Carcinogen Identification Committee Meeting
Part II: Assessment of Tumor Data from Animal Carcinogenicity Studies

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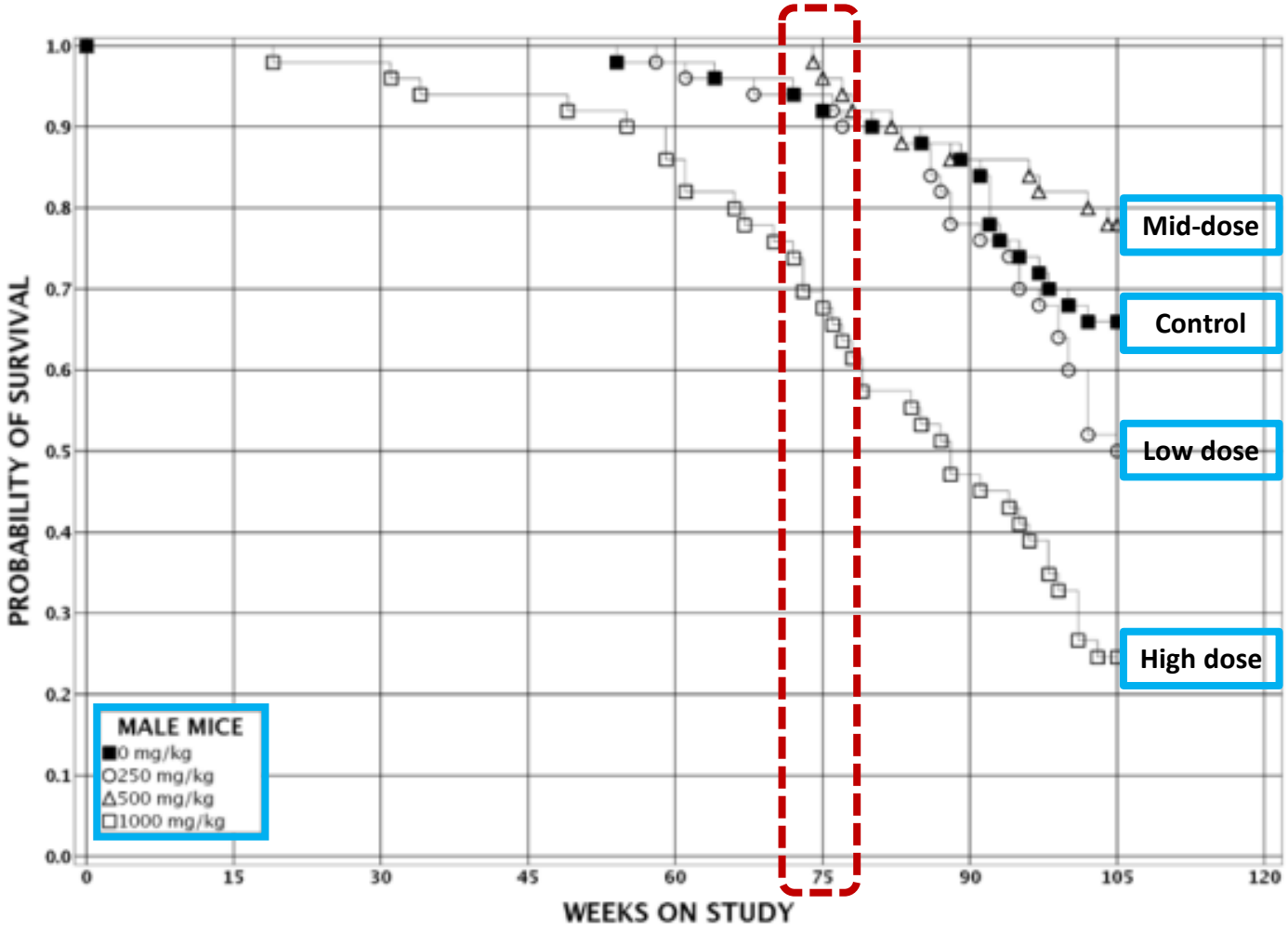
Overview

- Analysis approach for cancer bioassay data
- Using effective number to present animal tumor incidences
- Statistical methods in assessing significant tumor incidences
- Multiple comparisons
- Brief Q&A break
- Consideration of controls
- Assessment of rare tumors
- Brief Q&A break

Analysis and presentation of cancer bioassay data

- Study design considerations
- Increases in tumor incidence: reported and/or apparent
- Time of occurrence of tumors to capture animals at risk
- Individual animal survival data



