BIOSAFETY PLAN FOR PPRL

Working at PPRL presents a number of unique challenges to biosafety. Diseases may be carried by the research animals that can infect humans. Rodents and other wild animals around the grounds may also carry diseases that affect humans (hantavirus, bubonic plague). A number of research protocols involve fluids or tissues derived from human sources thus allowing the possibility of exposure to hepatitis B or human immunodeficiency viruses (among others). Poisonous plants, although not specifically covered under OSHA(Occupational Health and Safety Administration), CDC (Centers for Disease Control), or NIH (National Institutes of Health) standards, also present a health hazard. Most research involving animals entails collection of blood, other body fluids, and tissues, which exposes workers to accidental stickings with needles or cuts from scalpels. The purpose of this written program is to set down the work practices to be followed at PPRL when research involving any of these hazards is performed. Following these control practices will minimize the risk to laboratory workers from infection, maintain the integrity of the research being conducted, and protect the surrounding community from substances (plant toxins, spread of poisonous plants or noxious weeds) being studied.

OSHA defines a biohazard as any pathogenic microorganisms that are present in human blood (includes blood components and products made from blood) and can cause disease in humans. Despite the rigorous testing done on human-source products used in diagnostic kits, the possibility exists that pathogenic microorganisms could be present and use of these products would expose the worker to a possible infection. Likewise, much of the research involves animals that can carry zoonotic diseases that can infect humans (brucellosis, Q-fever). Many of the field studies are in areas known to have rodent populations carrying hantavirus and fleas with the bubonic plague bacterium. Bites from venomous spiders or snakes can also occur, especially when collecting plants.

The following sections are *general* guidelines for work involving the above-mentioned biohazards. Each project leader should determine the specific hazards involved in the research and select the appropriate practices for controlling exposure. These procedures and controls must then be passed on to the workers involved in the project. In addition, there are sections detailing medical surveillance and training requirements for this program.

ANIMAL HEALTH

Large and small animals are used in PPRL research. Large animals (cattle, sheep, goats, horses, pigs) are generally purchased from auction consignments, ranchers, or other USDA research stations. Small animals (rats, mice, rabbits, hamsters, gerbils) are purchased from laboratory animal supply facilities with disease controls in place.

Large Animals

All large animals brought onto the PPRL facility are quarantined for two weeks. They have no direct contact with other animals currently on the facility. Animals are inspected by the Veterinary Pathologist. Cattle are vaccinated with 8-Way (*Clostridium* spp.) and 3-Way (IBR, PI3, and BVD). Anthalmetics (Ivomec) and insecticides are administered. Sheep are purchased from the USDA Dubois station and are held in quarantine, vaccinated for clostridial diseases, and treated with Ivomec. After shearing (each spring), they are treated with Ectrin (arthropod control, always given topically); TBZ is administered in the fall. Goats are vaccinated and dewormed as the sheep are. Horses and pigs are occasionally used in research and follow a similar program of quarantine and vaccination before being used on experiments.

Newborn animals are also treated to control possible diseases. Calves will have their navels dipped in iodine and will be dehorned and/or castrated at 1-3 months of age. Lambs are given C&D shots and Bo-Se at 3-4 weeks of age. The lambs are boosted with C&D shots a second time (21-28 days later). Lambs are drenched with Ivomec at weaning.

Animals taken out on field studies are inspected before leaving the facility and appropriate tests are run. When they are brought back to the facility at the end of the field study, they are quarantined and observed for two weeks before being re-introduced to the herd. Most studies are conducted with the animals kept separate from any others present in the field. If they are allowed to run freely on the range, they are treated as new introductions to the herd when they return to the PPRL facility. For more detailed information, refer to the SOP for Large Animals.

Small Animals

All of the small animals (rats, mice, gerbils, hamsters, etc.) are purchased from lab animal suppliers who maintain disease-free facilities. Animals are held in quarantine and observed for any signs of disease before being used on an experiment.

HUMAN-SOURCE SUBSTANCES

A number of the diagnostic kits used in PPRL research are kits designed for human analyses that have been adapted for animal studies. Examples are the radioimmunoassay (RIA) kits used for analysis of progesterone, estradiol, testosterone, and cortisol. As such, the serum controls are drawn from human sources. In spite of extensive testing for any pathogenic microorganisms that may be present in the stock supply, the possibility exists for exposure to such when using the end product. A number of products used in microbiology procedures also represent health hazards. Following are basic safe laboratory techniques that will markedly reduce exposure to dangerous organisms.

