NTP
National Toxicology Program

NTP's Toxicology and Carcinogenesis Studies: Experimental Design, Statistical Analyses, and Hazard Determinations

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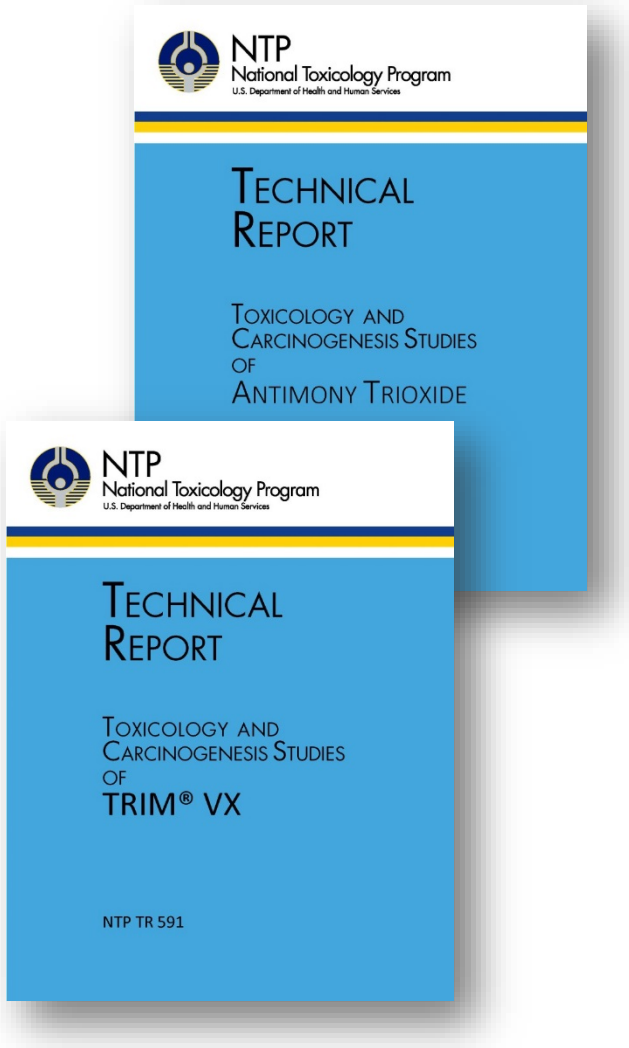
National Toxicology Program (NTP) Technical Reports

- As part of NTP's mission, NTP provides data from various studies for human health protection
- Chronic toxicity and carcinogenicity studies are conducted on agents of concern to identify potential hazards after long term exposure
- These studies are described in NTP Technical Reports, which include conclusions as "levels of evidence" for carcinogenic activity



Outline

- Provide brief overview on elements of these NTP studies
  - Design elements of NTP chronic toxicity and carcinogenicity studies
  - Factors in analysis and interpretation
  - Hazard Identification: Levels of Evidence of Carcinogenic Activity

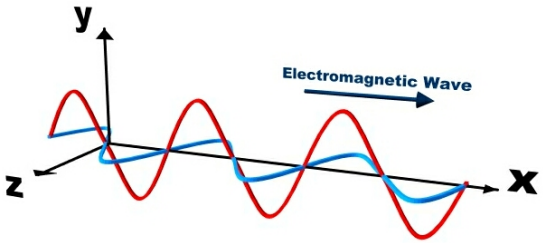
Design considerations

- Objective of these studies is to identify a potential increase in toxicity and/or neoplasms from long-term exposure to a test agent
- Several elements are considered in designing chronic toxicity and carcinogenicity studies, which include:
  - Selecting animal models and appropriate number of animals
  - Selecting exposure route, duration, and levels
  - Selection of endpoints to evaluate
  - Building of exposure systems and quantifying exposure during the study

Animal model and numbers

- NTP uses two rodent species to evaluate chronic toxicity and carcinogenic activity
  - Hsd:Sprague Dawley SD Rats
  - B6C3F1/N mice: F1 cross of C57BL/6N females and C3H/HeN males
- Typically 50 animals per sex are used per group
  - Due to concern of detecting low incidence tumors, 90 animals per group were used for these radiofrequency radiation (RFR) studies

Exposure language



Chemicals	→	**Waves of Electromagnetic energy**
Concentrations mg/kg, ppm, mg/cm^3	→	**Specific Absorption Rate (W/kg) power (Watts); field strengths (v/M)**
Material reactivity	→	**Interference of signal Absorption of energy Heating of metals**
Enantiomers	→	**Frequencies (900, 1900MHz) Modulations (CDMA, GSM)**
Route of exposure	→	**Exposure system**

Exposure route and duration

- The route of exposure is typically based on human exposure patterns, e.g., dietary exposure, occupational exposure, whole-body exposure
- Duration of exposure often daily throughout a two-year time frame, and with widespread exposure of particular agents, this typically means developing young are also exposed
- The two-year bioassay in rats includes a perinatal exposure component:
  - Includes the time periods of gestation and lactation
  - Two to three pups per sex per litter selected during weaning for continued RFR exposure

Exposure levels

- Selection of a study's exposure levels is based on balancing:
  - Tolerance of the animal model to agent of concern
  - Desire to challenge animal model for hazard identification
- To estimate tolerance, frequently short-term exposure studies are conducted by the NTP
- Based on these shorter studies, a maximum exposure level is selected and additional lower doses are spaced accordingly

