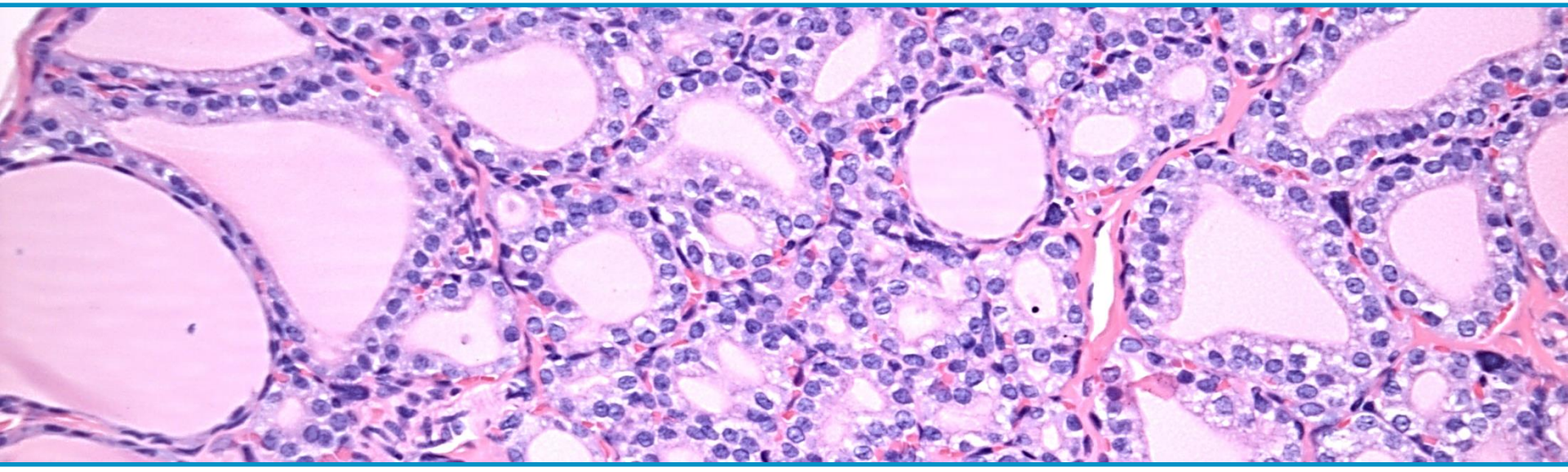
TOX'2019

Magyar Toxikológusok Társasága

Tudományos konferencia

Szeged, Október 09.-11.

Genotoxic, Equivocal or Non-genotoxic:
Evaluation and Interpretation of Carcinogenicity Studies from the View-point of a Toxicologic Pathologist



