Poisonous Plant Research Laboratory
REGISTRATION NUMBER: 87-G-0001
EXPLANATION FOR THE USE OF ANIMALS LISTED IN COLUMN E

Mice:
The 2018 Annual Report of Research Facility for the Poisonous Plant Research Laboratory indicates laboratory mice have been used in studies involving pain and distress for which the use of appropriate anesthetic, analgesic or tranquilizing drugs would have adversely affected the procedures, results or interpretation of the research. Research conducted at this institution involves experiments to determination the toxicity of plant compounds using a median lethal dose (LD_{50}) protocol in a laboratory mouse model. In the case of all LD_{50} experiments, animals are intensively monitored during the experiment. Previous experience with LD_{50} experiments in laboratory mice at this laboratory have provided specific criteria for each plant compound indicating likely death. Laboratory mice meeting these criteria are euthanized to minimize pain and distress. All compounds used with the animals in this report are extremely acute toxins, with death occurring within minutes after IV injection. All animals that survive are quickly euthanized after it is apparent they survived the treatment. Therefore, any pain and distress experienced by the animals is minimized by euthanizing the animals.

Some of the LD_{50} experiments performed at the Poisonous Plant Research Laboratory involve the investigation of plant compounds for which the toxicity and mechanism of action is not known. The experimental status of the plant compounds being tested means that little or no information is available regarding possible drug-drug interactions. Co-administration of pain relieving compounds alters their toxicity, and therefore would skew the true toxicity values of the compounds. Therefore, the use of pain relieving substances is avoided in these experiments.

Results from a literature search with PubMed suggests that commonly used pain medications such as opioids [1-3] and non-steroidal anti-inflammatory agents [4-6] may alter the response to bioactive molecules like plant compounds and other drugs. Furthermore, it is well documented that bioactive plant products in human medicine and veterinary medicine have the potential for adverse effects [7-9]. Due to the confounding effects of drug-plant compound interactions, pain relieving substances are not routinely administered to laboratory mice in LD_{50} experiments. Of the 231 Category E mice, 132 were related to LD_{50} experiments.

Of the 231 Category E mice, 98 were related to studies investigating the toxic principles of *Ipomoea asarifolia*. Plant-derived fungal-derived toxins are a major source of losses for the livestock industry world-wide. Included in this category is *Ipomoea asarifolia*, which contains various tremor-inducing alkaloids. These studies used mouse models, as a small animal model, to study intoxication in adult mice and in nursing pups, as little information was available about this tremorgenic syndrome. Mice were evaluated daily for decreased activity, abnormal postures, hunched back, poor grooming, decreased food and water intake, and loss of > 20% of body weight. All animals were observed daily and evaluated 3x/week for tremors and given a visual score based on severity of tremors. Animals showing the most severe tremors were euthanized. In addition, moribund animals were immediately euthanized; other animals were

evaluated according to the criteria listed above, and euthanized when their health was deemed compromised beyond a reasonable measure by the PI and AV.

One mouse on the DHPA study was found dead in its cage. It did not previously appear sick or show any indication of disease clinically or gross lesions at necropsy. Our experience shows that these P53 knockout mice are prone to develop leukemia which likely caused their sudden death.

1. Hedrich, W.D., et al., *Insights into CYP2B6-mediated drug-drug interactions.* Acta Pharm Sin B. 2016. 6:413-425.
2. Mouly, S. et al., *Is the clinical relevance of drug-food and drug-herb interactions limited to grapefruit juice and Saint-John's Wort?* Pharmacol Res. 2016. S1043-6618(16)30991-4.
3. Kohurt, S.J., In*teractions between nicotine and drugs of abuse: A review of preclinical findings*. Am J Drug Alcohol Abuse. 2016 Sep 2:1-16.
4. Russo, N.W., et al., *Aspirin, stroke and drug-drug interactions*. Vascul Pharmacol. 2016. S1537-1891(16)30269-5.
5. Lionetto, L., et al., *Choosing the safest acute therapy during chronic migraine prophylactic treatment: pharmacokinetic and pharmacodynamic considerations.* Expert Opin Drug Metab Toxicol. 2016. 12:399-406.
6. Supuran, C.T., *Drug interaction considerations in the therapeutic use of carbonic anhydrase inhibitors.* Expert Opin Drug Metab Toxicol. 2016. 12:423-31.
7. Campos, M., et al., *Herb-drug Interactions: An insight into cardiovascular diseases based on case reports.* Cardiovasc Hematol Agents Med Chem. 2016 Oct 7. [Epub ahead of print]
8. Singh, D., et al., *Herbs-are they safe enough? an overview.* Crit Rev Food Sci Nutr. 2012. 52:876-98.
9. Poppenga, R.H. *Herbal medicine: potential for intoxication and interactions with conventional drugs.* Clin Tech Small Anim Pract. 2002. 17:6-18.