Chapter 3. Health and Safety

Specifications for the Conduct of Toxicity Studies by the Division of Translational Toxicology at the National Institute of Environmental Health Sciences

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3. Health and Safety

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3.1. Administrative Controls

3.1.1. Regulations and Guidelines

The National Institute of Environmental Health Sciences (NIEHS) or its program representatives may inspect, sample, and monitor the laboratory and associated facilities used for its studies at any time to ensure that the requirements and applicable regulations and guidelines (described below) are being followed. Any deviations to these requirements shall be approved by the program.

All work shall conform to applicable local, state, and federal statutes in effect at the time of award and throughout the period of performance. These statutes include the following federal regulations and any updates:

Occupational Safety and Health Administration (OSHA)

- Standards for General Industry, 29 CFR 1910
- Hazard Communication, 29 CFR 1910.1200
- Respiratory Protection, 29 CFR 1910.134
- Occupational Exposure to Hazardous Chemicals in Laboratories, 29 CFR 1910.1450
- Occupational Exposure to Blood-borne Pathogens, 29 CFR 1910.1030
- Formaldehyde, 29 CFR 1910.1048 (applicable to the use of formaldehyde in histology, pathology, and anatomy laboratories)

Department of Justice (DOJ)

- Americans for Disability Act, Accessibility, Design Guidelines, 28 CFR, Title III, Part 36

Environmental Protection Agency (EPA)

- Clean Air Act, 40 CFR 50-80
- Clean Water Act, 40 CFR 100-140 and 400-470
- Resource Conservation and Recovery Act (RCRA) 40 CFR 240-271
- Comprehensive Environmental Response, Compensation and Liability Act (CERCLA, Superfund, SARA), 40 CFR 300

Department of Transportation (DOT)

- General Information, Regulations, and Definitions, 49 CFR 171

- Hazardous Material Table, Special Provisions, Hazardous Materials Communication Requirements and Emergency Response Information Requirements, 49 CFR 172
- Shippers, General Requirements for Shipments and Packaging, 49 CFR 173
- Carriage by Public Highway, 49 CFR 177

Nuclear Regulatory Commission (NRC)

- Standards for Protection against Radiation, 10 CFR 20
- Notices, Instruction, and Reports to Workers; Inspections, 10 CFR 19
- Recommendations described in the most recent version of the National Institutes of Health (NIH) Radiation Safety Guide

Drug Enforcement Administration (DEA)

- Federal Requirements for Controlled Substance, 21 CFR 1300

For contract work involving infectious agents, the Centers for Disease Control guidelines, Biosafety in Microbiological and Biomedical Laboratories (HHS Publication No. (NIH) 93-8395, 2009) and the NIH Guidelines for Research Involving Recombinant DNA Molecules (66 Federal Register 57970, 2001 and updates), shall be followed.

Where not superseded by this document, the American National Standard for Laboratory Ventilation, Z 9.5, published by the American National Standards Institute (ANSI), shall be followed. In many cases, the ANSI Z 9.5 provides requirements and recommendations that describe how to approach defining specific requirements for a given facility or program of work. Thus, the information and requirements presented here may be more specific; however, the overall philosophy presented in the ANSI Z 9.5 shall be applied to the health and safety program of the facility.

Other consensus standards and publications may include: the current edition of the Threshold Limit Values for Chemical Substances and Physical Agents & Biological Exposure Indices published by the American Conference of Governmental Industrial Hygienists (ACGIH), criteria documents for various substances and the recommended exposure limits published by the National Institute for Occupational Safety and Health (NIOSH), and the Workplace Environmental Exposure Levels (WEEL®) published by the American Industrial Hygiene Association. If the acceptable exposure levels conflict with the OSHA permissible exposure limit, the more stringent standard shall be used for workers' protection.

3.1.2. Health and Safety Plan (Chemical Hygiene Plan)

The scope of each health and safety plan shall address the organization's health and safety policies and occupational medical surveillance program, as well as potential chemical, physical, biological, and ergonomic hazards (e.g., acquisition of study materials, storage, and handling through ultimate disposal of contaminated wastes).

No contract laboratory will participate in studies without a health and safety plan that has been approved by the sponsor. An updated plan shall be submitted every 2 years for review. In addition, the contracting officer's representative (COR) shall be informed of any updates to the

plan immediately. If approval of the plan is not granted at the time of award, the laboratory must submit a revised plan for review within 30 days of the receipt of award notification. Revisions to the plan shall be clearly indicated to facilitate reviewer approval.

For all contract laboratories, a chemical hygiene plan as required under the OSHA "Laboratory Standard" may be used in place of a health and safety plan provided it meets or exceeds *all* of the requirements outlined in this chapter.

The health and safety plan shall include procedures for ensuring that subcontractors performing major aspects of work have appropriate health and safety procedures in place to perform required work. These procedures are expected to be commensurate with the nature of the work performed.