1. Wear proper protective equipment: latex gloves, lab coat, and safety goggles or glasses with splash guards at a minimum.
2. No mouth pipetting; use a pipet bulb. Do not blow out the pipettes when pipetting possible infectious material.
3. Inspect tubes for cracks or other signs of failure before centrifuging. Disinfect the centrifuge if a tube breaks.
4. Never leave waste infectious material unattended.
5. Sterilize all waste or leftover material that could be contaminated.

6. Keep your hands away from your mouth, nose, eyes, and face to avoid self-inoculation. Do not apply cosmetics or insert contact lenses while in the lab. Do not eat, smoke, or drink in the lab.
7. Unless contaminated by other hazardous materials, put the waste, leftover material, and any contaminated lab supplies (paper, kimwipes, etc.) into a container and autoclave it before disposal. Special bags with the OSHA-required labeling are available in the safety supply cabinets (Building 25 Surgery area) as well as biohazard tags for labeling other containers.

Rarely, a research project will require the use of potential pathogen-causing agents, none above a BSL-2 level, however. In those circumstances, the research protocol will be reviewed by Terrie Wierenga (Location Biosafety Officer) to ensure that the protocol includes handling precautions (PPE, decon, etc.) and waste treatment and disposal. These reviews will be documented and the review and protocol will be kept in the PPRL Safety Files.

RODENT CONTROL

In 1993 an outbreak of disease caused by hantavirus occurred in the southwestern U.S. The hantavirus is found most often in the deer mouse, although other rodent species have been identified as carriers. A person is infected by breathing in the airborne particles of rodent urine, droppings, or saliva. This may occur when cleaning or disturbing rodent nests, infected rodents, droppings, or burrows. The potential for exposure to the hantavirus occurs when PPRL employees are out in the field and have to camp out or when buildings are being cleaned, especially barns, sheds, and other outbuildings.

Nearly all of the field studies will have a sheep camp or trailer available for housing. Keeping rodents out of these will markedly reduce exposure risk. Certain plant collection trips will require that the employee camp out. Select the campsite with care, avoiding rodent burrows, nests, or traffic areas. Sleep in a self-contained tent and/or on a cot. Avoid raising dust that may be contaminated with rodent droppings.

When cleaning buildings, always ventilate the closed areas for at least 30 minutes. Place spring-loaded traps in the building for several days and treat the area with a flea killer (fleas don't carry the hantavirus but can transmit other diseases such as plague). Spray dead rodents, nests, droppings, and surfaces with a general purpose disinfectant such as Lysol or a dilute solution of bleach (3 tablespoons bleach to 1 gallon water). Let the disinfectant thoroughly soak in before cleaning further. Wear rubber gloves and place all contaminated items into a plastic bag. When the bag is full, seal it and place in a second plastic bag. Either bury the waste 2-3 feet deep or burn it. The local health department can also help in advising on appropriate disposal methods.

Birds and domestic pets (dogs, cats) can also carry diseases that will affect humans or other animals. Currently, the level of activity of these disease vectors is low enough that there are no controls on them. If incidents occur or the populations increase, control procedures will be developed.

Venomous snakes and spiders (rattlesnakes, black widow spiders) are also a concern. Spiders especially are brought back to the lab in plant collections. Snakes and spiders are often found at the collection and field study sites. In most cases, the best first aid is to get the injured person to a hospital or clinic as soon as possible. Don't use the old method of cutting the bite and

sucking out the venom!! Cellular phones are available to take out on trips to the field.

TOXIC BIOLOGICAL SUBSTANCES

Poisonous plants, although a biological material that can be acutely toxic to humans and animals, are not considered a biohazardous agent at this time. However, they will be classified as a biosafety concern for this laboratory. Exposure to the plant toxins generally occurs when the plant is being ground. The dust generated by this process can contain high levels of toxic substances that can be inhaled or absorbed through the mucus membranes (eyes, nose, mouth). Certain plants (waterhemlock) contain the most toxin in the plant "juices". In those cases, the most likely point of exposure occurs when the plant is being collected. Wearing basic protective equipment (gloves, dust masks or respirators, safety goggles, lab coat or coveralls) can prevent most exposures. In order to select the proper protective equipment, the toxin(s) must be known or at least the basic nature of its effects on animals. Plants high in selenium require a different type of protection from plants with the primary source of toxin in the fluids. Refer to the SOP for Grinding Poisonous Plants and/or the risk assessment form filed by your supervisor.

Most of the animal tissues or fluid samples are from animals held for study here at the lab, thus their exposure to diseases is known. However, samples are sometimes sent in to the lab or collected in the field for analysis. Generally, the disease exposure history is known. It is best, however, to treat these samples as possibly contaminated. Any excess animal tissue is incinerated at the USU Diagnostic Laboratory (with which we have a cooperative agreement).