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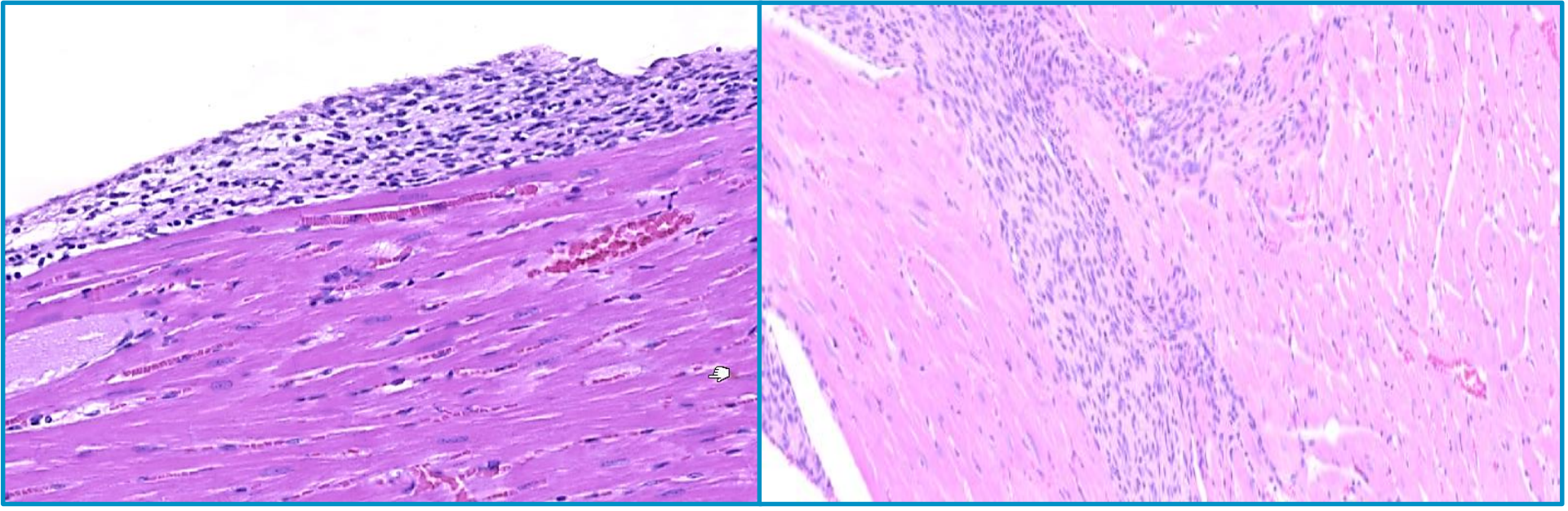
Disclaimer

All statements given in this presentation are the result of my continuous learning and my personal experience
(but maybe also my misinterpretation !)

They thus, do not necessarily reflect the opinion of my former employer or any regulatory agency

Furthermore, I received no financial support or grants for the authorship and preparation of this presentation
(except paying no registration fee)



Agenda

- Introduction / Definitions
- Why and when are Carcinogenicity Studies needed ?
- How are Carcinogenicity Studies performed ?
- How are Carcinogenicity Studies reported ?
- Alternatives to Conventional Carcinogenicity Studies
- Interpretation of Results (Example)
- Summary/Conclusion

Introduction

- Genotoxicity:
  The ability of an agent to damage or alter the genetic information (DNA)
- Carcinogenicity:
  The ability of a carcinogen to cause cancer, either directly or indirectly

