

## USE OF NOVEL COMPOUNDS AND EXTRACTS

**Summary/Purpose: To establish measures that help ensure justification of animal numbers and PI and IACUC oversight of pain and distress in drug discovery research.**

### **I. Rationale**

Unlike traditional hypothesis testing, drug discovery research on novel compounds and extracts 1) cannot always predict precisely how many compounds and extracts will be tested in a protocol and, therefore, cannot always predict the numbers of animals required, and 2) cannot always predict adverse effects that could produce pain or distress in animals. The purpose of this policy is to establish measures that help ensure justification of animal numbers and PI and IACUC oversight of pain and distress in drug discovery research.

### **II. Definitions**

#### **A. Novel Compounds and Extracts**

A Novel Compound and extract is any chemical entity that 1) has never been tested in animals; or 2) is in preliminary stages of animal testing. Novel Compound and extract status affects the evaluation of a compound for potential adverse side effects and the animal monitoring intensity level. Increased frequency animal monitoring will continue until the PI has collected a reasonable amount of data from observing animals at the maximum dose of a novel compound. If, at this stage, no adverse effects have been found, increased frequency monitoring can be reduced. If adverse effects have been found, monitoring will continue at a level necessary to prevent unnecessary pain or distress.

#### **B. Unanticipated Significant Adverse Effects**

Pain and distress may be an unavoidable part of some research protocols (e.g. pain studies, infectious disease models). Guidelines pertaining to these types of studies already exist in the current protocol application. This policy seeks to address unanticipated pain and distress that occurs as a result of administering substances with unknown effects on animals. Examples of Unanticipated Significant Adverse Effects include but are not limited to lethality, substantial weight loss, and collapse.

### **III. Background to Justification of Number of Animals**

Identification of novel compounds and extracts of interest occurs continuously but at varying rates in drug discovery laboratories. PIs test large numbers of novel compounds and extracts annually (e.g. 80-100) in search of drugs to cure disease, but the number of compounds and extracts that will be used for testing can rarely, if ever, be specified in advance. This prevents PIs from stating precisely the number of animals required in IACUC applications. However, based on the estimated number of compounds, extracts, and using an appropriate justification formula (see V.B.1. below), PIs can estimate the number of animals required per compound and extract, and the IACUC will review that estimate. The IACUC will monitor the justification formula through the annual update process: PIs will submit the number of compounds and extracts, and number of animals used for the year on the standard Protocol Annual Update Form.

#### **IV. Background to Recognition of and Minimizing Pain and Distress**

Most novel compounds and extracts are pre-screened for the biological activity of interest and for toxicity through *in vitro* tests. However, in some cases *in vitro* tests correlate very poorly with *in vivo* results. Also, in many cases the animal model used to test the novel compound and extract introduces known diseases and/or drugs that have significant pain or distress profiles. In contrast, novel compounds and extracts are developed with the aim to have no adverse effects.

Drug discovery procedures often have pilot research strategies built in. Initially, only a small number of animals receive each dose of a novel compound or extract. If Unanticipated Significant Adverse Effects are seen at higher doses, testing of those doses is terminated (unless scientifically justified).

Pain and distress concerns about novel compounds and extracts are dealt with in this policy in two ways. First, PIs indicate how prior data (if available) will be used to estimate potential for adverse side effects and to determine initial dose. Second, PIs submit a plan for monitoring adverse effects. This plan includes a determination of humane endpoints (see V.C.3. below).

#### **V. Procedures**

##### **A. Compound and Extract Names and Structures**

The names and structures of compounds and extracts are not required in protocol applications.

##### **B. Justification of Numbers**

1. PIs will provide numbers of animals/treatment/procedure. If the number of Novel Compounds and Extracts to be tested cannot be determined at the time of submission, PIs will provide the best estimate of compounds, extracts, and animals needed. As information is acquired, the number of animals may change and must be approved in an amendment.
2. Animal numbers will be tracked and reviewed for justification through the Protocol Annual Update Form.

