

APPENDIX A:

**ACTIVITIES AND IMPACTING FACTORS ASSOCIATED WITH THE
PROPOSED BIOLOGICAL SAFETY LEVEL-3 FACILITY**

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The Consortium for Advanced Radiation Sources (BioCARS) Biological Safety Level-3 (BSL-3) Facility is the National User Facility for Macromolecular Crystallography, where users from universities nationwide can safely conduct x-ray diffraction experiments on crystals of BSL-2 and BSL-3 agents. All experiments conducted at BioCARS are reviewed to ensure that the proper safety protocols are in effect.¹ Table A.1 gives the definitions of the four biosafety levels defined by the Centers for Disease Control and Prevention (CDC). Agents classified as BSL-2 involve a broad range of indigenous, moderate-risk agents that are present in the community and associated with human disease of varying severity. Agents classified as BSL-3 are indigenous or exotic agents associated with serious and potentially lethal human diseases as a result of exposure by the inhalation route. The infection does not spread easily to others, however, and preventive or therapeutic intervention is available (high individual risk but low community risk). Examples of the microorganisms assigned to this level include *Mycobacterium tuberculosis*, St. Louis encephalitis virus, and *Coxiella burnetii*. Which particular BSL-3 agents will be used at the BioCARS facility will depend on the scientific proposals of principal investigators conducting experiments at BioCARS. The quantities of the agents to be used at any time at the BioCARS facility are small and typically involve a total of tens of crystals, with the volume of each crystal typically ≤ 10 nL (≤ 0.2 mm³). Crystals are typically prepared at the user's home institution (itself a BSL-3 facility) and sent in accordance with U.S. Department of Transportation (DOT) regulations to the BioCARS facility, at most several days prior to the experiment. In some cases, it is necessary to prepare crystals at the BioCARS facility, in which cases a small amount of the agent solution is sent (≤ 1 mL, ≤ 10 mM).

In addition to the above categorizations, "The Antiterrorism and Effective Death Penalty Act of 1996" called for the definition of select agents that pose a severe threat to human health and safety and the regulation of the transfer thereof. Select agents were defined by the CDC in Title 42, Part 72, of the *Code of Federal Regulations* (42 CFR 72), Appendix A. Laboratories seeking to conduct research involving select agents must be registered with the CDC and undergo periodic CDC oversight audits. The currently proposed BSL-3 research agenda does not include the study of select agents. Thus, there are no immediate plans to seek CDC registration for the BSL-3 facility. However, it is possible that studies involving select agents will be proposed for the future. If decisions are made to accept research involving select agents into the BSL-3 facility, then CDC registration will be pursued. It is believed that the current engineering design of the BSL-3 facility will not require modification or enhancement in order to secure CDC registration.

¹ Centers for Disease Control and Prevention (CDC) guidance is available at its Web site: <http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm>.

TABLE A.1 CDC Definitions of Biosafety Levels

Level	Description	Precautions
BSL-1:	<p>“...viable microorganisms not known to consistently cause disease in healthy adult humans.”</p> <p>Examples: <i>Bacillus subtilis</i>, <i>Naegleria gruberi</i>, and infectious canine hepatitis virus.</p>	<p>... standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for handwashing.</p>
BSL-2:	<p>“...broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity.”</p> <p>Examples: Hepatitis B virus, the salmonellae, and human-derived blood.</p>	<p>...primary barriers should be used as appropriate, such as splash shields, face protection, gowns, and gloves. Secondary barriers such as handwashing sinks and waste decontamination facilities must be available...</p>
BSL-3:	<p>“...indigenous or exotic agents with a serious and potentially lethal infection.”</p> <p>Examples: <i>Mycobacterium tuberculosis</i>, St. Louis encephalitis virus, and <i>Coxiella burnetii</i>.</p>	<p>All laboratory manipulations should be performed in a Biological Safety Cabinet (BSC) or other enclosed equipment... Secondary barriers for this level include controlled access to the laboratory and ventilation requirements that minimize the release of infectious aerosols from the laboratory.</p>
BSL-4 (Included for completeness. Work at this level is NOT proposed)	<p>“...dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy.”</p> <p>Examples: Marburg virus and Congo-Crimean hemorrhagic fever virus.</p>	<p>...Class III BSC or in a full-body, air supplied positive-pressure personnel suit. The BSL-4 facility itself is generally a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems to prevent release of viable agents to the environment.</p>

Source: Available at CDC Web site: <http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm>.

Research at the BioCARS facility is, for the most part, basic research. Some proprietary research is conducted though, mainly to understand the interaction between drugs and their target proteins. The techniques of genetic engineering are used, along with other standard microbiological techniques, at the home institutions of the experimenters to produce samples for study at BioCARS. The facilities at BioCARS are not appropriate for the production of genetically engineered organisms.

Consistent with the normal operation of BioCARS, biological materials would be cultured at the home institution of the principal investigator. After the biological materials are cultured and purified, the process determining the optimal conditions for crystal growth commences. Proposals for beamtime at BioCARS usually do not take place before crystals have been obtained. Often the crystals can be frozen in liquid nitrogen and shipped by commercial carrier in accordance with U.S. Department of Transportation (DOT) regulations. Crystals that cannot, for whatever reason, be frozen, are shipped at room temperature or packed in dry ice. Occasionally crystals can be obtained but are too fragile to be shipped by any means. In this case, the purified biological material is transported by the appropriate means, and crystallization is initiated at BioCARS. In every case, the experiments are designed to minimize the handling of the biological material. This is done not only to protect the environment from the biological material, but also to protect the biological material from contamination from the environment.