BLOOD COLLECTION

Most PPRL animal experiments require blood to be collected for analysis at a later time. Proper restraint of the animals is critical to avoid injury (both to the animal and to the handler) and to ensure a correct sample is taken. General animal handling guidelines are given in the PPRL Safety Plan and the Animal Care and Use Plan. At the present time, we are allowed to dispose of small quantities of animal blood in the city and university waste systems. Once the serum or plasma has been drawn off, tubes are placed in a ziploc bag and then placed into the unregulated waste. Samples that could be contaminated with a biohazardous agent will be double bagged and incinerated.

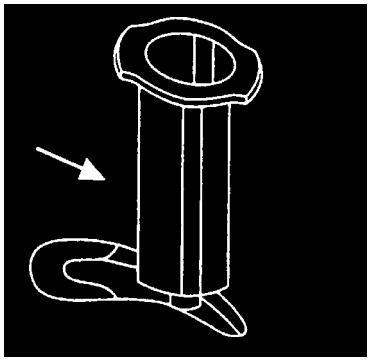
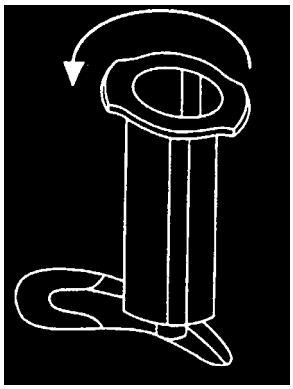
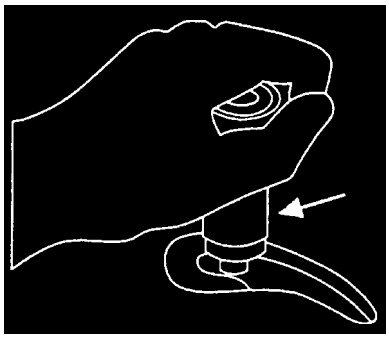
Sharps containers are available throughout the facility. Dispose of all needles, razor blades, scalpel blades, syringe and needle combinations, etc. in these containers. Do not resheathe needles. Use the needle removal slots in the tops of the sharps containers to remove needles from syringes. Used syringes are not to be disposed of in the general waste stream. They are to be disposed of in the biohazard/sharps containers. Past practices included a recommendation to crush, clip or cap the syringes; this is now discouraged. Figure 1 shows the proper procedure for removing needles from syringes. As containers are filled, Terrie will collect them for sterilization (autoclave for 30 min) before disposal in the Logan Landfill.

CONTROLLED SUBSTANCES

The ARS Safety, Health, and Environmental Management Program (Manual 230) has set the standards for acquisition, registration, recordkeeping, and security of controlled substances used in

research projects. Currently, Dr. Stegelmeier (Veterinary Pathologist) and Dr. Panter (Reproductive Physiologist) are licensed by the DEA to purchase and administer specific classes of controlled substances. The common uses of controlled substances at PPRL are for vaccinations, euthanasia, and surgery. An inventory (separate from the chemical inventory) is kept for all controlled substances. It indicates when the substance was acquired, when it was used, what it was used for, when it was disposed of (and how), and who used it. These substances are kept in a locked refrigerator and locked cabinet in a room that is also access-controlled. Copies of the current DEA licenses are on file in the office and with the Logan Purchasing Officer; all orders are confirmed with the license holder before being processed.

Figure 1. Needle Removal

		
Insert needle into tapered slot and move toward narrow end until hub fins are engaged.	Twist holder counterclock-wise to unthread needle (approximately 1½ turns).	Slide holder toward large end of tapered slot allowing needle to drop into collector.

Radioactive compounds present a biohazard as well. The location Radiation Safety Plan (located in the Safety Plans notebook) presents information on the hazards and the procedures used in handling these compounds. The USU Radiation Safety Handbook is located in Room 1A of the Meats & Physiology Laboratory; the radioactive materials license at PPRL is currently under the jurisdiction of USU. Terrie Wierenga is the Location Radiation Protection Officer.

EXPOSURE CONTROL

A number of control methods have already been mentioned. The first control to consider is administrative: is there a less hazardous alternative that can be used? If not, order only the quantity needed for the experiment. Even if you get a tremendous price break by ordering a large amount, the costs of management and disposal of the excess will always exceed any initial savings.

