

Chemical Containment Levels (CCL) for Animal Administration

May 2023 Revision

Introduction/Background:

This program is intended to provide a standardized, risk-assessment based approach to engineering controls, PPE, and husbandry in BU's animal spaces for work with rodents. Other animals (i.e., birds, aquatic species, sheep, pigs, non-human primates) will require additional risk assessment to determine an appropriate CCL.

This guide focuses on mitigating health hazards associated with the use of chemicals (including biological toxins) and drugs in animal research from the time the material is brought into the animal space until the animal and the material leave the space. Transportation of these materials shall follow the normal recommended safe use and transport guidelines based on the hazards. Chemical and hazardous drug preparation will generally be completed in the research laboratory or animal procedure room *not* the animal housing room.

Chemicals and hazardous drugs are classified into four risk-based categories (CCL1-4, with 4 reserved for the most hazardous) in order to protect researchers, animal care staff, and the environment. The majority of chemicals/ hazardous drugs with *known* toxicological data will fall within CCL-2. When determining a CCL, it is primarily the researcher's responsibility to provide data showing the material is non-hazardous, that a hazardous material is contained during and after dosing, or that a hazardous material is contained during dosing and all metabolites are non-hazardous. Researchers planning to euthanize the animal within two hours or less of dosing may apply for an exception to work at a lower CCL.

Definitions:

Chemical Containment Level One (CCL-1):

CCL-1 is the lowest containment level for **very low risk** materials that pose little-to-no potential risk to researchers, Animal Science Center (ASC) staff, or the environment during or post-administration to animals. There are no additional controls beyond what is required by the ASC to work at the approved Animal Biosafety Level (ABSL).

CCL-1 materials include the following:

- foods used in research,
- biodegradable, commercial nanoparticles,
- chemicals whose safety data sheet (SDS) states "not a hazardous substance or mixture" or without an assigned GHS pictogram,
- SDS signal word is usually none/not applicable or warning.

Examples include condensed milk, cereals, sugar, phosphate buffered saline (PBS), sodium chloride (NaCl), citric acid.

☐ **Chemical Containment Level Two (CCL-2):**

CCL-2 are materials that pose **low risk** to researchers, ASC staff, or the environment **during** administration to animals, *but do not present a hazard post-administration*.*

Routes of exposure during administration include accidental:

- inhalation,
- direct inoculation,
- splatter to mucous membranes or exposed skin, and/or
- ingestion

Because of these risks, workers require additional control measures while dosing the animal, see *Table 2: CCL PPE and Hazard Control Measures* below.

Post-administration, no additional controls are needed beyond what is required by ASC to work at the approved ABSL as there is no significant risk of excretion or shedding from the animal and/or all known metabolites are characterized as non-hazardous.

**Note oral, topical, or rectal dosing on cage card* as chemical/hazardous drug can fall off or be spit out and contaminate the inside the cage in the native (hazardous) form.

CCL-2 materials include the following:

- SDS signal word often warning but can be danger,
- Many classes of irritants

Examples include acetonitrile, 37% hydrochloric acid, Cefazolin, dimethyl sulfoxide (DMSO).

☐ **Chemical Containment Level Three (CCL-3):**

CCL-3 materials pose a **moderate risk** to researchers, ASC staff, or the environment during and post-administration to animals. This can be due to the type of chemical and/or the type of administration (e.g., in drinking water or food).*

Routes of exposure during administration include accidental:

- inhalation,
- direct inoculation,
- splatter to mucous membranes or exposed skin, and/or
- ingestion

and workers require additional control measures while dosing the animal, see *Table 2: CCL PPE and Hazard Control Measures* below.

Post-administration risks include:

- chemical excretion or shedding by the animal,
- metabolites are known to be hazardous and present in detectable quantities,
- metabolites and/or their amounts and routes of excretion are unknown.

Additional controls are required beyond what is typically used to work at the approved ABSL when performing animal husbandry after dosing, see *Table 2: CCL PPE and Hazard Control Measures* below.

