

3.23. Animal Work with Chemicals of High Toxicity – Safety Procedures

All researchers administering chemicals to animals must identify and understand the hazards of the chemicals used in their research, select the proper procedures, hazard controls, personal protective equipment, and provide protocol-specific training to protect those handling the chemicals. University Environmental Health and Safety (IUEHS) will provide the OSHA compliant laboratory chemical safety training.

The chemicals of concern include anesthetics, drugs, controlled substances, carcinogens, allergens, intoxicants, reproductive toxins, embryotoxins, chemical toxins, and novel substances such as synthesized experimental drugs, chemicals, or mixtures. Less hazardous substances such as saline solution and buffers are not chemicals of concern and should be handled using standard chemical handling procedures.

Principal investigators may use the “Principal Investigators Chemical Hazard Assessment for Animal Research” form in Appendix A to perform a chemical hazard assessment for the chemicals in use. Use the guidelines below to determine if the chemical is a “particularly hazardous substance” and follow the required procedures. IUEHS will perform a chemical hazard evaluation during the protocol safety review process and provide guidance on personal protective equipment, regulatory compliance and safety procedures based on the hazardous properties of the chemicals.

Note: Safety Data Sheets for all laboratory chemicals are required to be maintained in the laboratory or on-line. The pertinent information for chemical hazard analysis is found on the Safety Data Sheet (SDS) and in the *Laboratory Safety and Chemical Hygiene Plan*. The SDS for the exact chemical or mixture must be used and provided by the manufacturer of the product.

3.23.1. Particularly Hazardous Substances

If the chemical is a carcinogen, reproductive toxin, or a chemical with a high degree of acute toxicity, the Occupational Safety and Health Administration (OSHA) defines it as a “particularly hazardous substance” (*Laboratory Safety and Chemical Hygiene Plan, Section 3.8*). In addition, novel chemicals including those synthesized in research laboratories, that have not been tested explicitly for carcinogenic or toxic properties must be handled as “particularly hazardous substances” until the hazards have been evaluated because their hazards are unknown.

3.23.1.1. Carcinogen

A substance that either causes cancer in humans or, because it causes cancer in animals, is considered capable of causing cancer in humans. OSHA identifies those that pose the greatest carcinogenic hazards as “select carcinogens” and includes carcinogens identified by OSHA, the National Toxicology Program (NTP) or the International Agency for Research on Cancer (IARC). The list is provided in the *Laboratory Safety and Chemical Hygiene Plan, Appendix B*.

3.23.1.2. Reproductive Toxin

A substance that causes chromosomal damage or genetic alterations (mutagens) or substances that cause lethal or physical malformations or defects in a developing fetus or embryo (teratogens) and is given on the Safety Data Sheet (SDS).

3.23.1.3. Chemicals with a High Degree of Acute Toxicity

These chemicals include both “highly toxic” and “toxic” chemicals with acutely toxic effects and are based on the route of entry and lethal dose (LD50) or concentration (LC50) given on the Safety Data Sheet (SDS).

3.23.1.4. Chemicals with High Chronic Toxicity

These chemicals include both “highly toxic” and “toxic” chemicals under the OSHA Hazard Communication Standard prior to 2012 with chronic toxic effects. This group may include human carcinogens or reproductive toxins and therefore must be handled as “particularly hazardous substances” also.

Route of Entry	Highly Toxic	Toxic
Oral LD ₅₀ (albino rats)	≤ 50 mg/kg	>50-500 mg/kg
Skin Contact LD ₅₀ (albino rabbits, 24 hour)	≤ 200 mg/kg	>200-1000 mg/kg
Inhalation LC ₅₀ (albino rats, 1-hour) as vapor	≤ 200 ppm	>200-2000 ppm
Inhalation LC ₅₀ (albino rats, 1-hour) as dust, mist, or fumes	or ≤ 2 mg/liter	or >2-20 mg/liter

The current OSHA definition under to the Globally Harmonized System (GHS) of chemical classification and labeling would include GHS acute toxicity ratings of 1 or 2 to account for chemicals with ‘high acute toxicity’.

For practical purposes, these chemicals may also be identified using the National Fire Protection Associations (NFPA) health hazard classifications found on the bottle label and Safety Data Sheet (SDS). In general, a chemical with a 3 or 4 in the blue diamond of the NFPA label can be considered to be a “particularly hazardous substance” (excluding cryogenics and some corrosives).

Route of Entry	NFPA 704 Health Hazard Classifications				
	4	3	2	1	0
Oral LD ₅₀	0-5 mg/kg	>5-50	>50-500	>500-2000	>2000
Skin Contact LD ₅₀	0-40 mg/kg	>40-200	>200-1000	>1000-2000	>2000
Inhalation LC ₅₀	0-1000 ppm	>1000-3000	>3000-5000	>5000-10,000	>10,000



3.23.2. General Procedures

- **Access and facilities:** For large scale studies, special facilities with restricted access are preferable. IUEHS conducts annual chemical safety inspections of the facilities and evaluation of safety equipment. Animal facilities must be posted with a sign to indicate that particularly hazardous substances are used in the area and to provide safety instructions. The “Animal Facility Safety Information” sign found in Appendix A is an example that shows the chemicals present, personal protective equipment, and any other special requirements for entry.
- **Administration of the toxic substance:** When possible, administer the substance by injection or gavage instead of in the diet. If administration is in the diet, use a caging system under negative pressure or under laminar air flow directed toward HEPA filters.
- **Aerosol suppression:** Devise procedures, such as lightly spraying bedding with water, which minimize formation and dispersal of contaminated aerosols, including those from food, urine, and feces (e.g., use HEPA filtered vacuum equipment for cleaning, moisten contaminated bedding before removal from the cage, mix diets

in closed containers in a hood).