The standard and specific safety practices, the safety equipment (primary engineering barriers), and secondary barriers, such as administrative controls and standard operating procedures (SOPs) for work with the BSL-3 agents, are described in the CDC's "Biosafety in Microbiological and Biomedical Laboratories" guidelines (CDC 1999). The BioCARS implementation of these practices and engineering and administrative controls is described in the BioCARS BSL-3 SOP.²

The most important features of the facility involve the Class II, type B2 biological safety cabinet and the directional airflow, where the air from the facility is exhausted through the safety cabinet after high-efficiency particulate air (HEPA) filtering. HEPA filters remove 99.97% of particles in air with a diameter of 0.3 μm or larger. All sample manipulations are conducted in the safety cabinet. A sample (single crystal) is then transferred in a safe manner to the experimental station where the experiment is conducted. All waste generated during sample manipulation is collected and sent to incineration upon completion of the experiment. Because of the small quantity of the agents used and the engineering and administrative controls (described in the BioCARS SOP), the risk of an environmental release of agents is very low.

² The scope of the BioCARS SOP is comprehensive. In addition to addressing the health, safety, and environmental aspects of each experiment, the BioCARS SOP also addresses biological agent security issues, including chain-of-custody controls and packaging requirements for shipments, controls for researcher access to biological agents, engineering controls for access to biological agent storage areas, quality control features for all aspects of agent management (including disinfection), and training of personnel regarding appropriate safe handling practices and administrative controls. The BSL-3 SOP appears as Appendix T to the CARS Safety Plan. The CARS Safety Plan, which addresses all activities occurring within the facility, was first published in May 1995 and last revised in January 2003. It can be viewed at http://cars.uchicago.edu/safety/Safety_current.pdf.

Individual experiments at BioCARS typically last two to three days. Currently, the facility supports an average of three experiments per year involving BSL-2 organisms. This level of activity is expected to continue into the foreseeable future. In addition, once the Proposed Action commences, the facility will support an expected two experiments per year involving BSL-3 organisms. However, the facility is now being operated at capacity. Therefore, to accommodate any new experiments involving BSL-3 agents, a proportional number of experiments (involving noninfectious biological agents) would be eliminated.

Chemicals used to support research on biological organisms are those commonly associated with microbiological research and are used in very limited quantities. For each experiment involving either BSL-2 or BSL-3 organisms, typical waste types and quantities include standard salt buffer solutions in milliliter quantities; wash solvents (typically 95% ethanol) in milliliter quantities; the sample itself (normally a single crystal of microgram proportion); and decontamination solutions (aqueous hypochlorite solutions) in milliliter quantities. For each experiment conducted at BioCARS, including those involving BSL-3 agents, the largest single volume waste will be personal protective equipment (PPE) (gloves, laboratory coats, etc.) and rags and wipes. A few cubic feet of such waste per experiment is typical. All wastes associated with BSL-3 organisms would be captured, disinfected, and delivered to the Waste Management Operations Division (WMO) of Argonne National Laboratory-East's (ANL-E's) Plant Facilities and Services (PFS) for subsequent delivery to an appropriately permitted commercial facility for incineration. Provisions are in place for currently generated BSL-2 wastes to be handled in the same manner. However, for some BSL-2 wastes, the individual researcher has the option of having all wastes and excess chemicals returned to his/her home institution. In those instances, ANL-E will provide support for such shipments. Each experiment is expected to generate small amounts of wastewater, primarily from the disinfection of experimental solutions. Wastewaters directly associated with experiments will be captured and removed from BioCARS for further treatment or off-site disposal, as appropriate. Other wastewaters associated with routine operation of the BioCARS facility include small amounts of detergent wash solutions from cleaning of experimental equipment and glassware and handwashing activities. Because there is no potential for agent contamination in such wash waters, they are currently discharged via the ANL-E industrial sewer system to the ANL-E industrial waste treatment facility. This management scheme would not change under the Proposed Action. The volumes and character of such wash solutions also would not change appreciably under the Proposed Action. Finally, solutions containing radioisotopes are prohibited from introduction into the BioCARS facility, and the process of conducting experiments using x-ray beams cannot activate the samples. Consequently, no radioactive or mixed wastes are possible.³

As noted previously, under the Proposed Action, because the facility is now operating at capacity, some ongoing experiments involving noninfectious biological organisms would cease in order to accommodate an equal number of experiments involving BSL-3 agents. However, this would result in very little overall change to impacts from the BioCARS facility. Impacts to ambient air from building ventilation and biosafety cabinet exhausts should not change. Waste

³ Some sealed radiological materials would be present as reference standards. However, no wastes would result from the presence or use of these materials. Appropriate controls have been established for all such materials.

volumes from each typical BSL-2 or BSL-3 experiment are very small (on the order of milliliter to deciliter quantities of liquids, milligram to gram quantities of solids, as well as a few cubic feet of solid waste, such as PPE, rags, and wipes). Wastes from experiments involving noninfectious wastes are of the same types and general orders of magnitude. Consequently, overall, waste volumes can also be expected to undergo very little change from those presently generated once BSL-3 research begins. However, the character of the waste would change slightly, and, as described previously, separate management procedures for BSL-3-related wastes would be put into effect for experiments involving those agents. Management of BSL-3-related wastes in the manner described would represent only small increases to the overall amount of biological agent-related wastes already being delivered to WMO from this facility and would not exceed WMO's current waste handling capacity.

REFERENCE FOR APPENDIX A

Centers for Disease Control and Prevention, 1999, *Biosafety in Microbiological and Biomedical Laboratories*, U.S. Department of Health and Human Services, Public Health Service, CDC and National Institutes of Health (NIH), 4th Ed., Washington, D.C., April.