Animals dosed with chemicals and/or hazardous drugs classified as CCL-3 with known short metabolic half-lives, may be moved to CCL-2 housing and care after five (5) metabolic half-lives have passed and 97% of the hazardous material should have cleared. Researchers looking to have animals dosed with CCL-3 materials moved to CCL-2 housing after five half-lives have passed must provide documentation of the biological half-life of the chemical in the species being exposed.*

**Note oral, topical, or rectal dosing on cage card as chemical/hazardous drug can fall off or be spit out and contaminate the inside the cage in the native (hazardous) form.*

CCL-3 materials include the following:

- Many toxic, mutagenic, carcinogenic, and/or reproductive toxicant materials,
- BU HHCs that have not been classified as CCL-4

Examples include methanol, Beta-aminopropionitrile (BAPN), isoflurane, cyclophosphamide, streptozotocin, tamoxifen, bleomycin

☒ Chemical Containment Level Four (CCL-4):

CCL-4 materials pose **high risk** to researchers, ASC staff, or the environment during and post-administration to animals. These materials require special risk assessment from EHS and ASC prior to use and additional containment measures may be necessary. Therefore, CCL-4 materials may not be approved within the standard five (5)-day approval window.

Routes of exposure during administration include accidental:

- inhalation,
- direct inoculation,
- splatter to mucous membranes or exposed skin, and/or
- ingestion

Post administration risks include:

- chemical excretion or shedding by the animal,
- metabolites are known to be hazardous,
- metabolites and/or their amounts and routes of excretion are unknown.

Additional controls are required beyond what is typically used to work at the approved ABSL during administration and when performing animal husbandry after dosing *and may include specific PPE, administrative controls (SOP), and/or engineering controls not typically used in a laboratory setting, see Table 2: CCL PPE and Hazard Control Measures below.*

Animals dosed with chemicals and/or hazardous drugs classified as CCL-4 generally remain in quarantine housing until they are euthanized. They may be downgraded to a lower CCL after five half-lives have passed based on risk assessment. Documentation of the half-life of the chemical in the species being exposed must be provided.

CCL-4 materials include the following:

- Any chemical with GHS code H300, H310, and/or H330 and hazard statement begins “Fatal if...”
- Chemicals that, in the event of an exposure, require immediate medical intervention and/or antidote, including:
 - Hydrofluoric acid (HF)
 - Cyanides
- Chemicals that require specific PPE to handle safely in the research laboratory including:
 - HF
 - Dimethylmercury
- Chemicals that require specific storage (i.e., gas cabinet) or handling procedures to use safely in a research laboratory including:
 - Hazardous gases (with an NFPA rating of 3 or 4 in any hazard class (flammable, health, reactivity)) – i.e., chlorine.
 - Osmium tetroxide
- Certain BU HHCs including organomercury compounds and dioxins
- Certain neurotoxins including:
 - [MTPT/MPP+](#) (penetrates the blood—brain barrier and enters the brain cells causing Parkinson's disease like symptoms)
 - 6-OHDA
- Default for chemicals listed in [40 CFR 261.24 Subpart C- Characteristics of Hazardous Waste](#) including their derivatives, salts, compounds, and mixtures;
 - [powdered lead](#)
- [40 CFR section 261.33 P-listed and U-listed chemicals](#);
- Brand new chemicals without SDS or known metabolite data

Roles & Responsibilities:

🔗 IACUC:

- Notify EHS Research Safety when protocols for treating animals with potentially hazardous chemicals have been submitted.

