation appear?

13. Is biotest material transferred under controlled conditions?

14. Do any special studies or routes of administration pose procedural problems?

15. Are staff members aware of spill cleanup procedures?

C. CHEMICAL STORAGE Was test material transferred directly from receipt to storage or was it repackaged? If repackaged, was it done by an approved method?

D. CHEMISTRY

1. Was the responsible chemist provided with the background information (including analytical report) on all new chemicals?

2. Will the chemist follow the analytical protocols provided by the analytical contractor?

3. Has the chemist been provided with the mixing schedule for dose preparations and required analyses for all new chemicals?

4. Have there been any particular problems with the chemistry since the monitor's last visit?

E. FACILITIES AND TESTING (General)

1. Have there been any changes in assigned facilities?

   (a) Why?

   (b) Are the new facilities adequate for the present bioassay needs? For future needs for assigned chemicals?

2. Do bioassay areas actually have limited access?

3. Are protocols available in the laboratory for responsible technicians?

4. Observations:

   (a) Verification of twice daily observations

   (b) What times of day during the week are observations made?

   (c) What is the weekend/holiday schedule for observations?

   (d) How often is weighing done, what time of day, what is the experience of personnel? Is weighing combined with some other task? If so, what?

   (e) Clinical signs

      (1) Who observes clinical signs?

      (2) Do technicians understand terminology?

(3) Who verified the uniformity of terminology?

(4) Does a veterinarian spot check the accuracy of records? How often?

(f) Who determines food consumption? How? How often?

(g) Who determines water consumption? How? How often?

5. Are daily records kept on the number of dead animals? (Check sign-in book for weekends to determine the time of observation.)

F. ANIMAL CARE, HANDLING

1. How consistent is air flow and air direction?

2. Have any HVAC equipment malfunctions been reported?

3. Is personnel protective equipment appropriate (as specified in the health and safety plan and SOP's)?

4. How are animal logs and records handled?

5. Are room sanitation procedures adequate?

6. What are the weekend/off hour emergency procedures?

7. What is the highest and the lowest temperature recorded for this quarter?

8. Have any emergencies occurred this quarter? Explain.

9. Is there any evidence of vermin in the facility?

10. Are the maintenance procedures specified in the health and safety plan being properly implemented?

11. How are animal rooms and support corridors cleaned? How frequently?

12. What cleanup techniques are employed?

13. What are the procedures for room decommissioning after the testing phase?

14. Do members of the maintenance staff comply with the personnel protective requirements of the animal care staff?

15. Are contract maintenance personnel accompanied by program staff?

16. Are maintenance logs up to date?

17. Has emergency power been tested recently? How frequently? Is there a log of tests?

18. Are members of the maintenance staff familiar with program needs/requirements?

19. How is equipment serviced (e.g., HVAC, wastewater streams, etc.)?

20. Have any equipment malfunctions been reported?

G. DECOMMISSIONING (Specific Bioassay Test Terminations)

1. How is the chemical surplus handled/shipped?

2. Is all material accounted for?

3. Are special decontamination procedures required in specific work areas?

4. Where are dedicated cages disposed of? How?

H. DOSED FEED

1. Is food consumption dose related?

2. Do animals appear to waste excessive food?

3. Are dedicated feeders used for each chemical?

4. How are samples selected for analysis?

5. Who makes the selection?

I. WASH ROOM

1. Are control cages washed before cages used for dosed animals?

2. Is the water reservoir for the cage washer changed between chemicals?

3. When water bottles are washed, are the control bottles washed before the bottles used for dosed water?

4. How are treated cages marked? Is it easy to determine which cages were used for each chemical?

J. CAGE EMPTYING/WASHING AREA

1. Is adequate local exhaust ventilation being provided?

2. How are wastes handled? Do any chemical tests require special waste handling?

3. Examine the loading dock (for waste shipment) or onsite incinerator area (if present).

   (a) Are wastes double bagged?

   (b) Is refrigeration required?

4. Are washing and sanitizing equipment properly operated?

5. Are certain personnel dedicated to cage washing/dumping areas?

K. GAVAGE

1. Are records of accidental gavage inoculation (deaths per technician) kept as an indication of performance?

2. Is gavage performed under hoods with appropriate personnel protection?

L. SKIN PAINTING

1. Are animals housed as groups or as individuals?

2. What are the clipping procedures? What is the method and frequency?

3. What is the site of application?

4. How are descriptions of observations made?

   (a) Are individual animal diagrams used?

   (b) Are lesions measured?

   (c) How frequently are lesions recorded?

   (d) How is regression recorded?

5. Is there a buildup of material? What is the procedure for cleaning skin?

6. Are animals visibly systemically affected? What is the documentation?

M. GAVAGE/IP/SKIN PAINTING

1. Are hoods available? Are they in use?

2. Is there any history of technique problems?

N. DOSE PREPARATION AND STORAGE AREA

1. Were mixing protocols provided by the analytical contractor and, if so, are they being used?

2. How many different bioassay and other studies on chemicals are handled in any one day for dose preparation? Are hoods, blenders, etc., adequately cleaned between preparations of different doses?

3. Do the mixing operations yield acceptable dosage mixtures (i.e., homogeneous feed mixes, clear corn oil gavage solutions, etc.)?

4. Are the dosage mixtures properly stored and labeled? Do they follow the storage and handling procedures outlined in the analytical contractor's report?

5. How are dose levels prepared? Are the different levels weighed and mixed separately, or is the high dose mixed and then diluted for subsequent lower doses?

6. Is the transfer of dosed feed minimizing aerosol contamination?

7. Where are the feed hoppers filled? How?

O. CHEMICAL/VEHICLE ANALYSIS

1. Are the dosage mixtures being analyzed promptly?

2. What is the time frame between mixing and analysis? Do these operations occur before the animals are dosed?

3. Are the referee samples sent to the analytical contractor properly?

P. IS HOUSEKEEPING ADEQUATE?

Q. NECROPSY

1. Who decides when abnormal animals are to be killed?

2. Does the number of moribund animals killed seem reasonable?

3. Are unscheduled necropsies performed in less than 24 hours?

4. Are animals necropsied on weekends?

5. Where are dead animals stored until necropsy?

6. What is done with moribund animals on the weekend?

7. Is protective equipment/clothing worn?

8. Where/how are necropsy tissues stored? (Are there any contamination problems?)

9. Do the prosectors have adequate work areas?

10. How are animal records handled and transferred?

11. Does the necropsy staff have any problems that relate to working conditions?

12. Is a list of SOP's present in the necropsy room?

13. Is ventilation adequate? (Are formalin fumes or other odors obvious)?

14. Is dye present in the formalin bottles or jars?

R. HISTOPATHOLOGY

1. How many histology technicians are working in the laboratory?

2. Are SOP's readily accessible?

S. HISTOLOGY

1. What protective equipment is in use?

2. How are solvents stored and handled?

3. How is HVAC performing? (Are there strong odors?)

4. Is there any evidence of food or tobacco products in the area?

5. How are waste solvents handled?

6. How is area cleaned up? By whom?

7. Are necropsy samples decontaminated before being transferred to histology? (If required.)

8. How many slides, blocks, and tissues from the bioassay are currently in storage at the laboratory? Where are they located?

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