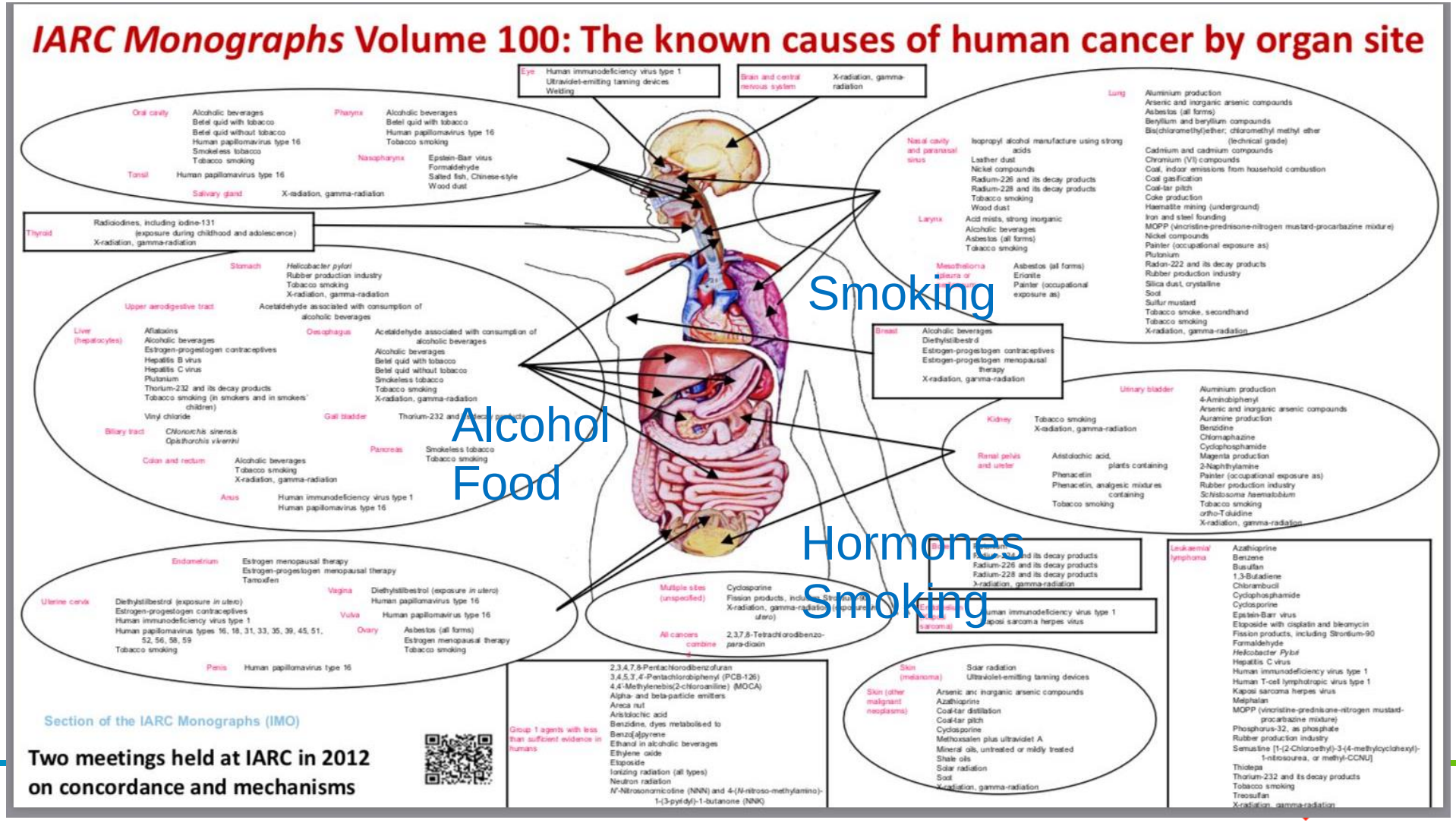
  A carcinogen is an agent whose administration to animals leads to a statistically significant increased incidence of **neoplasms** compared to untreated controls

  A **neoplasm** is a heritably altered, relatively autonomous growth of tissue
- Carcinogenicity can be a result of a genotoxic insult, but can also be induced by non-genotoxic mechanisms

Introduction

- Worldwide more than **3.000** chemical compounds in use
- About **1.700** have a full set of data in long term studies
- Many of the compounds are cancerogenic at or near to MTD
  - **Hazard Identification:**
  - Epidemiology: Low Exposure (General Community)
High Exposure (Production)
  - Life Time Rodent Bioassay
  - Results from Short Time Assays
  - Chemical Structure
  - **Risk Characterization:** Knowledge about potency
  - **Risk Reduction:** Information, Replacement

Human Carcinogens



Introduction: IARC Classification

IARC Monographs 100 (2012): Review of Human Carcinogens (Package of 6 Volumes: A,B,C,D,E,F)

- Pharmaceuticals (including hormons for contraception)
- Biological Agents (such as Epstein Barr Virus or Helicobacter Pylori)
- Arsenic, Metals, Fibers, and Dusts
- Radiation
- Personal Habits and Indoor Combustions
- Chemical Agents and Related Occupations

IARC Monographs 102 (2013): Non-ionizing Radiation, Part 2: Radiofrequency Electromagnetic Fields

IARC Monographs 112 (2017): Some organophosphate insecticides and herbicides (including Glyphosate)