🔗 BU Animal Science Center (ASC) Staff:

- Ensure that appropriate facilities, equipment, and PPE are available for the assigned chemical containment level in BUASC spaces;
- Ensure that researchers are following the appropriate procedures defined in the chemical containment level in BUASC spaces. Notify the principal investigator and Environmental Health and Safety if non-compliance is noted;
- Ensure that BUASC staff are appropriately trained and follow appropriate cage change procedures, that appropriate cages and bedding are used, and appropriate waste containers are available as required by chemical containment level;
- Supply cage cards and signage to the researchers in order to identify rooms and animals involved in procedures using chemicals as dictated by the chemical containment level;

- Ensure the appropriate signage was posted by the researcher on the door of the room when animals are being or have been treated with a chemical, as required by the chemical containment level;
- Assist in the determination of the CCL based on the individual risk of each project's chemicals, doses, animal metabolism and other factors and communicate the requirements for that level to the PI and EHS in a timely manner.

🔍 **BUASC Trainer:**

- Provide BUASC-specific training to all researchers and PI's working in BUASC.

🔍 **EHS Research Safety:**

- Assist in the determination of the CCL based on the individual risk of each project's chemicals, doses, animal metabolism and other factors and communicate the requirements for that level to the PI and BUASC in a timely manner;
- Update requirements outlined in the chemical containment levels as needed;
- Provide assistance to BUASC and PIs in training staff and researchers as requested;
- Coordinate review of IACUC protocols within EHS as needed.

🔍 **EHS Environmental Management:**

- Make waste determination of bedding, carcasses, cage wash effluent, etc.

🔍 **Principal Investigators:**

- Assign a preliminary CCL upon submission of an IACUC protocol;
- Provide information to allow EHS Research Safety Staff to perform an appropriate risk assessment for the chemical exposure in animals;
- Ensure that research staff are appropriately trained;
- Ensure that research staff follows the procedures prescribed by the chemical containment level for the project as specified by EHS;
- Notify Research Occupational Health (ROHP) of any suspected exposure to a potentially hazardous chemical.

🔍 **Research Staff:**

- Follow the procedures prescribed by the chemical containment level for the project as specified by EHS. Notify Environmental Health and Safety and BUASC staff if the procedures require modification;
- Notify Research Occupational Health (ROHP) of any suspected exposure to a potentially hazardous chemical;
- Obtain and post the appropriate signage on the door to the room when animals are being or have been treated with a chemical, as required by the chemical containment level;
- Use the appropriate cage card to identify animals that are involved in a project using chemicals, as required by the chemical containment level.

🔍 **Laboratory Safety Committee:**

- Ensure that all work at Boston University and Boston Medical Center is completed per the requirements outlined in the Chemical Hygiene Plan;
- Provide enforcement actions against laboratories that are not in compliance with this procedure.

☐ **Research Occupational Health Program (ROHP):**

- Provide medical surveillance for hazardous agents in compliance with relevant OSHA standards as indicated by EHS exposure assessment;
- Provide confidential reproductive counseling for laboratory workers;
- Provide immediate first aid care; post exposure risk assessment and guidance;
- Review research-related exposure to potentially hazardous chemicals.

Special Requirements:

☐ **Training:**

- All researchers performing research with animals must attend BUASC's New Researcher Orientation and Environmental Health and Safety's Laboratory Safety Training.
- Researchers must also be trained on the requirements set forth by the assigned CCL.
 - For all CCL's, the principal investigator must assure that the researchers are informed of the hazards associated with the chemical being used.
 - For CCL 2, BUASC will communicate the requirements beyond the standard BUASC requirements for non-chemical work to the researcher.

Table 1: CCL Classification Scheme by GHS Hazard Code:







