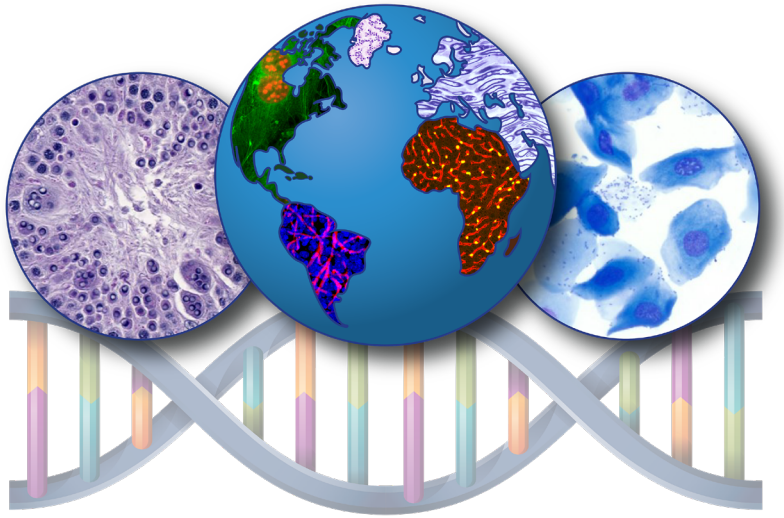
Pathology Data Interpretation of Carcinogenicity Studies at the Division of Translational Toxicology



Division of Translational Toxicology Global Toxicologic Pathology Training Program

National Institutes of Health • U.S. Department of Health and Human Services

The National Toxicology Program (NTP) – Background

- The NTP plays a role in providing the scientific basis for programs, activities, and policies that promote health or lead to the prevention of disease
- Founded in 1978, the NTP has been instrumental in generating, interpreting, and sharing toxicologic information about potentially hazardous substances in our environment
- The NTP is an interagency partnership of the Food and Drug Administration, National Institute for Occupational Safety and Health, and National Institute of Environmental Health Sciences (NIEHS)
- The NTP has evaluated almost 3,000 environmental agents for potential human health effects

NTP – Links for Further Information

- Mission & Goals
- History & Milestones
- Organization

Division of Translational Toxicology (DTT)

- An intramural division at the NIEHS (formerly known as the Division of the National Toxicology Program)
- Mission: to improve public health through data and knowledge development that are translatable, predictive, and timely
- Much of the work carried out by the DTT is in support of the NTP, including toxicology testing and contributions to various publications (https://ntp.niehs.nih.gov/publications/index.html)
- Important roles of the DTT pathologists include: directing, managing, evaluating, and interpreting all pathology data generated during the conduct of DTT toxicity and carcinogenicity studies (previously referred to as NTP studies)

DTT Carcinogenicity Studies Introduction

DTT Carcinogenicity Studies

- Carcinogenicity studies generally involve exposing laboratory animals (rats and mice) to a substance for a period of two years
- Studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected substances
- Substances selected for DTT toxicology and carcinogenesis studies are chosen primarily on the basis of human exposure and chemical structure
- Substance selection is not an indicator of its carcinogenic potential

Common Study Design for Carcinogenicity Studies

- Routinely consists of 50 rats or mice per dose group
- Generally, the dose groups are vehicle controls (receive the vehicle the test article is mixed with), low dose, mid dose, and high dose of the test article (200 males and 200 females per animal species)
- The test article can be given to the animal in various ways (route of administration):
  - Drinking water
  - Feed
  - Gavage
  - Inhalation (nose-only cone or whole-body chamber)
- After two years of being on a study, animals are humanely terminated
- Routine tissues are collected and processed to glass slides for microscopic evaluation

Lesions Diagnosed in Carcinogenicity Studies

- Non-neoplastic lesions
- Pre-neoplastic lesions
- Neoplastic lesions
  - Origin of neoplastic cells (i.e., epithelial or mesenchymal)
  - Benign (not cancerous)
  - Malignant (cancerous)
- Metastatic lesions – secondary location(s) of malignant tumors that have spread from the primary site (i.e., liver cancer that has spread to the lungs; the lung tumors are the metastatic lesions)

Key Factors for Evaluating Carcinogenicity Pathology Data

- Adequacy of experimental design and study conduct
- Occurrence of common versus uncommon neoplasia
- Progression (or lack thereof) from benign to malignant neoplasia and/or preneoplastic to neoplastic lesions
    - Neoplasia progression: normal ⇒ hyperplasia ⇒ benign neoplasia ⇒ malignant neoplasia
- Combining benign and malignant tumor incidences from the same origin/histogenesis known or thought to represent stages of progression in the same organ
- Multiplicity in site-specific neoplasia (more than one of the same tumors occurring in a particular site)
- Metastases

Key Factors for Evaluating Carcinogenicity Pathology Data (continued)

