

Poisonous Plant Research Laboratory**REGISTRATION NUMBER: 87-G-0001****EXPLANATION FOR THE USE OF ANIMALS LISTED IN COLUMN E****Justification for Category E study with cattle. FY2020.**

Some plants grazed by livestock on western rangelands cause an acute toxicosis such that animals are simply found dead. Larkspur is one plant that causes acute lethality in livestock species. For this research project, plant material was administered to cattle to determine and compare how much plant material containing different amounts of the primary toxins a cow must consume to become poisoned by larkspur. We anticipated that these doses would be sub-lethal. The experimental plan was that as soon as the animals showed clinical signs, they would be treated to alleviate any pain or distress. The cattle were monitored hourly after dosing. All efforts were made to ensure that no undue pain or distress was caused to the animals, the experimental protocol was followed with the appropriate monitoring; however, two cattle died unexpectedly of larkspur intoxication, thus they were reported as category E.

Justification for Category E study with goats. FY2020.

Some plants grazed by livestock on western rangelands cause an acute toxicosis such that animals are simply found dead. Larkspur is one plant that causes acute lethality in livestock species. For this research project, plant material was administered to goats to determine how much plant material a goat must consume to become poisoned by larkspur. We anticipated that these doses would be sub-lethal. The experimental plan was that as soon as the animals showed clinical signs, they would be treated to alleviate any pain or distress. The goats were monitored hourly after dosing. All efforts were made to ensure that no undue pain or distress was caused to the animals, the experimental protocol was followed with the appropriate monitoring; however, two goats died unexpectedly of larkspur intoxication, thus they were reported as category E.

Justification for Category E study with mice. FY2020.

Research conducted at this institution involves experiments to determination the toxicity of plant compounds using a median lethal dose (LD₅₀) protocol in a laboratory mouse model. In the case of all LD₅₀ experiments, animals are intensively monitored during the experiment. Previous experiences with LD₅₀ 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. If the toxins do not act acutely, the animals are quickly euthanized. Therefore, any pain and distress experienced by the animals is minimized by euthanizing the animals. For this experiment mice were dosed with extracts from different larkspur collections in order to compare the toxicity of the different plant species. Previous research has demonstrated that pain alleviating medicines cannot be administered as it will shift the amount of plant extract required to kill mice. Thus, in order to effectively perform an LD₅₀ study in mice for larkspur plants, no pain alleviating treatment can be administered. 56 mice treated in this experiment showed clinical signs of

poisoning but were not administered pain alleviating medication as it would have compromised the outcome.

An additional nine category E mice were part of a carcinogenicity study to determine if subclinical dehydropyrrolizidine alkaloid treatment increases neoplastic transformation. All nine mice were dosed with dehydropyrrolizidine alkaloids between 6 and 12 months prior to their deaths. These nine mice are part of more than 200 mice that were part of this experimental study. All animals on these studies were closely examined according to protocol. The protocol states that any animal that develops minimal clinical signs (weight loss, loss of appetite, failure to groom etc) are euthanized and necropsied. These 4 mice died suddenly without developing any evidence of clinical disease. Such deaths are encountered with similar incidence in our untreated breeding colony. The cause of death in our breeding colony is often not apparent at necropsy or from microscopic studies. This suggests some deaths are likely due to some arrhythmia or some other functional cardiac disease. Such deaths are probably the effect of aging and are natural causes of death in old mice. As microscopic studies of these 9 animals are not complete the cause of death is yet undetermined; however, these nine deaths are likely due to similar reasons as the aged mice in the breeding colony. However, as these mice were treated and found dead unexpectedly, we have classified them as category E.