Incorporation of Perinatal Exposures into NTP Bioassays

Paul M.D. Foster, Ph.D., A.T.S.
Toxicology Branch
National Institute of Environmental Health Sciences

NTP Technical Report Review Panel,
June 25th, 2015
Overview

- NTP workshops
  - Major conclusions and outcomes
- Perinatal Bioassay design
- Statistical analysis of data
Alternative, sensitive animal models are needed for detecting specific types of tumors.

Additional endocrine responsive endpoints are needed in test guidelines.

A new rat strain is needed that is more sensitive to endocrine endpoints and has a lower background tumor burden than the F344.

The importance of developmental programming in hormonally dependent tissues leading to pre-neoplastic events and tumors must be addressed.

Outcomes from this workshop

- NTP moved from the inbred F344 to an outbred rat stock. This was also a recommendation of the “stocks & strains workshop”
  - Extensive survey of rat stocks, originally selected the Wistar Han and currently use the Harlan Sprague-Dawley
    - less prone to spontaneous testicular interstitial cell tumors and mononuclear cell leukemia than F344
    - Robust breeder: can be used for all NTP rat studies

- Refined our procedures in new versions of the NTP specifications for carcinogenicity and reproductive toxicity testing.
  - New endpoints; better recording of gross findings; improved histology of reproductive tissues.

- Changed default exposure paradigm to include pregnancy and early life exposures in rat cancer bioassays
NTP Rat Cancer Bioassays

Conventional Study (50 /sex/dose)
- Continuous Exposure
- ~ 6 weeks
- 2 yrs

Perinatal Study (25 litters/dose)
- Gestation
- Continuous Exposure
- Birth
- Lactation
- Selection (2♂ & 2♀/litter)
- Weaning
- 2 yrs
Better characterize early life exposure and cancer outcome

Exposure would occur in critical windows for the development of certain hormonal cancers (e.g., testis, mammary, prostate)

Evaluate developmental basis of adult disease/lifetime risk

NTP has conducted “perinatal cancer bioassays” in the past, but their conduct required special justification. The new default is to undertake such a study unless there is a scientific reason not to do so.

Before undertaking such a study, some preliminary dose-range finding information would normally be required to ascertain dose levels, using perinatal exposure.
Preliminary Design prior to a Carcinogenicity Study

- Ascertain dose levels for continuous exposure that would allow:
  - Dams to successfully carry their offspring to term
  - Deliver their offspring
  - Successfully raise their offspring to weaning
  - After weaning, determination of any target organ toxicity in resulting adults that would preclude the successful conduct of a carcinogenicity study (equiv. 90 d study – 10/sex/group)
Analysis of Data

• In the NTP perinatal design, we are selecting multiple pups from each exposed litter, which are not independent.

• Two important issues arise in the statistical analysis of perinatal exposure studies:
  – Pups within a litter tend to be more like each other than they are like pups from other litters; i.e., there may be within-litter correlation.
  – There is likely to be both litter-to-litter variation and within-litter variation.

• Several approaches are possible:
  – Select only one pup/sex/litter for the study or analysis.
  – Average the outcomes of all pups in a litter; analyze the averages.
  – Use mixed effects models using outcomes from all pups.
NTP’s approach to analyzing these data is to use mixed effects models.

- For binary outcomes, such as lesion incidences, NTP uses mixed effects logistic regression (which can include additional covariates, such as length of survival).

- For continuous outcomes, such as organ weights, NTP uses mixed effects analysis of variance.

This approach uses data from each pup while accounting for within-litter correlations.

- Does not overstate the significance of differences (i.e., p values too small) which would occur if pups are assumed to be independent.

- Achieves better power than using one pup or one average per litter.