Guidelines for the Skin Painting of Mice

Mice have been integral in the study of skin cancer. Many skin cancer studies involve skin painting, the topical application of chemical carcinogens to induce neoplasia. Generally, a single application of an initiator (e.g., DMBA) is used to damage the DNA; this is followed by repeated applications of a tumor promoter (e.g., TPA) to incite tumor development. Painted animals will develop benign papillomas, (round pedunculated tumors arising from epithelial cells), which may be large and/or numerous, but generally don't have deleterious effects on the physiology of the animal. If the papilloma tumor burden is excessively large or fast-growing the animal may be at risk for anemia related to blood supply to an increasing amount of tissue. Large tumor burdens may be increasingly common with the conduct of skin painting studies in genetically engineered mouse strains. Over time some papillomas will convert to carcinomas; these tumors are invasive, growing downward, losing their blood supply and often ulcerating. Lymph node swelling and generalized debilitation may be rapid sequelae to the development of these malignant tumors.

While the *Guidelines Involving Experimental Neoplasia Proposals in Mice and Rats* provide appropriate guidance for the conduct of most cancer studies, they fail to adequately address the intricacies of skin painting. The goal of these guidelines is to aid investigators and facility staff in the design and conduct of skin painting studies, and to address and ensure animal welfare concerns.

LAM Notification:

LAM should be notified when a skin painting study is started; cages with painted animals should be clearly marked. Skin-painted animals are housed in rooms with a respirator requirement.

Clipping:

To facilitate the contact of the tumor inducing chemicals with the skin, haired mice are clipped prior to initiator application.

- To avoid repeated fur clipping an attempt should be made to clip the fur on the dorsum of the mice when the majority of it is in the telogen, (resting), phase of the hair cycle. This generally occurs between 7 and 10 weeks of age, although it is strain specific and may be seasonally variable.
- Fur should be clipped well in advance of skin paint application to allow any clipper burn to subside prior to the application of skin paint, (which is usually dosed in an irritating acetone vehicle). Ideally the operator should clip the animals and monitor for hair regrowth for 2-3 days to ensure that the telogen phase was captured.
- If clipper injury occurs, lesions should be treated with a topical antibiotic ointment/analgesic, (e.g. Triple Antibiotic with Pain Relief), and skin painting should be postponed until healing is complete.

Tumors:

The keratinized surface of papillomas may appear dark or necrotic; this is characteristic of these tumors and is not grounds for treatment or euthanasia. Open, bleeding wounds on the skin or tumors must be treated or addressed.

- Mice engaging in cannibalism of tumors should be separated to their own cages.
- Polycarbonate enrichment devices, (tubes and igloos), are not recommended for mice with skin tumors because papillomas, (which are only attached to the mouse by a thin stalk), may catch on the edges of these objects and be torn off.
- If open wounds occur at any time during the course of the study, skin painting should be temporarily halted and lesions should be treated until they heal.

Anemia:

Signs of anemia in mice include pale skin and mucus membranes, (peri-ocular tissue, ear pinnae, feet); late stage signs may include lethargy and rapid breathing.

- If signs of anemia are noted a small blood sample should be taken on a weekly basis for hematocrit determination. (Normal hematocrit values for the mouse approximate 40%).
- The veterinary staff should be notified if signs of anemia are noted in skin painted animals; they will provided assistance in hematocrit determination and follow-up care.

Endpoints:

Study endpoints vary by investigator and may include the age of the animals, the number or size of papillomas, or the conversion of papillomas to squamous cell carcinomas.

- If excessive tumor burdens are unexpectedly generated, endpoints should be adjusted accordingly.
- Where a carcinoma endpoint is required, the development of a single carcinoma should be used as an endpoint unless scientifically justified.
- In cases of extended endpoints, twice daily monitoring is required and the use of analgesics is strongly encouraged.
- Specific extended endpoint criteria should be provided, including the maximum time period an animal will be maintained with carcinomas.
- For mice maintained with carcinomas, daily palpation of the tumors is required to assess invasiveness. When a carcinoma and surrounding skin is no longer freely movable, it has invaded the underlying tissues and the mouse should be euthanized unless scientifically justified.

Please address any questions concerning these guidelines to the facility manager or veterinary staff.