GUIDANCE ON ALTERNATIVE ENDPOINTS

1. Alternative endpoints for Infectious Disease Animal Models

a. Hypothermia – Monitoring body temperature should be part of the study design utilizing an infectious disease animal model. Lowered body temperature, hypothermia, has been shown to be an accurate indicator of a deteriorating condition in animals. A significant decrease (>4-6°C) has been correlated with death and has been shown to be useful as an endpoint in numerous infectious disease models including influenza and bacterial septicemia. There are both non-invasive and invasive means of measuring temperature in small laboratory animals including the use of infared temperature scanners, tympanic infared thermometers or implanted thermistor microchips.

b. Acute Phase Proteins (APPs) – Increases in serum levels of cytokines and acute phase proteins can be used as predictors of severity and outcome. Elevations in cytokines can be transient; however, APPs rise in response to increased cytokine production and high levels of major APPs correlate well with the presence and severity of infectious disease.

c. Physiologic and Behavioral Changes – These include, but are not limited to weight loss (10-20%), decreased activity, and inappetance/anorexia. These clinical signs are typically secondary to the effects of alterations in cytokine levels. Establishing an observational checklist of these deviations may be helpful in identifying the expected progression through these deviations and can be used to determine criteria indicative of an endpoint.

2. Alternative Endpoints for Hematopoietic/Lymphatic Cancer Research

Increases in circulating tumor cells can forecast the onset of clinical signs; however, when human leukemia cells are engrafted into immunodeficient mice the number of circulating cancer cells is less predictive. In the absence of reliable laboratory-based assays, animals with leukemia/lymphoma should be observed for early clinical signs such as anemia, weight loss (>20%) or impaired respiration. An evaluation system is recommended to assess the severity of the disease. This system would be based on a combination of physiologic, biochemical and hematologic parameters.

3. Alternatives for Vaccine Potency Testing

a. In-vitro serologic testing – To replace the challenge procedure, in vitro serologic testing has been used to assess antibody responses of animals after immunization. For example, a good correlation has been shown between the titer of antibodies induced and the level of protection after challenge has been confirmed for toxoid and some of the clostridial vaccines.

4. Alternatives for Median Lethal Dose (LD50) Test

a. Up-and-Down Procedure – Dosing is performed in individual animals in a staircase fashion. The next dose depends on the results from the previous animal, i.e. if the animal survived, the dose is higher and if the animal died, the next dose is lower. (ICCVAM 2001) Additionally, there is a revised version of this procedure which provides additional guidance on how this procedure can be utilized to reduce the number of animals as compared to the traditional LD50 test.

5. References

- Alternatives to Animal Testing on the Web (2004), *Humane Endpoints Database*. (http://altweb.jhsph.edu/) Johns Hopkins Center for Alternatives to Animal Testing. Baltimore.
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- Hendriksen, CFM and Steen, B. *Refinement of Vaccine Potency Testing with the Use of Humane Endpoints*. ILAR 41(2), 2000.
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