Endpoint evaluation

- Typical biological endpoints evaluated in this study type
  - Survival, body weight, and clinical observations
  - Gross and histopathologic evaluation of 40 tissues
- Interim evaluations provide additional information of potentially ongoing toxicity
  - Body and organ weights
  - Clinical pathology (clinical chemistry and hematology)
  - Reproductive endpoints (andrology and vaginal cytology)
  - Histopathology
  - Genetic toxicology (micronucleus assay and comet assay)

Interpretation of response

- Statistics
- Dose-relationship
- Common versus uncommon lesions
- Concurrent and historical control data
- Multiplicity
- Latency
- Progression: benign to malignant and metastases
- Pre-neoplastic lesions
- Survival
- Body weight effects
- Structure-activity correlations
- Genetic toxicology
- Findings in the other sex or species
- Combinations of neoplasms in the same tissue of origin

Statistical analysis

- Analysis of neoplasms and nonneoplastic lesions take into account survival and littermates
  - Neoplasms usually take time to develop and survival will affect incidence
  - Littermates may have a genetic or environmental component in their response leading to an intra-litter correlation

Summary table: No adjustment

	Sham Control	Low Dose	Medium Dose	High Dose
Number of animals with tumor	x_0	x_{LD}	x_{MD}	x_{HD}
Total number of animals	n_0	n_{LD}	n_{MD}	n_{HD}
Proportion of animals with tumor	$p_0 = x_0/n_0$	$p_{LD} = x_{LD}/n_{LD}$	$p_{MD} = X_{MD}/n_{MD}$	$p_{HD} = x_{HD}/n_{HD}$

Cochran-Armitage (trend) $H_0: p_0 = p_{LD} = p_{MD} = p_{HD}$

Fisher's exact (pairwise) $H_0: p_0 = p_{LD}$

$H_0: p_0 = p_{MD}$

$H_0: p_0 = p_{HD}$

Survival-adjusted analysis

- NTP uses a survival-adjusted statistical analysis (Poly-k) to analyze lesion incidence and account for differential mortality
  - For a given site, each animal is assigned a risk weight
  - If an animal dies prior to terminal euthanasia, and does not have a lesion, risk weight is a fraction of the study time it survived raised to the kth power (k = 3)

w_{ij} = 1 if the *jth* animal survives duration of study (t_{max})

$w_{ij} = \left({}^{t_{ij}}/_{t_{max}}\right)^{K}$ if *jth* animal survival time $t_{ij} < t_{max}$

$n_i^* = \sum_j w_{ij}$

Litter adjustment

- The use of littermates (siblings) may lead to an intra-litter correlation and thus overstate the effective sample size (a small p-value)
- To adjust, a Rao-Scott approach was used to estimate effective sample size by using ratio of variance with litter effects and without litter effects

Design Effect:

$$\widehat{D}_i = \frac{\text{estimated variance of } proportion\ of\ animals\ with\ a\ tumor \text{ considering litter}}{\text{estimated variance of } proportion\ of\ animals\ with\ a\ tumor \text{ without considering litter}}$$

Effective Sample Size:

$\tilde{n}_i = \frac{n_i}{\widehat{D}_i}$ (adjusted across all dose groups)

Rao-Scott example

- For example, the variance of tumor incidence is estimated two ways:
  - 25 litters of 2 siblings each
  - 50 independent animals
- The ratio of these two variances is the factor by which the sample size, 50, is reduced to yield the effective sample size
- The effective sample size is used in the Poly-3 test instead of the actual sample size

Historical control

- Concurrent control of a study considered the most appropriate
- Historical control data from previous NTP studies within five-year window are used to provide context of response in current study
- Species, strain/stock, feed, route of exposure can all vary the background incidence of specific neoplasms
- The route of exposure is unique for these RFR studies

Historical control

- NTP historical control database for Hsd:Sprague Dawley SD Rats consists of four completed studies
  - This database includes the concurrent control
  - Database of brain neoplasms consists of three studies due to change in histopathological evaluation (increased sections of the brain from three to seven)
  - Studies at contract lab were reviewed to determine consistency of background response in brain and heart neoplasms (Wyde *et al*. 2016)
- NTP historical control database for B6C3F1/N mice consists of eleven completed studies

Hazard identification

- NTP conducts these studies to identify an agent's potential chronic toxicity and carcinogenic activity
- The strength of the neoplastic response or lack of the response is categorized into Levels of Evidence
- These levels are meant to communicate the confidence in interpreting the carcinogenic response of the agent of interest *under the conditions of the study*

Levels of Evidence of Carcinogenic Activity

- **Clear evidence of carcinogenic activity**
  - Dose related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy
- **Some evidence of carcinogenic activity**
  - Agent related increased incidence of neoplasms in which the strength of the response is less than that required for clear evidence
- **Equivocal evidence of carcinogenic activity**
  - Marginal increase of neoplasms that may be agent related
- **No evidence of carcinogenic activity**
- **Inadequate study**

Summary

- Several elements are taken into account when designing chronic toxicity and carcinogenicity studies
- Variety of factors are used in interpreting the responses from these studies
  - Appropriate statistical analysis
  - Historical control database
- The strength of the carcinogenic response is reflected in the Levels of Evidence categories

Questions?