Introduction: Key Characteristics of HC

Key Characteristics of Human Carcinogens	Examples of relevant evidence
Is electrophilic or can be metabolically activated	Parent compound or metabolite with an electrophilic structure (e.g. epoxide, quinone, etc.) formation of DNA and protein adducts
Is genotoxic	DNA damage (DNA-strand breaks, - protein cross-links, - unscheduled synthesis), intercalation, gene mutations, or cytogenetic changes such as chromosome aberration or micronucleus formation
Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g topoisomerase II, base excision or double-strand break repair.
Induces epigenetic alterations	DNA methylation, histone modification, micro RNA expression
Induces oxidative stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules such as DNA or lipids
Induces chronic inflammation	Continuously elevated wbc, myeloperoxidase activity, altered cytokine and chemokine production
Is immunosuppressive	Decreased i-surveillance or IS-dysfunction
Modulates receptor-mediated effects	In/activation of receptors (e.g. AhR, ER, PPAR) or modulation of endogenous ligands (including hormones)
Causes immortalization of cells	Inhibition of senescence, cell transformation, or altered telomeres
Alters cell proliferation, cell death, or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cycle control, or angiogenesis.

IARC Classification

Group 1: Chemicals that should be detected as positive in *in vitro* mammalian cell genotoxicity tests. Chemicals in this group are all *in vivo* genotoxins at one or more endpoints, either due to DNA-reactive or non-DNA-reactive mechanisms. Many are known with a mutagenic MoA, but a subclass of probable aneugens has been introduced.

Group 2: Chemicals that should give negative results in *in vitro* mammalian cell genotoxicity tests. Chemicals in this group are usually negative *in vivo* and non-DNA-reactive. They are either non-carcinogenic or rodent carcinogens with a non-mutagenic MoA.

Group 3: Chemicals that should give negative results in *in vitro* mammalian cell genotoxicity tests, but have been reported to induce gene mutations in mouse lymphoma cells, chromosomal aberrations or micronuclei, often at high concentrations or at high levels of cytotoxicity. Chemicals in this group are generally negative *in vivo* and negative in the Ames test. They are either non-carcinogenic or rodent carcinogens with an accepted non-mutagenic mode of action. This group contains comments as to any conditions that can be identified under which misleading positive results are likely to occur.

Introduction

Genotoxic damage ⇒ repair or cell death

Initiation ⇒ elimination of genotoxically damaged or initiated cells

(Mutation, of oncogenes – *ras, myc, sis, HER2* –
or tumorsuppressor-genes – p53, wt1, BRCA1,2, APC)

Promotion (growth related genes)

Progression ⇒ elimination of cells progressed in malignancy

Invasion and Metastasis ⇒ Elimination of healthy cells

Introduction

- Genotoxic Agents:
  - Polycyclic Aromatic Hydrocarbons (DNA-adducts)
  - Aromatic Amines (Arylamines)
  - Nitrosamines
  - Acrylamid
  - Anorganica (metals, fibers like asbestos, etc.)
  - Aflatoxin1B, Ochratoxin A (DNA-adducts)
  - ROS (Base modifications, DNA-strand breaks)

- Compound-induced DNA damage:
  - DNA crosslinking: Aldehydes, Cisplatin, Mitomycin
  - DNA intercalation: Daunomycin,
  - Used as therapeutic principle: metallo-protein-intercalators

Introduction

Genotoxic DNA-damage (covered by Genotox battery; ICH-S2)

- Ames test
- Mammalian cell assay
- *in vivo* assay on chromosome aberration

➡ Presumption: Any genotoxicant will be carcinogenic unless proven otherwise

Question for the Future: (addressed at a meeting of the IARC WG)

If a chemical (whatever nature) possesses multiple key characteristics, can we classify it as a possible/probably human carcinogen without any animal bioassay or epidemiological data ?

Introduction

So what about

Equivocal or Non-genotoxic compounds:

- Tumor promoters (1,4-dichlorobenzene),
- Endocrine-modifiers (17 beta-estradiol)
- Receptor-mediators (2,3,7,8-tetrachlorodibenzo-p-dioxin),
- Immunosuppressants (cyclosporine) or
- Inducers of tissue-specific toxicity and inflammatory responses (metals such as arsenic and beryllium).

⇨ 2-year studies in rodents at high exposure (MTD or Multiple of AuC)

Carcinogenicity testing:

What is tested?

