TITLE: BROMODEOXYURIDINE (BrdU) TREATMENT

I. Use

Bromodeoxyuridine (BrdU) is a synthetic thymidine analog that gets incorporated into a cell's DNA when the cell is dividing (during the S-phase of the cell). BrdU is commonly used in the detection of proliferating cells in living tissues or as a cell cycle marker. It is administered to animals either shortly before euthanasia or as a chronic treatment. A common reason for chronic administration is to monitor the proliferation of experimentally administered stem cells.

When possible, a safer, less toxic alternative to BrdU should be used.

II. Toxicity

BrdU is a mutagen, teratogen and cytotoxin.

III. Personal Protective Equipment (PPE)

When working with BrDU outside of LASC, PPE should include, at a minimum, a lab coat, Nitrile gloves, and ANSI Z-87 compliant protective eyewear that provides chemical splash protection and appropriate lab attire (full-length pants, closed toe shoes, etc.)

Whenever the potential for aerosolization of prepared BrdU solutions exists, an N95 respirator should be worn. Note that the use of an N95 respirator requires medical clearance and fit testing to comply with 29 CFR 1910.134. Contact the Office of Environmental Health and Safety prior to purchasing or wearing respiratory protection.

When working with BrDU within LASC spaces, PPE should be the same as when working at ABSL2. This includes a head cover, eye protection, N-95 respirator, wrap-around gown, double gloves, and shoe covers. All ABSL-2 PPE is single use and should be disposed of in the designated biowaste container upon exiting LASC space. This PPE is required any time the microisolator cages are open (see section V).

IV. Research Animal Procedures

Because BrdU is a mutagen, a teratogen and a cytotoxin, it must be handled carefully. Pregnant women should not be exposed to or handle this chemical in any form.

To assure the safety of research staff solutions of BrdU should be handled and prepared inside a chemical fume hood that has recently been tested by a third-party vendor that has been approved by the Office of Environmental Health and Safety. Contact the Office of Environmental Health and Safety for more information. Following preparation of BrDU, the work area should be thoroughly cleaned with soap and water. Any visible contamination or spills should be neutralized with 10% fresh bleach solution to inactive the chemical prior to cleaning.

It is recommended that animal bedding should be made of corn husks (Bed-o-Cobs is commonly used for rodents), or other similar material to minimize dust generation. Cardboard, paper or other friable materials should be avoided whenever possible. A plastic liner which can simply be rolled up and disposed of should be placed under the bedding when cage changes won't take place in a cage change cabinet.

1) Acute administration

Acute administration is typically conducted by injecting a specified dose of BrdU IV or IP into the animal subject a few hours before euthanasia.

Used needles must be disposed of in an approved sharps container immediately after use. Used needles should not be set on the bench, sheared, bent or re-capped prior to disposal.

Whenever feasible, self-sheathing needles should be used to avoid the potential for accidental needle stick injuries.

To avoid the potential for inhalation of aerosols, the injection of BrdU into smaller research animals should always be conducted in a certified Class II or III biosafety cabinet. For work with non-human primates, N95 respirator use is recommended.

2) Chronic Administration

BrdU may be administered to animals via IV or IP injection, supplied in the drinking water or via an osmotic pump.

Chronic administration of BrdU via IV or IP should follow the same injection procedures as acute administration above.

Mice may be given BrdU in the drinking water (0.25-1 mg/ml) daily for up to 6 weeks or as daily i.p. injections (~2 mg in 100µl of sterile saline) for up to 1 week. BrdU-containing water bottles are shielded from light to prevent BrdU degradation and the water is replaced twice per week. 1% glucose may be included in the drinking water during the first week to overcome taste aversion. Total duration of the procedure is 1 hr to 6 weeks, after which animals are euthanized. Drinking water solutions of BrdU must be prepared

in an appropriate chemical fume hood. Waste drinking water should be diluted to a 10% final solution of fresh mercury-free bleach, and may then be disposed in the drain. Water bottles should be washed with a 10% solution of fresh, mercury-free bleach. Appropriate PPE should be worn (see section III).

Osmotic pumps should be prepared in an appropriate chemical fume hood. Pumps should only be handled with gloved hands. Appropriate PPE should be worn (see section III).

3) Monitoring toxicity in animals during chronic administration

BrdU, because of its action on dividing cells, is primarily directed to the immune system including the bone marrow and also the GI tract. Possible side effects include interference with bone marrow progenitor cells, which in turn may result in leucopenia or anemia. Possible side effects on the gastrointestinal mucosa include bloody stool or diarrhea.

Mice must be evaluated 3-4 times a week for the first week, and weekly thereafter for general appearance, ability to move normally around cage and reach food and water, ability to eat and drink, grooming habits, skin color, lethargy, aggression (animals will be housed individually if aggressive behavior is shown). Mice will be evaluated by the Body Condition scoring index.

Larger animals on chronic BrdU administration, in addition to monitoring of appetite, body weight and demeanor require monitoring via a CBC and a chemistry profile on a regular basis.

V. Animal Housing during Chronic Administration

Smaller animals being housed during the course of chronic BrdU administration must be kept in filter-top microisolator cages to minimize the aerosolization of potentially contaminated bedding and excreta. Animals kept in microisolator cages may be housed in the same room as other animals at ABSL 1 containment.

VI. Disposal of Bedding and Carcass Waste

Research on whether BrdU is present in animal excreta is inconclusive. Therefore, all bedding should be disposed of as regulated medical waste and packaged for incineration. Likewise, all animal carcasses should be packaged for medical waste incineration after euthanasia.

VII. Bedding Changes and Cage Washing

Bedding changes and cage washing should be conducted in an approved cage wash facility that is maintained under negative pressure. Cage changing personnel must wear appropriate PPE as described above. Where feasible, used bedding should be changed inside a cage change cabinet. If no such device is available, or the cages are too big to fit in the cabinet, an absorbent pan liner should be used beneath the bedding material and rolled to facilitate bedding changes and minimize dust generation.

VIII. Chemical Spills

1) Major spills of stock solutions

Isolate area and report spill to the Office of Environmental Health and Safety (617-414-6666 on the Medical Campus, or 617-353-7233 on the CRC). Direct contact with the stock solution should be avoided.

2) Minor spills

Contain spill and clean up bulk material using paper towel or absorbent pads from nearest chemical spill kit. For spills of powder, it may be helpful to wet the absorbent material. Wash the area with a 10% fresh bleach solution and then soap and water.

In addition to the PPE described in section III above, an additional layer of Nitrile gloves or equivalent should be used whenever spills are handled.