	Signal Word	Pictogram	Hazard Code	Hazard Statement	Category 29 CFR 1910.1200
CCL-1	None or Warning	None or 	H303	May be harmful if swallowed	Category 5
			H313	May be harmful in contact with skin	Category 5
			H333	May be harmful if inhaled	Category 5
			H315	Causes skin irritation	Category 2
			H316	Causes mild skin irritation	Category 3
			H319	Causes serious eye irritation	Category 2A
			H320	Causes eye irritation	Category 2B
			H335	May cause respiratory irritation	Category 3
			H336	May cause drowsiness or dizziness	Category 3
CCL-2	Warning or Danger	 and/or 	H302	Harmful if swallowed	Category 4
			H312	Harmful in contact with skin	Category 4
			H332	Harmful if inhaled	Category 4
			H305	May be fatal if swallowed and enters airways	Category 2
			H314	Causes severe skin burns and eye damage	Category 1A, 1B, 1C
			H317	May cause an allergic skin reaction	Category 1, 1A, 1B
			H318	Causes serious eye damage	Category 1
			H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled	Category 1, 1A, 1B
CCL-3	Danger	 and/or 	H301	Toxic if swallowed	Category 3
			H311	Toxic in contact with skin	Category 3
			H331	Toxic if inhaled	Category 3
			H304	May be fatal if swallowed and enters airways	Category 1
			H340	May cause genetic defects	Category 1A, 1B
			H341	Suspected of causing genetic defects	Category 2
			H350	May cause cancer	Category 1A, 1B
			H351	Suspected of causing cancer	Category 2
			H360	May damage fertility or the unborn child	Category 1A, 1B
			H361	Suspected of damaging fertility or the unborn child	Category 2
			H370	Causes damage to organs	Category 1
			H371	May cause damage to organs	Category 2
			H372	Causes damage to organs through prolonged or repeated exposure	Category 1
			H373	Causes damage to organs through prolonged or repeated exposure	Category 2
CCL-4	Danger		H300	Fatal if swallowed	Category 1, 2
			H310	Fatal in contact with skin	Category 1, 2
			H330	Fatal if inhaled	Category 1, 2

Table 2: CCL PPE and Hazard Control Measures:

	CCL-1 Very Low Risk	CCL-2 Low Risk	CCL-3 Moderate Risk	CCL-4 High Risk
Researcher PPE (administration)	<ul style="list-style-type: none"> Normal for ABSL 	<ul style="list-style-type: none"> Normal for ABSL with Eye protection 	<ul style="list-style-type: none"> Normal for ABSL with Eye protection 	<ul style="list-style-type: none"> Normal for ABSL with Eye protection Additional PPE as needed after risk assessment
Researcher PPE (post-administration)	<ul style="list-style-type: none"> Normal for ABSL 	<ul style="list-style-type: none"> Normal for ABSL 	<ul style="list-style-type: none"> Normal for ABSL with Eye protection 	<ul style="list-style-type: none"> Normal for ABSL with Eye protection Additional PPE as needed after risk assessment
ASC PPE (post-administration)	<ul style="list-style-type: none"> Normal for ABSL 	<ul style="list-style-type: none"> Normal for ABSL <ul style="list-style-type: none"> Respiratory protection for dirty cage wash 	<ul style="list-style-type: none"> Normal for ABSL with Eye protection <ul style="list-style-type: none"> (Respiratory protection for dirty cage wash) 	<ul style="list-style-type: none"> Not applicable – researchers will do all animal and cage handling
Engineering Controls (administration)	<ul style="list-style-type: none"> Normal for ABSL 	<ul style="list-style-type: none"> Preparing stock solutions and/ or neat substances require chemical fume hood or Type II hard ducted biosafety cabinet, otherwise normal for ABSL 	<ul style="list-style-type: none"> All administration requires chemical fume hood or Type II hard ducted biosafety cabinet 	<ul style="list-style-type: none"> All administration requires chemical fume hood or Type II hard ducted biosafety cabinet Additional engineering controls after risk assessment
Engineering Controls (post-administration)	<ul style="list-style-type: none"> Normal for ABSL 	<ul style="list-style-type: none"> Normal for ABSL 	<ul style="list-style-type: none"> Chemical fume hood or Type II hard ducted biosafety cabinet Environmental monitoring for cage dump as needed 	<ul style="list-style-type: none"> Chemical fume hood or Type II hard ducted biosafety cabinet Additional engineering controls after risk assessment
Additional Practices & Procedures	<ul style="list-style-type: none"> Normal for ABSL 	<ul style="list-style-type: none"> Use appropriate engineering controls Sharps precautions Spill/emergency response supplies 	<ul style="list-style-type: none"> Cages are packaged and transported according to ASC guidelines Highly hazardous chemicals (HHC) require SOP Use appropriate engineering controls 	<ul style="list-style-type: none"> Use appropriate engineering controls Agent / HHC specific SOP Sharps precautions Spill/emergency response supplies