- **Personal protection:** When working in the animal room, wear plastic or rubber gloves, fully buttoned laboratory coat or jumpsuit and, if needed because of incomplete suppression of aerosols, other apparel and equipment (shoe and head coverings, respirator).
- **Waste disposal:** Package contaminated animal tissues and excreta appropriately for disposal by IUEHS or waste disposal vendor. See “Waste Disposal and Handling” below.

3.23.3. Chemical Handling

- These chemicals must be used in a designated area. This includes live animals and open cages with chemically contaminated bedding. Signs must be posted to designate that the entire laboratory or portion of the laboratory such as a specific fume hood, glove box, or adjacent room as the designated area for that chemical use. This may be accomplished by using the emergency door sign and identifying the chemical groups used in the laboratory (i.e., carcinogen, reproductive toxin, highly toxic or toxic chemical).
- The chemical must be handled in a containment (if decanted or exposed to the open air) or within a closed system. This includes live animals and open cages with chemically contaminated bedding. Containment devices include chemical fume hoods, glove boxes, or biosafety cabinets that are vented outside of the building. Closed systems include plumbing within instruments, cannulas, syringes, gavages, etc., as long as the chemical is not exposed to the atmosphere. If no containment is available then personal protective equipment assigned to the protocol during the safety review must be utilized by all personnel in the area.
- Only the minimum quantity of the material should be used.
- Appropriate personal protective equipment (e.g., gloves, lab coat, and eye protection) must be used when handling these hazardous substances.
- Procedures for waste removal must be established prior to use. Follow standard IUEHS chemical disposal procedures. Follow biological waste disposal guidelines below for animals and bedding.
- Decontamination procedures must be developed for the tools and area.
- The principal investigator listed on the protocol is responsible for establishing the experimental procedure, determining the hazard controls to be utilized, and providing protocol-specific training for the staff. Prior approval from the principal investigator or supervisor is required for the experiment to begin (see Laboratory Safety and Chemical Hygiene Plan Section 2.1.1 Prior Approval of Hazardous Operations).
- University Environmental Health and Safety (IUEHS) will provide the OSHA compliant laboratory chemical safety training. All personnel that handle any chemicals in the laboratory must attend chemical safety training required by the OSHA Laboratory Standard from IUEHS. Only laboratory personnel that have received IUEHS laboratory chemical safety training and protocol specific training from the principal investigator may work with these substances, and only within the designated area. These chemicals may not be decanted or exposed to the atmosphere outside a containment or closed system.

3.23.4. Chemical Storage and Labeling

- Acutely toxic chemicals, carcinogens and reproductive toxins must be stored designated areas for “particularly hazardous substances.”
- Storage areas should be clearly marked with the appropriate hazard warning signs.
- All containers of these substances (even in small quantities such as 0.1%) must be clearly labeled with the chemical name or components of the mixture and should be labeled with hazard information.
- Chemical storage areas should be secure to avoid spills or broken containers.
- Storage areas or laboratory rooms must be locked when laboratory personnel are not present.
- For more information, refer to [Section 3.2](#), Procedures for Proper Labeling, Storage, and Management of Chemicals.

3.23.5. Waste Disposal and Handling

- Waste handlers must wear standard personal protective equipment (PPE) required for laboratory work, lab coat or gown, safety glasses, gloves and closed toed shoes.
- Respiratory protection is not required if procedures do not produce aerosols or if a fume hood, ventilated cage dump station, or biosafety cabinet is utilized but may be used voluntarily to protect against fugitive dust emissions.
- All waste including biological tissues and fluid, chemicals, contaminated materials, sharps, and other items must be properly disposed as detailed in the [IU Biosafety Manual](#).
- Empty containers, with the exception of acutely toxic waste, may be triple rinsed and disposed of as sanitary waste.
- Chemical waste and contaminated materials are disposed of in accordance with the [IU Waste Management Program](#).
- All animal carcasses and tissues are disposed of as medical waste to prevent unacceptable conditions in the sanitary waste containers and to ensure that transgenic animals are disposed of as regulated medical waste.
- Animal use protocols are reviewed by the IUEHS staff to identify any rare or unusual circumstances that would affect waste disposal or occupational safety.
- Animal carcasses must be frozen for disposal. Place materials in a biohazard bag with a biohazard symbol. Double bag if necessary to prevent perforations. Place the bag in a freezer and [contact IUEHS](#) for your respective campus for pickup (except for the IUPUI campus and IUSOM locations where the carcasses are picked up for disposal by an approved vendor).
- Animal bedding is sanitary waste and is disposed of with other sanitary waste from the facility.
- Even though animal bedding is not typically an EPA hazardous waste or biohazardous waste it must be handled carefully for occupational safety purposes. Animal bedding from chemically dosed animals may contain dosed uneaten food or water and shedding or excretions that contain very small amounts of hazardous chemicals or their metabolites.
 - a) If the bedding is known to contain small amounts of chemicals in dosed food or otherwise, the bedding must be handled using fully buttoned lab coats or gowns, gloves, safety glasses in a ventilated dump station, biosafety cabinet, fume hood or with respiratory protection.
 - b) If it is unknown or inconclusive that the bedding contains these hazardous substances then the bedding must be handled as if it does using fully

buttoned lab coats or gowns, gloves, safety glasses in a ventilated dump station, biosafety cabinet, fume hood or with respiratory protection.

- c) If the researcher can demonstrate the bedding or excreta does not contain hazardous amounts of these substances then the waste handling requirements may be modified.
- Empty cage bedding from dosed animals into a waste bag using a ventilated dump station or within a chemical fume hood or biosafety cabinet. Place the cage within the bag. Remove the lid. Empty the cage into the bag and remove the cage from the bag. Close and secure the bag.