- Supporting information from hyperplastic lesions in same site of neoplasia or in other studies (same lesion in another sex or species)
- Presence/absence of dose response
- Statistically significant increase ($p \leq 0.05$) in observed neoplasm from treated group(s) compared to concurrent control group
- Concurrent control tumor incidence and historical control data (HCD)/variability for a specific neoplasm
- Survival-adjusted analyses
- Other (toxicokinetic data; genetic toxicology)

DTT Historical Control Database (HCD)

- DTT has gathered significant HCD on chronic toxicity and carcinogenicity studies
- Done to evaluate study results and to follow changes in the biology of test species
- Compiles carcinogenicity information about control animals that have not received treatment
- Generally, includes incidences of all tumor types in the most recent DTT carcinogenicity studies within a five-year window

DTT Carcinogenicity HCD

- For meaningful comparisons, the conditions for studies in the HCD must be generally similar
- Due to a variety of factors that can influence response, HCD may be identified by:
  - Species
  - Sex
  - Route of Administration
  - Vehicle
  - Study Type
- Is most useful for determining whether
  - Uncommon/rare tumors are biologically significant
  - Common neoplasms are biologically insignificant

DTT Carcinogenicity HCD

- Tumor incidence is defined as the number of animals exhibiting a tumor type divided by the number of animals examined and is expressed as both raw counts and percent
- Present the mean and standard deviation of tumor incidence among studies, along with the number of studies summarized
- The DTT Carcinogenicity HCD for mice and rats is located online

Select Factors for Evaluating Carcinogenicity Pathology Data

Combining Tumors

Appropriate Combinations of Neoplasms

- Combine neoplasms of differing origin/histogenesis?
  - Liver – hepatocellular carcinoma (epithelial origin) and Liver – hemangiosarcoma (mesenchymal origin)
  - Lung – alveolar/bronchiolar carcinoma (epithelial origin) and Lung – fibrosarcoma (mesenchymal origin)

NO

Appropriate Combinations of Neoplasms

- Combine neoplasms of similar origin/histogenesis?
  - Liver – hemangiosarcoma (malignant endothelial) and Heart – hemangiosarcoma (malignant endothelial)
  - Liver – hepatocellular adenoma (benign epithelial) and Liver – hepatocellular carcinoma (malignant epithelial)

YES

Select Factors for Evaluating Carcinogenicity Pathology Data

Using HCD

HCD: Male Mice Treated with Sodium Tungstate Dihydrate in Drinking Water (Technical Report 599)

	Control	Low Dose	Mid Dose	High Dose
Kidney – Renal tubule adenoma	0/50 (0%)	0/50 (0%)	1/50 (2%)	0/50 (0%)
HCD – Renal tubule adenoma	2/50 (4%)			

The mean renal tubule adenoma percentage in the mid dose sodium tungstate dihydrate group (2%) is within the historical control mean of this lesion in male mice from drinking water studies (4%), and this would be considered in determining if sodium tungstate dihydrate is carcinogenic.

HCD: Male Mice Treated with Antimony Trioxide via Inhalation (Technical Report 590)

	Control	Low Dose	Mid Dose	High Dose
Lung – Alveolar/Bronchiolar (A/B) carcinoma	4/50 (8%)	18/50 (36%)	20/50 (40%)	27/50 (54%)
HCD – A/B carcinoma	33/250 (13.2%)			

The mean historical control database (HCD) percentage of Alveolar/Bronchiolar (A/B) carcinoma is 13.2% in male mice from inhalation studies and the percentages of this tumor in the three antimony trioxide treatment groups in male mice is well above the historical control mean; therefore, this would be considered in determining if antimony trioxide is carcinogenic.

Other Select Factors for Evaluating Carcinogenicity Pathology Data

Female Mice Treated with Pentabromodiphenyl Ether Mixture via Gavage (Technical Report 589)

	Control	Low Dose	Mid Dose	High Dose
Liver – Eosinophilic Focus (Preneoplastic)	3/50 (6%)	2/49 (4%)	16/50 (32%)	15/49 (31%)
Hepatocellular Adenoma (Neoplastic)	5/50 (10%)	7/49 (14%)	32/50 (64%)	46/49 (94%)

Progression of a Proliferative Lesion

There are increased preneoplastic and neoplastic lesions in the liver of female mice, notably in the mid dose and high dose Pentabromodiphenyl Ether Mixture groups. This progression is crucial when evaluating carcinogenicity studies.

Male and Female Mice Treated with Cobalt Metal via Inhalation (Technical Report 581)

	Control	Low Dose	Mid Dose	High Dose
Males: Lung – A/B Carcinoma	11/50 (22%)	38/49 (78%)	42/50 (84%)	46/50 (92%)
Females: Lung – A/B Carcinoma	5/49 (10%)	25/50 (50%)	38/50 (76%)	43/50 (86%)

Dose Relationship

There are increased incidences of A/B Carcinoma in both male and female mice in the low-, mid-, and high-dose Cobalt Metal groups when compared to the respective concurrent control groups. The tumor incidences increase with increasing dose. Dose-response relationships are critical in predicting carcinogenicity.