- Agrochemicals
- Chemicals
- Food additives
- Veterinary pharmaceuticals
- Human pharmaceuticals for long term use
- …

Carcinogenicity – Cancerogenicity – Oncogenicity studies ????

… and Guidelines, guidelines, guidelines…

Chemicals: OECD Guideline (most recent)

https://read.oecd-ilibrary.org/environment/test-no-451-carcinogenicity-studies_9789264071186-en

OECD/OCDE 451

Adopted: 25 June 2018

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Carcinogenicity Studies

…This Test Guideline is designed to be used in the testing of a broad range of chemicals, including pesticides and industrial chemicals. It should be noted however that some details and requirements may differ for **pharmaceuticals** (see International Conference on Harmonisation (ICH) Guidance S1B on Testing for Carcinogenicity of Pharmaceuticals).

Agrochemicals:
US Environmental Protection Agency

United States Environmental Protection Agency | Prevention, Pesticides and Toxic Substances (7101) | EPA 712–C–98–211 August 1998

EPA

Health Effects Test Guidelines
OPPTS 870.4200
Carcinogenicity

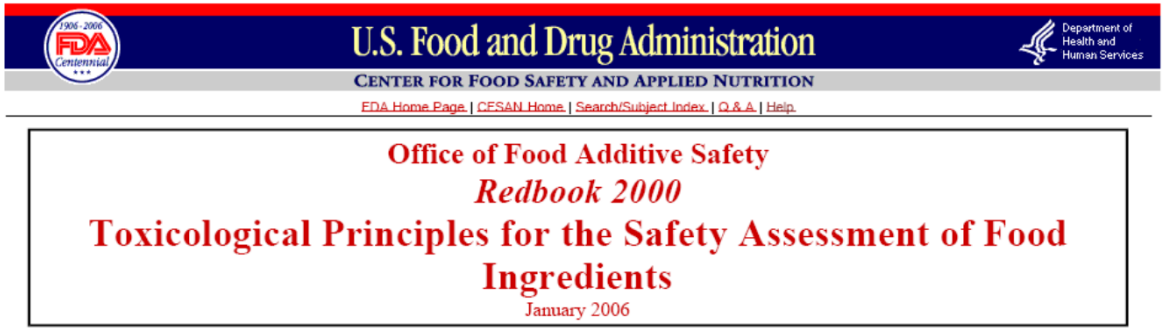
United States Environmental Protection Agency | Prevention, Pesticides and Toxic Substances (7101) | EPA 712–C–98–212 August 1998

EPA

Health Effects Test Guidelines

OPPTS 870.4300
Combined Chronic Toxicity/Carcinogenicity

Animal Products: The « US FDA Redbook »



U.S. Food and Drug Administration

Department of Health and Human Services

CENTER FOR FOOD SAFETY AND APPLIED NUTRITION

FDA Home Page | CFSAN Home | Search/Subject Index | Q & A | Help

Office of Food Additive Safety

Redbook 2000

Toxicological Principles for the Safety Assessment of Food Ingredients

January 2006

IV.C.6. Carcinogenicity Studies with Rodents

e.g. “Delaney Clause” (i.e. Food Additives Amendment of 1958):

“the Secretary of the FDA shall not approve for ***use in food*** *any chemical additive found to induce cancer in man,* ***or, after tests, found to induce cancer in animals****”*

e.g. Male Rat Kidney: D-limonene
Male Rat Urinary Bladder: Saccharin
Female Uterus: Toltrazuril (Coccidiostatic)

https://www.fda.gov/downloads/food/guidanceregulation/ucm222779.pdf

Pharmaceuticals: EMA / ICH

S1A Guideline on the need for carcinogenicity studies of pharmaceuticals (CPMP/ICH/140/95)