Written Policies. In addition to the standard operating procedures (SOPs) outlined below, the health and safety plan shall address (but not be limited to):

- Health and safety responsibilities, policies, and organization
- Record keeping and archiving
- Initial and periodic employee training
- Engineering controls
- Personal and environmental monitoring
- Medical surveillance and biological monitoring
- Respiratory protection program
- Personal protective clothing and equipment
- General housekeeping
- Eating and smoking policies and areas
- Precautionary signs and labels
- Chemical and biological storage
- Fire protection and prevention
- Emergency and evacuation contingencies
- Locations (with schematic diagrams) of fire control equipment, and plumbed eyewash stations and emergency showers
- Laboratory safety inspection
- Waste management and disposal
- Other pertinent personnel, operational, and administrative practices, and engineering controls necessary for the containment and safe handling of chemical, physical, biological, and radiological hazards
- Entry and exit to restricted areas
- Visitors

3.1.3. Standard Operating Procedures

The laboratory shall be required to have written SOPs that have been reviewed and approved by NIEHS for at least the following activities:

- Visitors access to test areas
- Employee training
- Medical surveillance and biological monitoring
- Respiratory protection, mask fit, cleaning/maintenance, and inspection
- Eye and face protection
- Personal protective clothing and equipment
- General housekeeping practices
- Ventilation system maintenance
- Storage, receipt, transport, and shipping of study materials
- Hazardous material handling (e.g., in analytical chemistry labs)
- Dose/exposure formulation preparation (as applicable)
- Entry and exit from the limited access areas (including traffic patterns of formulation preparation facility and room(s) used for housing, handling, or evaluating animals, as applicable)
- Spill cleanup, accident, emergency response and evacuation (including natural disasters), and fires/explosions
- Use of radio-labeled material, infectious agents, or controlled substances (if applicable)
- Hazardous/nonhazardous waste management and disposal

3.1.4. Exposure Evaluation and Control

Permissible Exposure Limits/OSHA-regulated Substances

All laboratories shall ensure that employees' exposures to hazardous substances do not exceed the permissible exposure limits (PELs) specified by OSHA in 29 CFR 1910, subpart Z. In addition, initial monitoring of employees' exposure to any substance regulated by a standard (e.g., 29 CFR 1910.1001-1101), which requires monitoring, shall be conducted if there is reason to believe that exposure levels for that substance routinely exceed the action level (or in the absence of an action level, routinely exceed the PEL). If this initial monitoring reveals that an employee's exposure exceeds the action level or the PEL, the testing laboratory shall comply with the exposure monitoring provisions of the relevant standard.

If a PEL has not yet been established for a study material, alternative acceptable exposure standards (e.g., TLV®, REL, WEEL®) shall be used (refer to Section 3.1.1). In situations for which there is no known exposure standard for a proposed test article, a suitable interim exposure standard that is based on current toxicology and industrial hygiene literature shall be established when feasible.

Formaldehyde Monitoring

Histology, pathology, and anatomy laboratories must comply with the formaldehyde OSHA standard, 29 CFR 1910.1048. The use of formaldehyde in all other laboratories shall be carried out in accordance with the OSHA formaldehyde standard.

Histology, necropsy, tissue storage, and tissue trimming operations shall be conducted in a manner that employs engineering controls to ensure that airborne concentrations of formaldehyde do not exceed 0.75 ppm as an 8-hour time-weighted average (TWA) or 2 ppm as a 15-minute short-term exposure limit (STEL). Monitoring shall be performed to evaluate exposure levels (both TWA and STEL) of workers potentially exposed to formaldehyde hazards. If the results of initial monitoring indicate exposure levels exceeding either 0.5 ppm as an 8-hour TWA (action level) or 2 ppm as a 15-minute STEL, additional monitoring must be performed. If the TWA exceeds the action level, sampling must be repeated every 6 months. If the STEL exceeds 2 ppm, sampling must be repeated annually. The repeat sampling may be discontinued when two consecutive sampling rounds are below the STEL and action level as described in the OSHA formaldehyde regulation, 29 CFR 1910.1048. In addition, the testing laboratory shall adhere to all other provisions of the OSHA formaldehyde standard.

Test Article/Positive Control Monitoring

Exposure monitoring shall be routinely conducted where both test article and controls are handled and when the test article/positive control has an established exposure standard, such as the PEL, TLV®, REL, or WEEL® of 10 ppm or less, or 0.1 mg/m^3 or less. This exposure monitoring shall be performed at least once during initial dose preparation and once during initial dose administration, and at the midpoint of the study (for prechronic studies) or every 6 months (for chronic studies). When there is no known exposure standard for a proposed test article, the testing laboratory shall perform exposure monitoring at the same frequency stated above. Determination of exposure and adoption of controls shall be based on a predetermined interim exposure standard, when feasible.

3.1.5. Occupational Medical Surveillance

An occupational medical surveillance program shall be implemented to cover personnel who will be working with study test articles or animals. The frequency of surveillance as well as the scope of medical examination shall be specified in the laboratory's health and safety plan. Persons who are required to wear respirators must obtain written medical clearance from an occupational health service provider (e.g., an occupational medicine physician, a physician assistant, or nurse practitioner who is supervised by the physician) for use of this equipment.

