NTP
National Toxicology Program

Incorporation of Perinatal Exposures into NTP Bioassays

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Overview

- NTP workshops
  - Major conclusions and outcomes
- Perinatal Bioassay design
- Statistical analysis of data

"Hormonally induced reproductive tumors- relevance of rodent bioassays": Major Conclusions

- 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.

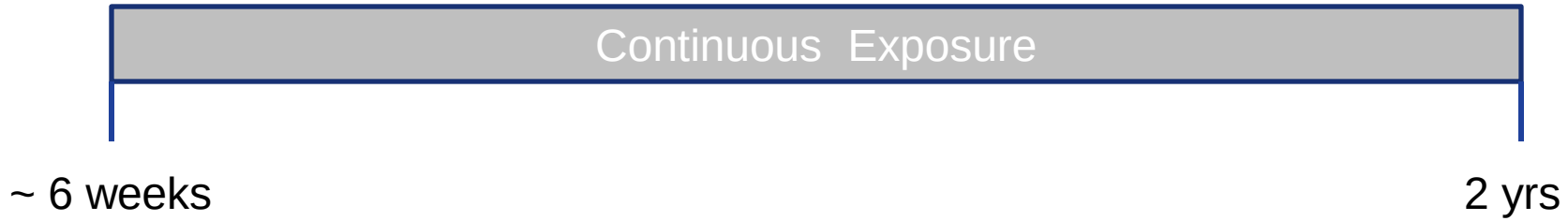
Thayer & Foster (2007) *EHP* 115: 1351-6

Outcomes from this workshop

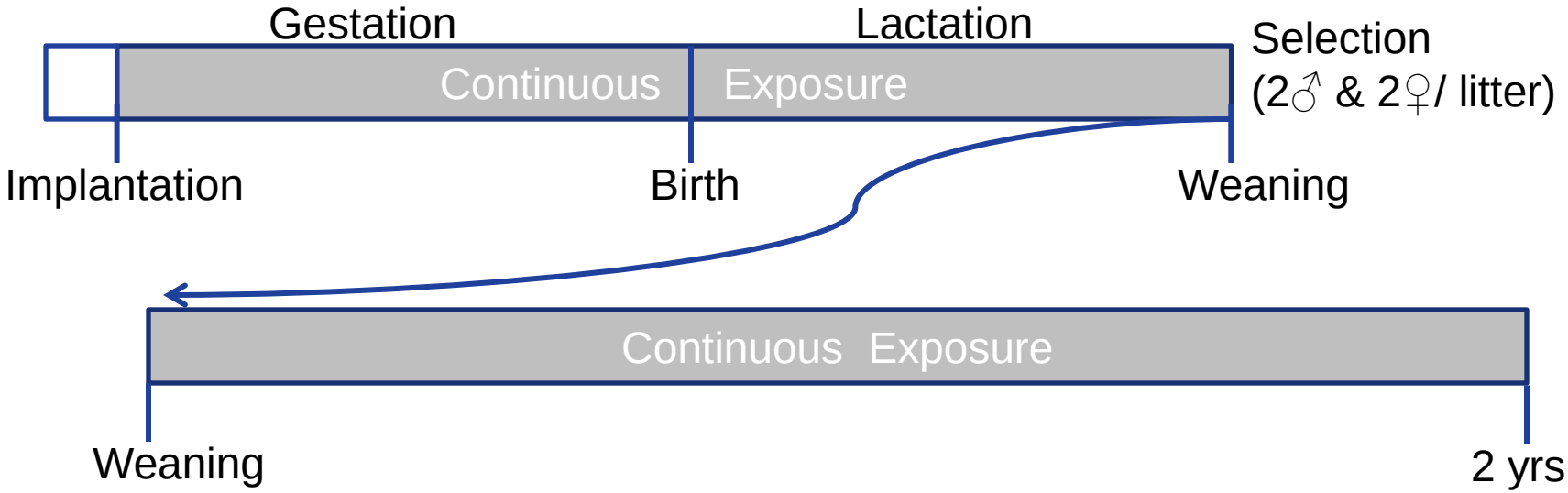
- NTP moved from the inbred F344 to an outbred rat stock. This was also a recommendation of the “stocks & strains workshop”
  - Extensive survey of rat stocks, originally selected the Wistar Han and currently use the Harlan Sprague-Dawley
    - less prone to spontaneous testicular interstitial cell tumors 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)



Perinatal Study (25 litters/ dose)



Perinatal Bioassay

- 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.

Statistical Methods

- 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.