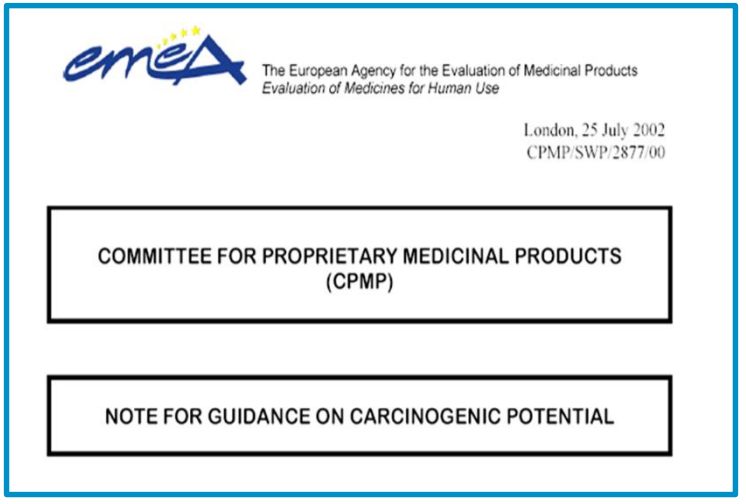
S1B Carcinogenicity: testing for carcinogenicity of pharmaceuticals (CPMP/ICH/299/95)

S1C Carcinogenicity: dose selection for carcinogenicity studies of pharmaceuticals (CPMP/ICH/383/95)

S1C(R) Addendum to 'dose selection for carcinogenicity studies of pharmaceuticals': addition of a limit dose and related notes of pharmaceuticals (CPMP/ICH/366/95)

S1(R1) Rodent Carcinogenicity Studies for Pharmaceuticals – Position Paper

- Revision of S1A-C
- More integrated


emea
The European Agency for the Evaluation of Medicinal Products
Evaluation of Medicines for Human Use

London, 25 July 2002
CPMP/SWP/2877/00

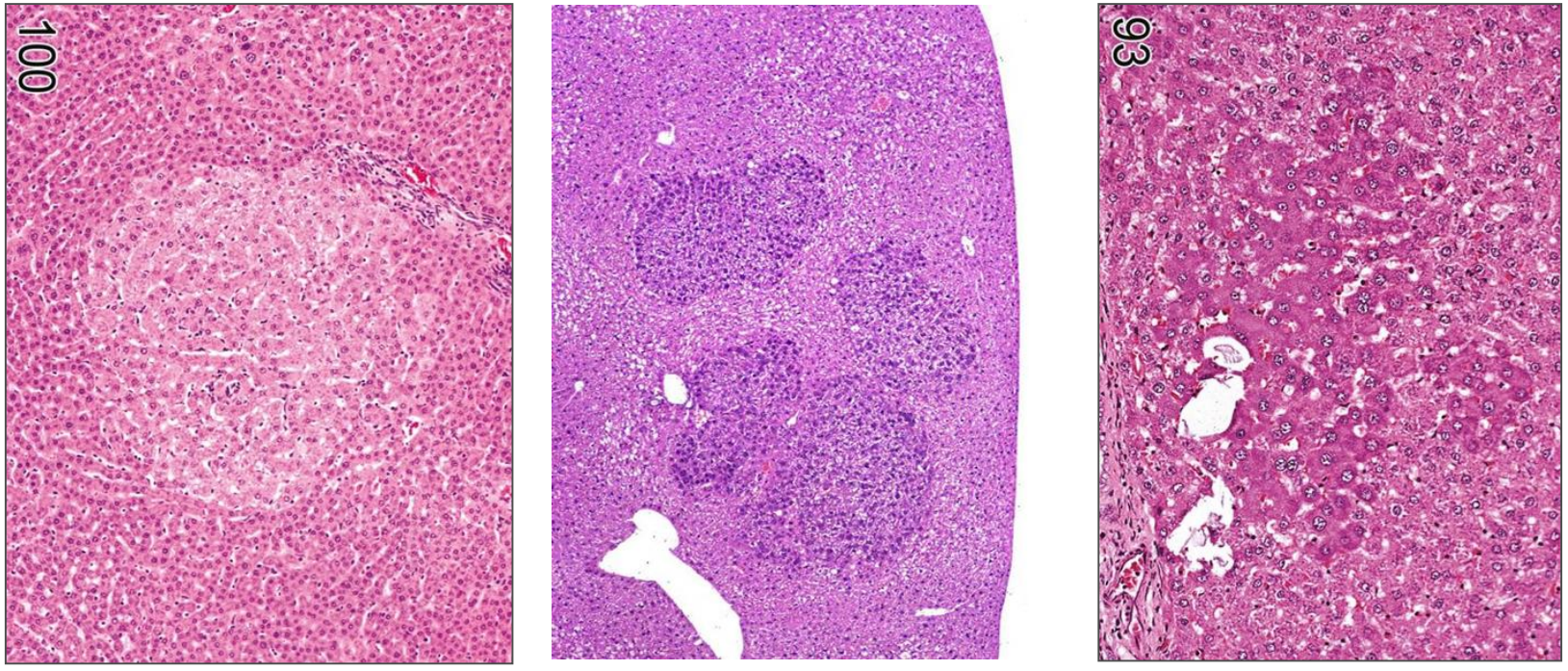
COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS (CPMP)