Female Mice Treated with β-Picoline in Drinking Water (Technical Report 580)

	Control	Low Dose	Mid Dose	High Dose
Liver – Hepatocellular Carcinoma	11/49 (22%)	20/50 (40%)	26/50 (52%)	23/50 (46%)
Lung – Hepatocellular Carcinoma, Metastatic	1/50 (2%)	5/50 (10%)	8/49 (16%)	4/49 (8%)

Metastases

Metastatic hepatocellular carcinomas are observed at a greater frequency in the lungs of all groups of females exposed to β-picoline. Metastatic lesions are a factor in predicting carcinogenicity.

Male and Female Mice Treated with β-Picoline in Drinking Water (Technical Report 580)

	Control	Low Dose	Mid Dose	High Dose
Males: Lung – A/B Carcinoma, Multiple	0/50 (0%)	2/50 (4%)	0/50 (0%)	3/50 (6%)
Females: Lung – A/B Carcinoma, Multiple	0/50 (0%)	2/50 (4%)	2/49 (4%)	4/50 (8%)

Multiplicity in Site-specific Neoplasia

Incidences of A/B Carcinoma, Multiple occur in most male and female β-picoline exposed mice. Multiplicity of neoplasms is important in determining carcinogenicity.

Levels of Carcinogenicity Evidence

Levels of Evidence (LoE) of Carcinogenic Activity

- Clear Evidence (CE) = showing a 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 (SE) = showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence
- Equivocal Evidence (EE) = showing a marginal increase of neoplasms that may be chemically related
- No Evidence (NE) = showing no chemical-related increases in malignant or benign neoplasms
- Inadequate Study (IS) = because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity
- For explanations of LoE of carcinogenic activity: https://ntp.niehs.nih.gov/ntp/test_info/cartox_loe_508.pdf

Positive Results (Clear Evidence and Some Evidence)

- Clear Evidence and Some Evidence means a chemical or agent is carcinogenic in laboratory animals under the conditions of the study
  - Indicate that exposure to the chemical has the potential for hazard to humans
  - Clear Evidence and Some Evidence are considered “positive” results
  - In a study with Clear Evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed Some Evidence are indicated as "were also related" to chemical exposure
- Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence
  - This can include animal studies such as those conducted by the DTT, epidemiologic studies, and estimates of exposure
- The actual determination of risk to humans from chemicals found to be carcinogenic (Clear Evidence and Some Evidence) in laboratory animals requires a wider analysis

Equivocal Evidence

- Equivocal Evidence of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may or may not be chemically related
- In studies with Clear Evidence or Some Evidence of carcinogenic activity, other responses that alone might be termed Equivocal Evidence are indicated as "may have been" related to chemical exposure

Select Examples of LoE for DTT Studies

Clear Evidence of Carcinogenic Activity

- **Clear Evidence (CE)** – a dose related increase in:
  a) malignant neoplasms
  b) benign and malignant neoplasms
  c) marked increase in benign neoplasms that have the ability to progress

Pentabromodiphenyl Ether Mixture – CE in Female Mice (Technical Report 589)

	Control	Low Dose	Mid Dose	High Dose
Liver – Hepatocellular adenoma (benign)	5/50 (10%)	7/49 (14%)	32/50 (64%)	46/49 (94%)
Liver – Hepatocellular carcinoma (malignant)	4/50 (8%)	2/49 (4%)	6/50 (12%)	27/49 (55%)
Combined (most severe lesion counted if animal had both)	**8/50 (16%)**	**8/49 (16%)**	**33/50 (66%)**	**47/49 (96%)**

Some Evidence of Carcinogenicity

- **Some Evidence (SE)** – an increase of
  - a) benign neoplasms or
  - b) malignant neoplasms or
  - c) both benign and malignant neoplasms in which the strength of the response is less than that required for CE

Ethylbenzene – SE in Male Mouse (Technical Report 466)

	Control	Low Dose	Mid Dose	High Dose
Lung – Alveolar/Bronchiolar (A/B) adenoma (benign)	5/50 (10%)	9/50 (18%)	10/50 (20%)	16/50 (32%)
Lung – A/B carcinoma (malignant)	2/50 (4%)	1/50 (2%)	5/50 (10%)	3/50 (6%)
Combined (most severe lesion counted if animal had both)	**7/50 (14%)**	**10/50 (20%)**	**15/50 (30%)**	**19/50 (38%)**

Equivocal Evidence of Carcinogenicity

- **Equivocal Evidence (EE)** – a marginal increase of neoplasms that may be chemically related