3.1.6. Injury and Incident Reports

A record shall be kept of all injuries or illnesses, including animal bites. In addition, any record of an OSHA recordable incident shall include a full description of the incident, the test article/positive control involved, the medical attention required, any remedial actions taken, and planned follow-up to minimize the likelihood, or eliminate the potential for, reoccurrence (if pertinent). Copies of such incident reports shall be forwarded to NIEHS. The program COR shall be notified *immediately* if a serious (as defined by OSHA) accident or incident occurs. All occupational injuries and illnesses shall be recorded and reported according to the OSHA recording system.

3.2. Test Article/Positive Control Handling and Safety Policies

3.2.1. Receipt/Handling/Storage

A log shall be maintained that will include the date of test article receipt and a continuous balance of the remaining amount of test article.

Weighing of the test article/positive control shall be done using the smallest quantity needed. An analytical balance shall be used whenever possible to preclude the need for handling large amounts of chemical. At all times, this balance shall be placed in an effective laboratory hood or a vented enclosure exhausted to the outside (see Section 3.3.3). Protocols shall be designed to use the minimum possible quantities of "neat" chemical in preparing solutions.

A nonbreakable, secured secondary container shall be used for transfer of any test article/positive control.

Volatile test articles shall be handled properly (e.g., keeping lids on container when not in use, segregating from unintended contact with heat or high pressure) and stored in an area with adequate ventilation that is directly vented to the outside. All other test articles shall be stored in a secured, designated storage area(s). Flammable liquids must be stored, however, in a nonvented flammable liquid storage cabinet (see Section 3.5.1).

3.2.2. Hazard Communication

Training

Personnel who handle (receive, store, weigh, dilute, transport, package, or administer) hazardous agents shall be provided with written material and trained on the associated hazards of these agents including the contents of the material safety data sheet (MSDS). This training shall be conducted by the health and safety officer (HSO) or a program approved by the HSO and shall be properly documented. Training shall include the recommendations for handling carcinogens and reproductive, developmental, or neurobehavioral toxicants. In addition, training in accordance with the requirements of applicable regulations shall be conducted.

Labeling

Warning signs and labels shall be used wherever test articles are used or stored (e.g., on primary and secondary containers, affixed to entrances to work areas, refrigerators, and on containers holding hazardous waste). These signs and labels shall be conspicuous (especially for containers to minimize handling) and shall indicate the presence of suspected carcinogenic, mutagenic, and other hazards, as required by OSHA.

Health and Safety Documents

The testing laboratory must have health and safety documentation available for each study agent and positive control that includes, but is not limited to, the supplier's MSDS, which includes information on the material's hazards, properties, and appropriate control measures. If a chemical is produced for a user outside of the laboratory, the laboratory is required to develop an MSDS. All employees handling the study material or the positive control, or both, must be trained on the contents of the agent-specific health and safety data document.

The MSDSs shall be accessible at all times at designated locations known by the appropriate employees.

3.3. Engineering Controls

3.3.1. General Facility Requirements

Safety showers, drench hoses, and eyewash stations shall be located throughout the facility as required by local, state, and federal regulations and must be located in close proximity to where potentially hazardous chemicals are stored or used. Only plumbed eyewashes are permitted.

3.3.2. Isolation and Access Restriction

General Requirements

An isolated, posted, restricted access laboratory (or laboratories) separate from other laboratory facilities shall be designated for unpacking, storing, weighing, and diluting of test articles/positive controls and where necropsy, tissue trimming, tissue processing, embedding, microtoming, and staining are performed.

Administration of test articles and positive controls shall be performed in a limited access area that has air supply under negative pressure with respect to connecting laboratories and hallways. This area shall be a separate laboratory from the area described above dedicated to unpacking, storing, weighing, and diluting.

Each laboratory shall have a room inspection program providing monthly checks of the airflow directionality. Relative pressures of laboratory areas shall be checked monthly with smoke tubes to verify that the air flows from relatively clean to relatively dirty areas. Monthly inspections shall be documented.

A record shall be kept of all personnel entering and exiting any limited access area(s).

Barrier Systems

The dose/exposure formulation preparation shall be isolated from general traffic, which can be accomplished by locating this area within the animal facility limited access barrier system, or by establishing a separate limited access area for dose preparation. If the latter approach is used, all areas into which laboratory workers might bring used protective equipment (including gloves, shoes, head covers, and clothing), respirators, or containers of dosed feed or water, etc. shall be behind the barrier. Also, any hallways used by workers for reaching the shower facility shall be considered to be behind the barrier (e.g., limited access area).

Personnel who enter the formulation preparation area, or an area requiring a complete set of clean protective clothing and equipment (e.g., a disposable laboratory suit, safety goggles, disposable gloves with permeation-resistant properties specific to the test article, disposable boots, disposable shoe covers or sneakers or rubber boots, and disposable head covering), must shower out before leaving the barrier facility at the end of the day.

Within the shower facility, the “clean” and “dirty” sides must be physically separated by the shower or by another physical barrier. The facility design and procedures shall be arranged so that it is not necessary to enter the clean side before showering and to prevent returning to the

dirty side after showering (e.g., to store or retrieve items such as shoes, towels, respirators). (See Chapter 2. Facilities.)