Secondary control measures are engineering designs that allow protection to the workers and their surroundings. Experimental design should be the first area considered. Can the procedure used be modified or replaced by one that will result in less of an exposure risk? Work with biohazardous agents can be carried out in hoods or in biological safety cabinets. The CDC-NIH Biosafety in

Microbiological and Biomedical Laboratories booklet provides an excellent resource for determining what level of protection is required. The April 1996 issue of Lab Animal is a good source for guidelines on working safely with research animals. Both of these are located in the Safety Files. Sharps containers are located in all animal handling areas and in the laboratories for disposal of needles, syringes, etc. Plastic bags and tie-on tags labeled according to OSHA requirements for biohazardous material are available. Always wash your hands when you are leaving the lab or finished working with the animal.

The final control method is personal protective equipment (PPE). All required protective equipment will be provided by PPRL. The Chemical Hygiene Plan (Chapter 1 of the PPRL Safety Plan) provides detailed guidelines for the selection and use of protective equipment. The Safety Files contain a number of references for selecting, using, and fit-testing PPE. As a general practice, wear gloves and protective clothing (lab coat or coveralls) when working with animals or hazardous substances. Respirators may also be required; refer to the PPRL Respirator Management Program for further details. A variety of gloves in various sizes is provided to protect workers' hands. Goggles, safety glasses, and face shields are available. Goggles or glasses with sideshields should be worn whenever there is a splash hazard. Face shields must be worn in addition to goggles or glasses when working with certain substances. The Principal Investigator is responsible for determining the type of PPE needed and providing it to the employee. The Safety Team can also help in determining appropriate PPE.

MEDICAL SURVEILLANCE

Although it is a separate program from medical surveillance, the Occupational Medical Surveillance Program (OHMP) provides a good base for monitoring unknown exposures. Health exams are offered each year under this program that are specific to the hazards each employee works with. The Location Safety Officer consults with the providing physicians to determine what exposures may have occurred. In the case of a known exposure to a biohazard, the same procedure as required in a chemical exposure will be followed (PPRL Chemical Hygiene Plan). The supervisor is to be informed immediately of the exposure and the employee will seek medical aid (all costs will be paid by the employer). Follow-up treatment or examinations are at the discretion of the physician and will also be provided at no charge to the employee. The incident will be investigated by the PPRL Safety Team and results/ recommendations reported to the supervisor and the research leader. In certain instances, pre-exposure exams and tests will be run; these will be determined on a case-by-case basis.

TRAINING

Training on this program will be conducted annually and whenever an employee starts work at the laboratory. The topics covered are listed in Appendix A. Instructors will be (but are not limited to) the Veterinary Medical Officer, PPRL Safety Team, or outside experts in the biohazard/ biosafety field. The written program will be evaluated at least annually by the Safety Team and updated according to regulatory requirements.

BIBLIOGRAPHY

ARS Manual 230: Safety, Health, and Environmental Management Program (1999). Section DV:215.

CDC (1993). Hantavirus Infection - Southwestern US. MMWR 42(RR-11):7-17.

CDC-NIH (1988). Biosafety in Microbiological and Biomedical Laboratories, 2nd Ed. HHS Publication No. (CDC)88-8395.

Kasting, G (1996). Revisiting medical surveillance in research animals facilities. *Lab Animal* 25(4):27-31.

NIH (1985). Guide for the Care and Use of Laboratory Animals.

OSHA (1996). 29 CFR 1910.1030 Bloodborne Pathogens Standard.

Richardson, JH and JR Broderson (1996). Biosafety guideline - an annotation of animal biosafety level 2. *Lab Animal* 25(4):32-34.

APPENDIX A. TRAINING TOPICS

I. Biosafety/Biohazard Program at PPRL
   - A. OSHA (Bloodborne Pathogen Standard, 29 CFR 1910.1030)
   - B. CDC-NIH Biosafety in Microbiological and Biomedical Laboratories

II. General Guidelines
   - A. Animal Health
     1. Quarantine
     2. Vaccination program
     3. Animal health program
   - B. Human Source Substances
     1. Analysis kits (DPC, serum enzyme)
     2. Microbiology supplies
   - C. Rodents and Other Animals
     1. Control methods
     2. Hantavirus
     3. Plague
   - D. Toxic Biological Substances
     1. Poisonous plants
     2. Animal tissues and fluids
   - E. Blood Collection (from animals)
   - F. Controlled Substances

III. Exposure Control
   - A. Administrative
   - B. Engineering (hoods, sharps containers, handling procedures)
   - C. Personal Protective Equipment

IV. Medical Surveillance
   - A. Monitoring for Exposure
   - B. Occupation Health Examinations
   - C. Pre-Exposure Monitoring
   - D. Exposure Response

V. Training Requirements