	CCL-1 Very Low Risk	CCL-2 Low Risk	CCL-3 Moderate Risk	CCL-4 High Risk
			<ul style="list-style-type: none"> Sharps precautions Spill/emergency response supplies 	
Cages	<ul style="list-style-type: none"> Micro-isolator 	<ul style="list-style-type: none"> Micro-isolator 	<ul style="list-style-type: none"> Micro-isolator 	<ul style="list-style-type: none"> <i>Disposable</i> cage
Bedding	<ul style="list-style-type: none"> Normal for ABSL 	<ul style="list-style-type: none"> Normal for ABSL 	<ul style="list-style-type: none"> Low dust bedding 	<ul style="list-style-type: none"> Low dust bedding
Housing	<ul style="list-style-type: none"> Normal for ABSL 	<ul style="list-style-type: none"> Normal for ABSL 	<ul style="list-style-type: none"> Quarantine room (May move to CCL-2 housing after 5 metabolic half-lives) 	<ul style="list-style-type: none"> Quarantine room
Signage & labeling	<ul style="list-style-type: none"> Normal for ABSL 	<ul style="list-style-type: none"> CCL-2 cage & door card 	<ul style="list-style-type: none"> CCL-3 cage & door card 	<ul style="list-style-type: none"> CCL-4 cage & door card
ASC Post-Admin. Animal Husbandry & Cage Cleaning	<ul style="list-style-type: none"> ASC Staff 	<ul style="list-style-type: none"> ASC Staff 	<ul style="list-style-type: none"> ASC Staff 	<ul style="list-style-type: none"> Researchers
Waste Management	<ul style="list-style-type: none"> Normal for ABSL 	<ul style="list-style-type: none"> Residual chemicals- dispose per hazardous waste guidelines Sharps- dispose per sharps guidance 	<ul style="list-style-type: none"> Residual chemicals- dispose per hazardous waste guidelines in SAA Sharps- dispose per sharps guidance 	<ul style="list-style-type: none"> Residual chemicals- dispose per hazardous waste guidelines in SAA Sharps- dispose per sharps guidance
Bedding Waste	<ul style="list-style-type: none"> Normal for ABSL 	<ul style="list-style-type: none"> Normal for ABSL 	<ul style="list-style-type: none"> EHS to make hazardous waste determination if characteristically hazardous reagents or HHCs are present in the bedding waste. 40 CFR 261.24 Subpart C- Characteristics of Hazardous Waste 	<ul style="list-style-type: none"> EHS to make hazardous waste determination if characteristically hazardous reagents or HHCs are present in the bedding waste. 40 CFR 261.24 Subpart C- Characteristics of Hazardous Waste
Carcass Waste	<ul style="list-style-type: none"> Normal for ABSL 	<ul style="list-style-type: none"> Normal for ABSL 	<ul style="list-style-type: none"> EHS to make hazardous waste determination if characteristically hazardous reagents or HHCs are present in the carcass waste. 40 CFR 261.24 Subpart C- Characteristics of Hazardous Waste 	<ul style="list-style-type: none"> EHS to make hazardous waste determination if characteristically hazardous reagents or HHCs are present in the carcass waste. 40 CFR 261.24 Subpart C- Characteristics of Hazardous Waste
ROHP	<ul style="list-style-type: none"> Normal 	<ul style="list-style-type: none"> Standard agent specific 	<ul style="list-style-type: none"> Standard agent specific 	<ul style="list-style-type: none"> Agent specific. Additional monitoring may be required.