Facility Design for Barrier Systems

Air exhausted from formulation preparation areas involving the particulate form of the test materials shall be passed through HEPA filters. If volatile chemicals are handled, charcoal filters shall also be used. These filtration systems shall be periodically monitored and maintained and personnel performing maintenance shall wear the protective clothing described for neat test article handling (see Section 3.4.1).

The relative location of external air intakes and exhausts for both local and general ventilation systems must be arranged to minimize the risk of re-entrainment of exhaust air. Documentation (e.g., schematic diagram) shall be provided that indicate the location of intakes and exhausts, stack height, discharge velocities, and the direction of prevailing winds. The use of weather caps shall be approved by the COR before implementation. No other obstructions shall be in the path of vertical discharge.

Within the barrier facility, walls, floors, and ceilings shall be sealed around all incoming and outgoing pipes, conduits, and other utilities to prevent release of contaminated material to surrounding areas. Animal rooms and formulation rooms shall be constructed of wall, floor, and ceiling materials that form chemical-tight surfaces. Animal room doors shall include windows to permit observation of workers within each room.

3.3.3. Hoods and Vented Enclosures

Where not superseded by requirements in this section, all work shall conform to the current edition of the Laboratory Ventilation Standard, Z 9.5, published jointly by ANSI and the American Industrial Hygiene Association. Effluent exhaust concentrations shall not exceed federal, state, and local air pollution emission requirements.

Operations and Requirements

The following operations, unless otherwise noted below, shall be performed in a laboratory hood or other enclosure:

- All dose preparation operations (e.g., weighing, premix, micro encapsulation, mixing of dosing solutions), as well as diluting or administering (gavage, dermal, intra-peritoneal injection, inhalation chamber administration) of study materials/positive controls
- Test article weighing in laboratories (e.g., analytical laboratories)
- Transfer/filling of dosed-feed containers
- Unpacking, analysis, and other handling operations involving test article/positive control or other hazardous agents
- Necropsy, tissue trimming, tissue processing, and staining
- Handling tissues, fluids, and exhaled air collected from animals for evaluation
- Cage and feed container dumping

- Plastic-backed absorbent matting shall be secured inside of any hood wherever the test articles/positive controls (including dilutions) are being handled. After each working session in the hood, or sooner if there is known contamination, this matting shall be disposed of as hazardous waste.

NOTE: Operations that cannot be performed within a laboratory hood or other enclosure due to the size of the containers or equipment will be conducted using other engineering controls (e.g., local exhaust, enclosed systems), administrative controls (e.g., restricted access during operations), additional personal protective equipment (PPE), or a combination of controls that will provide equivalent protection of employees. The HSO shall make the determination of appropriate controls.

Weighing, Diluting, or Administering Test Articles/Positive Controls

Laboratory hoods for diluting and administering test articles/positive controls (including gavage, dermal, intra-peritoneal injection and dosed-feed hoods) shall provide sufficient contaminant and containment capture velocities (an average air flow velocity of 100 ± 20 fpm at the operating sash height with no individual point <80 fpm or >120 fpm unless it can be demonstrated by testing—e.g., yearly use of smoke candles—that values >120 fpm provide adequate capture and do not cause turbulence). In addition, face velocities of balance enclosures shall be at least 50 fpm.

Biological safety cabinets used for dilution or administration of toxic agents shall recirculate no more than 30% of their air.

Automatic Tissue Processing/Staining

An effective exhausted enclosure or hood for automatic tissue processing or staining machines with exposed solvent systems shall supply sufficient capture velocities (e.g., 50 fpm minimum), as evaluated by a combination of velometer and smoke tube tests. Exhausted enclosures for automatic processors having exposed solvent systems shall be provided with a fire protection system or emergency power backup, or both.

Necropsy, Tissue Trimming, Manual Tissue Processing, and Manual Staining

An effective exhausted enclosure or hood for necropsy, tissue trimming, manual tissue processing, and manual staining as well as for all handling operations involving tissues, fluids, and exhaled air collected from animals considered to be contaminated with test article/positive control shall provide capture velocities of 80 ± 10 fpm (with no individual point <70 fpm or >90 fpm unless it can be demonstrated by testing [e.g., yearly use of smoke candles] that values >90 fpm provide adequate capture and do not cause turbulence).

Venting

Hoods and glove boxes used for weighing, diluting, or administering test articles/positive controls shall be exhausted to the outside.

Effluent exhaust vapor from sample oxidizers and analytical instruments (e.g., gas chromatograph, atomic absorption spectrophotometer) shall be vented to the outside.

Motors for hoods and enclosures exhausted to the outside shall be mounted outside the building such that all ductwork shall be under negative pressure.

Recirculation of air from local exhaust systems into occupied spaces shall not be permitted. The only exception to this requirement will be for dosed-feed container-filling hoods, cage-dumping hoods, or vented enclosures for studies involving nonvolatile, solid test articles. If recirculation is desired in this case, the air discharged from hoods or vented enclosures must be equipped with HEPA filtration to clean air before its discharge to the study room. The HEPA filter shall be disposed of as hazardous waste (see Section 3.7.2).