##### **C. Evaluation and Observation of Pain and Distress**

###### **1. Compound Effects**

The PI should provide the following information in the protocol application:

- a. Indicate which of the 3 following scenarios applies to the compound(s) and extract(s). (Appropriate literature or structure searches should be included [provide search strategy]. This information should be provided in Appendix VII.)
  1. *In vitro* or *in vivo* data exist and indicate no known toxicity.
  2. *In vitro* or *in vivo* data exist and indicate probable toxicity. This

information can come from testing the compound and extract in question or compounds and extracts with similar structure. It may include information such as chemical class (stimulant, depressant, etc.), mechanism/site of action, or cytotoxicity. The PI should submit this information with the protocol application.

3. There are no *in vitro* or *in vivo* data available to the PI.
  - b. Describe and indicate how the following are determined: the initial dose and route of administration to be used; the intervals for increasing the dose (e.g. half log); any reasons for deviating from this plan; the rationale for target dose (e.g. adverse effects and/or dose needed to treat the disorder). If compounds or extracts are unavailable at the time of initial protocol application, indicate the decision tree that will be used to determine initial dose, intervals, route of administration, increments of increasing doses, reasons for deviating from this plan, and rationale for target dose.
  - c. Provide a plan for monitoring for adverse effects in test animals. This plan should include a checklist of signs of pain and distress relevant to the species, any anticipated signs based on known information, and frequency of monitoring. (If the PI has collected a reasonable amount of data from observing animals at the maximum dose of a novel compound and extract and no adverse effects have been found, routine monitoring can be initiated. If adverse effects have been found, monitoring should continue at a level necessary to prevent unnecessary pain or distress.)
2. **Notification of Attending Veterinarian**  
The PI will notify the Attending Veterinarian in a timely manner when Unanticipated Significant Adverse Effects are observed. The PI will then complete the relevant monitoring sheet and submit a copy of it to the Animal Care Office in NCNPR.
  3. **Humane Endpoints**  
Ideally, humane endpoints (predictive signs, indicators of an irreversible deteriorating condition) should be established prior to beginning animal work. Realizing the difficulty in predicting these for studies that involve compounds and extracts with unknown adverse effects, the PI should revise humane endpoints if severe pain, severe distress, or death is observed. Guidelines should be in place to euthanize animals 1) when humane endpoints are reached, 2) when the study objectives have been realized, 3) if it becomes clear that they cannot be realized, or 4) whenever the degree of suffering is not required or justified by the protocol.
  4. **Cooperative Projects**  
In cooperative projects, PIs should make every attempt to obtain or provide information on adverse effects that may be associated with any substance. Often this may be unpublished data collected recently by another PI.

**5. Laboratory Monitoring**

To better educate the IACUC and to monitor attention to pain and distress, the Attending Veterinarian and one or two IACUC members may observe laboratory procedures under this policy at random intervals. PIs will be consulted in advance.

**6. Pain and Distress Category**

The pain and distress category of novel compounds and extracts will begin at B, unless procedures or anticipated toxicity dictates other categories. As information is acquired about the compound and extract, the category will be changed accordingly and reported in an amendment.

**D. Record Keeping**

PIs must include the following information in the Protocol Annual Update Form: number of compounds and/or extracts used; number of animals used; number of animals categorized as something other than the original pain category; and the number of animals euthanized to achieve the humane endpoint.

All animal monitoring forms should be kept in a location accessible to the Attending Veterinarian and animal care staff. PIs will notify the Veterinarian of Unanticipated Significant Adverse Events and submit a copy of the relevant monitoring sheet to the Animal Care Office in NCNPR.

**VI. Additional Points**

**A. Occupational Health and Safety**

The Department of Health and Safety has included unknown compounds and extracts in chemical safety training. This training satisfies the occupational health and safety need regarding potentially hazardous unknown agents.

**B. Standard Operating Procedures (SOPs)**

PIs are encouraged to provide SOPs for procedures involving animals, such as, animal models that will be used for testing the unknowns (e.g. Malaria model, Anxiety model). Once developed and approved, these SOPs can be referred to in new protocol applications. [*Note: An SOP is not a protocol substitute.*] SOPs should be updated as procedures change. Annual reports of SOPs should be submitted yearly for review.