Effective number

- When detailed information is available, incidence is calculated and reported by OEHHA using effective number of animals in the denominator

$$\text{Incidence} = \frac{\text{Number of tumor–bearing animals}}{\text{Number of animals alive at first occurrence and examined at the site}}$$

- When this information is not provided, denominators may be reported differently
  - Number of animals examined at the site, if reported by study authors
  - Total number of animals in the treatment group
- This definition is consistent with the IARC Preamble: "…the effective number of animals (alive at the time that the first tumour was discovered)…"

Assessing dose-response significance

- One-sided Fisher's exact test for pairwise comparisons
- Exact conditional Cochran-Armitage test for linear trend
  - The test originally derived by Cochran and Armitage relies on a Normal approximation
    - Performs well for large and balanced sample sizes
  - Williams (1988) demonstrated that using the exact conditional distribution of the test statistic improves the accuracy of the test
  - The algorithm used to derive the exact p-value is described in Mehta et al (1992)

Multiple Comparisons

- OEHHA performs tests for tumor sites where an increase is apparent
  - Typical NTP Technical Report: 4 experiments × 30 sites × 4 tests = 480 statistical tests ✗
- Problem of multiple comparisons in data analysis
  - Many simultaneous inferences
- Multiple testing and animal cancer bioassay data
  - Low spontaneous frequency for most tumor types (Haseman 1983)
  - Other considerations when assessing carcinogenicity (Haseman 1983)
  - Lack of evidence for substantial false positive problem (Rusyn et al. 2020)
- OEHHA summarizes the available data and relies on the CIC members to provide their expert opinions

Effective Number Example

Table 8 Incidence of treatment-related tumors in male B6C3F$_1$ mice administered coumarin via gavage 5 days/week for 103 weeks (NTP 1993)

Tumor site	Tumor type	Day of first tumor	Gavage dose (mg/kg)				Trend test p-value
			0	50	100	200	
Lung	Alveolar/bronchiolar adenoma	558	14/48	8/49	14/46	24/45*	$p = 0.001$
	Alveolar/bronchiolar carcinoma	716	1/45	1/47	2/43	1/37	*NS*
	Combined adenoma and carcinoma	558	14/48	9/49	15/46	25/45**	$p < 0.001$

Tumor incidence is expressed as the number of tumor-bearing animals over the number of animals alive at the time of first occurrence of the tumor and examined at the site.

Treatment group tumor incidence with asterisks indicates significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$, ** $p < 0.01$. Exact trend test conducted by OEHHA.

Q&A Break

Consideration of controls

- Concurrent controls
  - "The **concurrent control** group is generally the most appropriate comparison group for statistical analysis" (IARC Preamble)
  - US EPA, FDA, and NTP agree with IARC regarding concurrent controls

Application of historical control data

- Historical control data -Tumor incidence observed in control animals of a given species/strain/sex in previous studies
  - Useful to determine tumor types that are rare in untreated animals of a given species/strain/sex (NTP, IARC[a], US EPA, FDA)
    - Rare tumors – defined as those with incidence rates of less than 1% in untreated animals (Haseman 1983)
  - Provides context when assessing biological significance of rare tumors observed in treatment groups

[a]IARC 2019 preamble referred to these tumors as uncommon.

Appropriate historical control data

Closely resemble concurrent controls in terms of animals, animal care and environment, time period, etc.

- Factors specifically mentioned in the IARC Preamble:
  - Species
  - Sex
  - Strain
  - Basal diet
  - General laboratory environment
- Factors specifically mentioned by US EPA (2005):
  - Same laboratory
  - Same supplier
  - Data gathered within 2 or 3 years one way or the other of concurrent controls
- Additional considerations (e.g., NTP)
  - Route of administration
  - Length of experiment

Rare tumors

- Example from HID on Nitrapyrin (OEHHA 2015)
  - Two-year dietary study in male B6C3F1 mice conducted from 1994 to 1996 (Stebbins and Cosse, 1997)
    - Observations of 3 forestomach squamous cell carcinomas in the high-dose group
  - No *laboratory* historical control data available
  - Historical control data available from *NTP studies* conducted in the same strain of male mice during the early 1990s (1990 – 1996) (Haseman et al. 1998)

Tumor site	**Tumor type**	**Administered dose in feed (mg/kg/day)**			**Trend test P-value**	**Historical control** Haseman et al. (1998)
		0	125	250		
Forestomach	Squamous cell carcinoma (r)	0/43	0/49	3/38 (7.9%)	<0.05	2/1355 (0.1%)

Q&A Break