Monitoring

Exhaust enclosures shall be smoke tested using smoke tubes to demonstrate no leakage of smoke out of the enclosure during normal operating procedures.

All ventilation systems shall be routinely monitored. During chronic studies, laboratory hoods and all other local ventilation enclosures shall be quantitatively monitored on a quarterly basis. For studies of 90 days or fewer duration, each hood or vented enclosure shall be verified within 45 days before the beginning of the study unless monitoring data indicate a different frequency.

The sash height at which the face velocity has been measured shall be marked on each hood along with the date of the last measurement, the measured flow, and name of the person performing the monitoring.

The HSO shall maintain records of ventilation system checks. The records shall indicate for each hood, room, and area, at a minimum, when air was tested, what was found, who conducted the test, and what equipment was used.

3.4. Personal Protection Equipment Selection

3.4.1. Selection

Handling Neat Test Article/Positive Control and Activities in Animal Rooms

Where the neat test article/positive control (as neat material or in formulated doses) is stored and weighed in dose-formulation rooms and in animal-study rooms, or areas into which personnel directly exit when leaving animal study rooms (e.g., dirty side of the barrier), the following minimum personal protective clothing shall be worn at all times:

- Disposable full-body Tyvek® (or equivalent) suit and disposable head covering, unless Tyvek® suit includes a hood
- Gloves: If chemical-specific gloves cannot be identified, two pairs of dissimilar, disposable gloves (e.g., N-Dex® or equivalent, PVC, latex, natural rubber) will be worn when handling test article/positive control (as neat material or in formulated doses); both pairs of the two dissimilar gloves shall be changed after any known chemical contact and after every 2 hours of handling test article/positive controls or dose formulations
- Respirator: Appropriate NIOSH-approved respirators
- Eye protection: Splash-proof safety glasses, goggles, or other eye protection specified by OSHA and ANSI

- Footwear: Disposable shoe covers, disposable boots, or facility-dedicated rubber boots

Operations Not Involving Neat Chemical/Positive Control

For laboratory operations not involving the handling of neat test article/positive control (e.g., chemical analysis, histology, tissue trimming, and necropsy on the clean side of the barrier), the following shall be worn:

- Single pair of disposable gloves
- Laboratory coat
- Splash-proof safety glasses, goggles, or other eye protection specified by OSHA and ANSI

Animal Barrier "Clean" Corridor

All staff entering the clean corridor with the intention of entering animal rooms must follow PPE requirements as defined above for activities in animal rooms.

All staff entering the clean corridor for purposes other than entering animal rooms must wear disposable suits, scrubs, lab coats, or other launderable clothing dedicated to the facility, and disposable head and shoe covers.

3.4.2. Respiratory Protection

Where specific engineering controls (e.g., vented enclosure for test article/positive control weighing) have been demonstrated to be effective in controlling exposure levels, the need for respiratory protection shall be determined by the HSO.

The HSO in accordance with OSHA regulations and NIOSH Respirator Decision Logic recommendations shall select suitable, NIOSH-approved, task-specific respirators. Where air-purifying respirators (APRs) are used (e.g., with gas/vapor and particulate combination cartridges), written provisions shall describe when cartridges are to be changed and the logic used to make this determination. The date and time of installation shall be marked on all cartridges. Where air supplied devices are used, breathing air is to be analyzed periodically to ensure that the quality of air meets human breathable air standards. Personnel who are required to wear respirators shall be medically cleared, trained, and mask-fitted before they are allowed to wear the respirator.

A respirator program that meets the requirements of OSHA 29 CFR 1910.134 shall be implemented for routine and emergency use of respirators.

Any respirator cartridge used during a cleanup of spilled chemical shall be disposed of as hazardous waste.

3.4.3. Usage and Storage Practices

All protective equipment used in a particular laboratory shall be stored in accessible and convenient locations as dictated by the barrier design or procedures.

Disposable protective clothing shall not be worn out of the laboratory/test work area where neat chemical is handled.

Work clothing shall be removed upon exit from the laboratory on a daily basis. Disposable clothing shall not be reused.

Nondisposable items are to be stored in covered containers until washed. If laboratory personnel do the washing, they shall wear gloves and disposable suits while handling contaminated items. If washing is done by an outside service, they shall be notified in writing that they are handling items with potential contamination.

3.5. Fire Safety

NOTE: Fire safety requirements for inhalation studies are described in Section 3.8 below.

The facility and operations shall comply with applicable federal, state, and local fire and building codes.