Molybdenum Trioxide – EE in Male Rats (Technical Report 462)

	Control	Low Dose	Mid Dose	High Dose
Lung – A/B adenoma (benign)	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Lung – A/B carcinoma (malignant)	0/50 (0%)	1/50 (2%)	1/50 (2%)	1/50 (2%)
Combined (most severe lesion counted if animal had both)	**0/50 (0%)**	**1/50 (2%)**	**1/50 (2%)**	**4/50 (8%)**

No Evidence of Carcinogenicity

- **No Evidence (NE)** – no chemically related increases in malignant or benign neoplasms

Dietary Zinc – NE in Female Rats (Technical Report 592)

There were no neoplastic effects in female rats, therefore, the call of NE of carcinogenicity.

Inadequate Study

- **Inadequate Study (IS)** – because of major limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity

Triethanolamine – IS in Male and Female Mice (Technical Report 449)

The presence of a *Helicobacter hepaticus* infection complicated interpretation of the relationship between triethanolamine administration and liver neoplasms.

Summary of Pathology Data Interpretation from Carcinogenicity Studies

- Findings within an individual study dictate the most accurate interpretation
- Use of historical control data is important in interpretation
- Key factors in evaluating carcinogenicity pathology (i.e., tumor progression, tumor combining, metastases, dose relationships, etc.) are crucial in interpreting pathology results
- Pathology data plays a huge role in determining the level of evidence of carcinogenicity for DTT studies

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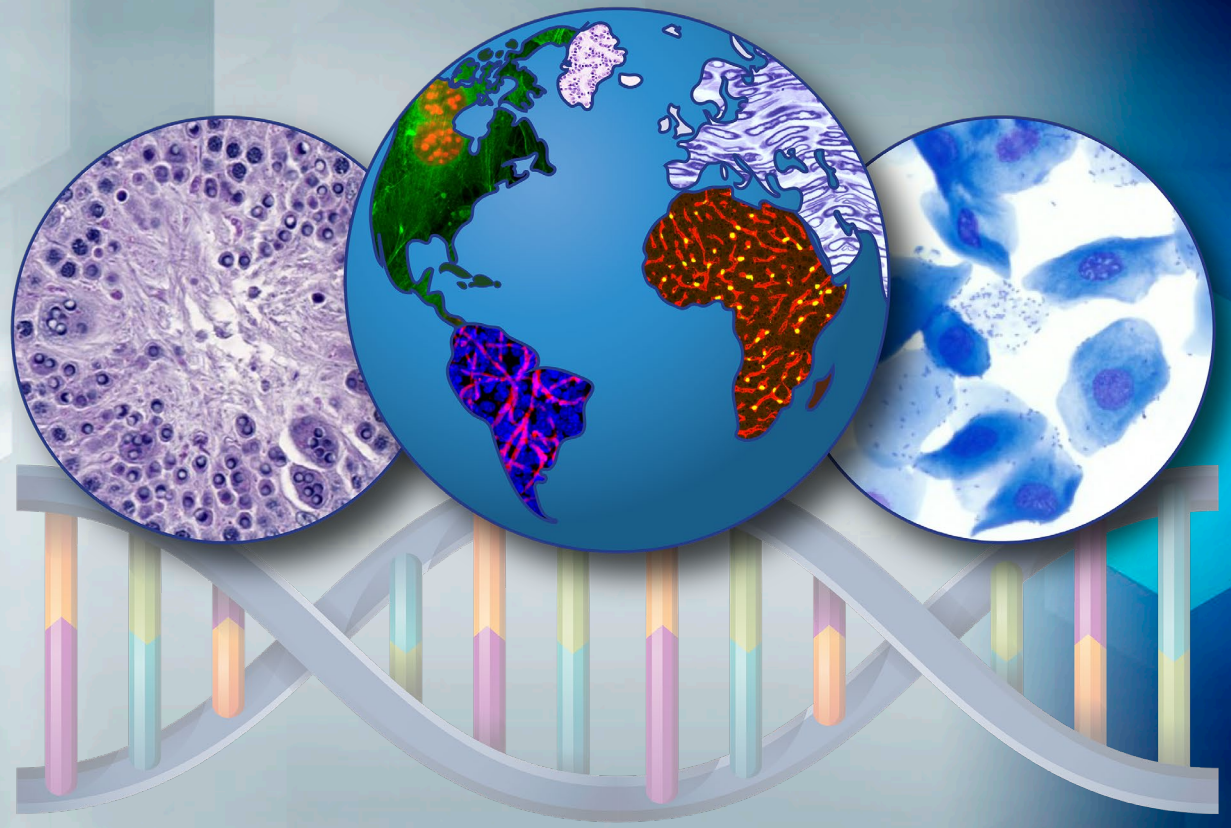

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