3.5.1. Storage and Handling

Flammable liquids shall be stored and handled in a manner that will reduce the risk of fire and explosion. This conduct includes:

- All nonworking quantities of flammable liquids shall be stored in storage cabinets approved by Underwriters Laboratories or Factory Mutual, or in a designated flammable liquids storage room with suitable fire protection, ventilation, spill containment trays, and with equipment meeting the requirements of OSHA. In either storage arrangement, the flammable liquids shall be segregated from other hazardous materials such as acids, bases, oxidizers, etc.
- Flammable storage cabinets shall not be vented unless required by a chemical-specific OSHA regulation or by local authorities. Metal bung caps shall be used in place of flash arrestor screens. If it is necessary that venting be provided, the following instructions shall be followed: (1) Remove both metal bungs and replace with flash arrestor screens. The top opening shall serve as the fresh air inlet. (2) Connect the bottom opening to an exhaust fan by a substantial metal tubing having an inside diameter no smaller than the vent. The tubing shall be rigid steel. (3) Ensure that the fan has a nonsparking fan blade and nonsparking shroud. It shall exhaust directly to the outside where possible. (4) The total run of exhaust duct shall not exceed 25 feet.
- Class I flammable liquids shall not be stored in conventional refrigerators/freezers. If flammable liquids must be kept at low temperatures, they shall be stored in Underwriters Laboratory (UL)-listed/Factory Mutual (FM) Global-approved refrigerators/freezers designed for flammable storage. In a potentially flammable or explosive atmospheric environment, only those explosion-proof refrigerators/freezers listed for Class I. Division 1, Group C and D, and listed by UL as a “Special Purpose Refrigerator and/or Freezer” shall be used. All explosion-proof refrigerators shall be labeled as such.

- Whenever flammable liquids are stored or handled, ignition sources shall be eliminated. Smoking is prohibited.
- Flammable liquid transfer shall be done in the designated storage room or over a tray within an effective laboratory hood. In the former location, all transfer drums shall be grounded and bonded and shall be equipped with pressure relief devices and dead man valves.
- Safety cans shall be used when handling small (e.g., no more than 2 gallons) quantities of flammable liquids, unless chemical purity requirements require otherwise (e.g., distilled-in-glass grade).

3.5.2. Fire Safety Equipment

Fire extinguishers: Fire extinguishers shall be conspicuously located where they will be readily accessible and immediately available in the event of fire as required by local, state, and federal regulations. Placement of portable fire extinguishers shall conform to OSHA 1910.157. The specific type and size of extinguisher shall be selected with consideration for the hazards to be protected and the strength of the personnel who might use the extinguishers. For most laboratory applications, water and aqueous film forming foam (AFFF) extinguishers shall have a capacity of 2.5 gallons. Dry chemical, carbon dioxide, and foam extinguishes shall have 20–30-pound capacity.

Safety showers: Safety showers shall be located in the immediate vicinity of every laboratory where flammable liquids are stored/used. Fire blankets may be used if available.

3.5.3. Training

All personnel shall receive training in fire safety. Course material shall include hazard awareness, proper techniques for the handling and storage of flammable liquids, and a briefing on the alarm system and emergency evacuation preplanning. In addition, “hands-on” training for appropriate personnel on fire extinguishers is encouraged.

3.6. Emergency Procedures

The written set of general safety policies shall include actions to be taken in case of fire or explosion. They will address personnel assignments, evacuation routes, and notification procedures. The National Fire Protection Association Life Safety Code, Number 101, and existing manual pull-box locations shall be considered when establishing means of egress.

A written set of emergency/evacuation procedures to be followed by all project personnel in the event of a spill or leak involving the test article/positive control shall be developed and posted in each laboratory. Personnel shall be instructed to call for appropriate help (e.g., inhouse emergency group or poison control center) in case of an emergency. This plan shall address the storage, use, and maintenance of emergency protective equipment.

The location and phone number of the nearest poison control center and any other emergency phone numbers shall be prominently posted in each laboratory.

Emergency protective equipment shall not be stored in the laboratory where test articles are stored and handled.

3.7. Waste Disposal/Test Article Shipment

3.7.1. Disposition/Shipment of Surplus/Residual Test Article

The practices described below shall be adhered to concerning the disposition of surplus/residual test article.

Thirty days before shipment, the testing laboratory shall notify the program COR of its intention to ship surplus or residual test article, including the amount to be shipped, and complete details of the shipping procedures, including the contractor that will be used.

Upon completion of testing and after receiving approval from the program COR, the testing laboratory shall immediately ship excess quantities of test article after the final bulk chemical analysis has been completed. In addition, a 100-gram aliquot of each batch of the test article is to be reserved and shipped separately to the sponsor-designated chemistry support contractor after the final bulk chemical analysis has been completed. For reactive chemicals gases, or other test articles that may be difficult to store long term, the program COR shall be contacted to determine if any test article is to be reserved and shipped.

The following requirements for packaging these test articles are made to minimize the possibility of exposure to personnel involved in the packaging, transportation, and receipt of these test articles. The requirements shall be consistent with the Department of Transportation (DOT) regulations (or International Air Transport Association [IATA] regulation for contractors outside the USA) as outlined in 49 CFR, parts 100 to 199.

Test articles shall be shipped in primary containers compatible with the physical and chemical properties of the substances that prevent contamination of the study material. Each primary container must be securely sealed to prevent leakage during transport. After being sealed, the exteriors of each primary container must be decontaminated and labeled with all pertinent information (including chemical name, lot number, amount, date, and source). Test articles that are gases or liquefied gases in cylinders shall be shipped without additional packing and according to appropriate transportation procedures.

All primary containers shall be sealed in double plastic bags to prevent leakage and exposure if broken, surrounded by absorbent material, and placed in secondary containers. Larger amounts of liquids may be shipped in 5-gallon metal drums, which shall be individually packaged and which shall meet all DOT regulations. These 5-gallon drums must be overpacked in larger drums with absorbent material, securely sealed, and fully labeled. All overpacked drums shall be fully filled, securely sealed, and completely labeled on the outside.

Outside containers must be free from extraneous and ambiguous labels. Labeling must include a directional label to indicate the top of the container, appropriate warning labels (e.g., SUSPECT CANCER AGENT, FLAMMABLE), and all required DOT labels and identification. All shipments shall be made in compliance with DOT regulations (or IATA regulations where applicable) and accompanied by a completed Shipper Certification Form for Hazardous Materials. A detailed packaging list must be placed on the outside of the shipping container identifying each chemical fully by name, amounts shipped, and lot numbers of each chemical. The sponsor shall be consulted if the quantity or type of substance to be shipped renders these requirements inappropriate.

3.7.2. Potentially Contaminated Material

All potentially contaminated material (e.g., dose formulations, bedding, used personal protective clothing and equipment, absorbent materials for handling test materials, disposable cages, lab ware, filters, respirator cartridges) shall be incinerated or disposed of in a licensed hazardous waste landfill, in a manner consistent with federal, state, and local regulations. Animal carcasses, blood samples, animal tissues, or any other materials that are grossly contaminated with blood, including sharps and syringes, shall be collected and disposed of by incineration. The laboratory shall indicate whether it plans to fulfill this requirement with its own incinerator, or by use of a licensed waste disposal firm. If the laboratory's incinerator is to be used, specifications (e.g., temperatures and residence times), operating procedures, and information on licensing by local regulatory authorities shall be provided for evaluation. If a contract disposer is to be used, complete information on the firm’s licensing and hazardous waste transporter shall be provided.

Computer terminals used to enter laboratory animal data into an electronic data capture system shall be decontaminated after each use and when removed from an animal room, using a chemical-specific solution. Terminals must be disconnected from any electrical power sources before decontamination, and care will be taken to ensure that any solvents used do not damage the plastic parts of the computer terminal.

Vacuum lines, including water aspirators, used when working with test article/positive control shall be protected with an absorbent or liquid trap and a HEPA filter.

3.8. Inhalation Studies

The following requirements apply to inhalation studies and supplement the requirements described in other parts of this chapter.

3.8.1. General Requirements

The test atmosphere generation apparatus and flow meters through which test atmospheres pass shall be contained in enclosures exhausted to the outside (see Section 3.3.3). All connections in the piping and ducting between the test atmosphere generator and the exhaust air filters shall be either compression-fitted, threaded, welded, or enclosed and vented and leak-tested before use. All equipment through which test article flows shall be electrically grounded and bonded according to the provisions of the National Electrical Code and the National Fire Protection Association Standard 77, “Recommended Practice on Static Electricity.” Use of plastics, such as PVC, is not permitted. All piping, ducting, and other materials must be compatible with the test article.

A full description of all safeguards, safety procedures, alarms, shutdowns, emergency plans, cleanup procedures and disposal methods must be reported to the program COR and be in place before the start of all studies.

At least one sampling port connected to the test article concentration monitoring system shall be located in each animal room involved in the study. Test article monitoring strategy must be submitted and be based on the physical properties of the test article for the exposure room. Exposure rooms do not have to be monitored during nonexposure periods.

All exhaust air from the inhalation chamber must be cleaned with HEPA or charcoal filters (depending on physical form) or other air cleaning devices (e.g., scrubbers, incinerators, electrostatic precipitators), unless the laboratory provides written documentation that local and state air pollution regulatory agencies have been informed of both the laboratory's operating practices and the potential hazards of the test articles in use. Compliance with all federal, state, and local air pollution laws and regulations is required.

Trained inhouse staff or emergency response HAZMAT personnel shall don appropriate self-contained breathing apparatus if emergency entry into a study room following a leak is required. If available inhouse, these units shall be maintained and inspected as required under 29 CFR 1910.134 of the OSHA respiratory protection standard.

The personal protection requirements for inhalation studies as specified in Section 3.4. shall apply except as follows:

When the test article is a gas or vapor and the ambient sampling port indicates that the air in the study room is not contaminated, personnel entering the study room need not wear respirators and disposable overgarments. However, when the exposure chambers are open, personnel entering the exposure rooms must wear appropriate respirators, gloves, eye protection, disposable overgarments, and head and foot coverings.

When the test article is a particulate, personnel transporting animals and necropsy personnel shall wear the same APRs equipped with P-100 filter cartridges and disposable overgarments, which are required at all times in the exposure facility unless all animals are bagged or otherwise enclosed and the containers are only opened under a vented enclosure. Necropsy shall be performed in an enclosure vented to the outside.

3.8.2. Combustible/Flammable Test Articles

When a test article is flammable or explosive, NIEHS requires that the test system minimize the probability of, and the consequences associated with, fire or explosion. The laboratory shall provide data on its test system that includes equipment and techniques for reducing the fire or explosion hazard. As NIEHS recognizes that there could be alternative approaches for minimizing the risks due to fire and explosion, the program COR may grant approval to test system configurations that differ from the following provisions on a site-specific basis.

If the exposure concentration is below 25% of the lower flammable limit or the minimum explosive concentration, the following provisions shall be made:

- Flow monitoring equipment shall be used to determine variations in the flows of the test article and carrier air. In the event that there is a 10% change in flow, system shutdown shall occur with an audible alarm signaling such action at a manned location.
- The instrumentation used to continuously monitor the inhalation chamber and chamber room for the test article shall be equipped with an audible alarm that signals a manned location when a concentration equal to 25% of the lower flammable limit or the minimum explosive concentration is detected.

- A flame arrestor shall be installed on the gas or vapor supply line. If the test article is a combustible dust, an optical flame detector must be located in the supply line and connected to trip a fast acting shut-off valve upstream.
- An alarm shall be in place to indicate when the air flow through the vented enclosure, which surrounds the test article generation devices(s), falls below 85% of its nominal value.
- All equipment through which the test article flows shall be electrically grounded and bonded according to the provisions of the National Electrical Code and the National Fire Protection Association Standard 77, "Recommended Practice on Static Electricity."

If the exposure concentration is ≥25% of the lower flammable limit or the minimum explosive concentration, the following provisions shall be made in addition to those stated above:

- The inhalation chamber study room shall be isolated from the other operations by walls with a fire resistance of 1 hour such that it is solely dedicated to the testing of the flammable or explosive study material.
- Explosion venting shall be installed on each inhalation chamber. The recommended vent ratio is 1 ft^2/10 ft^3.
- All electrical equipment shall be suitable for a Class I, Division II (flammable gas or vapor) or Class II, Division II (combustible dust) location as defined by the National Electrical Code.

If the exposure concentration is ≥100% of the lower flammable limit or the minimum explosive concentration, the following provisions shall be made in addition to those stated above:

- The inhalation chamber study room shall be located such that one wall of the room is common to an area outside of the building that is typically unoccupied. Explosion venting shall be installed in that wall. National Fire Protection Association 68, Guide for Explosion Venting, shall be used for reference when designing the installation.

3.8.3. Reporting Requirements for Inhalation Studies

The following information shall be included in the prestart inhalation report:

Effluent Exhaust Monitoring

- Description of the method(s) to be used for effluent exhaust treatment during generation runs at the protocol-required concentrations, in all chambers under actual animal exposure conditions.
- Data demonstrating the effectiveness of the effluent exhaust treatment unit immediately after the effluent treatment unit or at the point of exhaust from the building.
- Percent efficiency of the exhaust treatment—the effluent exhaust treatment must be effective in removing the test article to an acceptable concentration (e.g., >90% efficiency of removal by the treatment system and <50% of the TLV, if a TLV exists), or written documentation for a waiver from appropriate air regulatory agencies must be provided.

- Determination of the lifetime expectancy of any proposed filtration/treatment units and the amount of treatment media that will be required.
- Confirmation that none of the exhausted test article is re-entrained.

Room Air Monitoring

- Description of the test method(s) for room air monitoring during generation runs at protocol-required concentrations in all chambers under actual animal exposure conditions.
- Definition of the lower limit of detection of the monitoring method(s).
- Documentation that this level provides an adequate safety margin for personnel.

Special Requirements for Aerosol Studies

When a test article is an aerosol (e.g., particulate or liquid), NIEHS requires that the test system minimize the probability of, and the consequences associated with, fire or explosion. The laboratory shall provide data on its test system that includes equipment and techniques for reducing the fire or explosion hazard. The laboratory shall also provide complete information on the test article that includes determination of the following: the minimum explosion concentration, minimum spark ignition energy, explosion severity, minimum ignition temperature of a layer, and the volume resistivity. As NIEHS recognizes that there may be alternative approaches for minimizing the risks due to fire and explosion, the program COR may grant approval to test system configurations that differ from the following provisions on a site-specific basis.

Demonstration of containment of particulate study material during generation of protocol-required concentrations in all chambers under actual animal exposure conditions, description of the monitoring strategy including methodology, frequency of sampling, and results are to be provided.

Standard Operating Procedures

All SOPs that are specific to the study are to be attached to the prestart inhalation report.

3.9. Peer Review

The Division of Translational Toxicology (DTT) conducted a peer review of chapters 1, 2, 3, 4, 11, and 12 within the draft *Specifications for the Conduct of Toxicity Studies by the Division of Translational Toxicology at the National Institute of Environmental Health Sciences* by letter in February 2022 by the expert listed below. Reviewer selection and document review followed established DTT practices. The reviewer was charged to:

1. Peer review the following chapters within the draft Specifications for the Conduct of Toxicity Studies by the Division of Translational Toxicology at the National Institute of Environmental Health Sciences.
    - Chapter 1: General Personnel Requirements
    - Chapter 2: Facilities
    - Chapter 3: Health and Safety

- Chapter 4: Quality Program
- Chapter 11: Data Collection and Submission
- Chapter 12: Report Formats and Guidance

2. Comment on the completeness of each chapter.

DTT carefully considered reviewer comments in finalizing this document.

Peer Reviewer

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