NOTE FOR GUIDANCE ON CARCINOGENIC POTENTIAL

ICH / S1A
When are carcinogenicity studies needed?

- Continuous use of the drug for at least 6 (3) months or longer
- Repeatedly used in an intermittent manner (e.g. allergic rhinitis, depression, and anxiety)
- Concern from positive genotoxicity findings
- Concern from short-term carcinogenicity tests (e.g. 6 month study)
- Concern from exposure levels in specific organs
- Cause for concern, e.g.
  - carcinogenic potential in product class (hormones)
  - structure-activity relationship suggesting risk
  - preneoplastic lesions in repeat-dose toxicity studies
  - (e.g. increased No. of Foci of Hepatocellular Alterations)
  - drug retention in tissue

When are carcinogenicity studies needed? Expl.: Cause of Concern



Examples of preneoplastic foci of hepatocellular alteration

When are carcinogenicity studies needed?

Carcinogenicity studies conducted in rats and mice are another important source of information about the effects of chronically used drugs.

Although the **goal** of these studies is to assess the drugs' **carcinogenic potential**, organ changes assessed by histopathology also give **information** about **potential endocrine effects**. For example, persistent disruption of the hypothalamic-pituitary adrenal/gonadal/thyroidal axis(es) can result in various neoplasms.

Histologic, organ weight, clinical chemistry, or hematologic data evaluated together can form ***recognized patterns of changes*** associated with hormonal effects that precede the neoplasm. Often the available data are sufficient to identify the hormonal axes involved.

Nonclinical Evaluation of Endocrine-Related Drug Toxicity

Guidance for Industry (Sept. 2015)

When are carcinogenicity tests NOT necessary?

- Infrequent use or short term duration
  (e.g. anesthetics, radio-labelled imaging agents)
- Unequivocally genotoxic compounds (e.g. Quinolones)
  (due to assumption of trans-species carcinogens)
  (➔ consider conducting 1y chronic toxicity, if use is less than 1 year)
- Limited life-expectancy (e.g. 2-3 years)
  (e.g. for oncolytic or anticancerous agents)
  but if used for non-cancer indications ➔ testing is needed

- Topically applied drugs with poor systemic exposure

Antibiotics Carcinogenicity testing?

NO

- If only short-term exposure (10 days)
- if < 3 months, accumulated

YES

- if long-term exposure
- if often repeated intermittently
- less severe indications

ICH / S1B Testing for Carcinogenicity

- **Basic principle**
  - 1 long-time Carcinogenicity Study (2 yrs)
  - 1 other study type
  - Initiation-promotion assay
  - *Transgenic or neonatal rodent models*
  - 2-yr CS in 2nd species accepted
  - If genotox or 1 Ca Study indicate a carcinogenic potential, an additional study is not needed
- **Species selection**
  - Pharmacology, repeat dose toxicity
  - Metabolism, TK
  - Route of administration
  - Rat is 1st choice if no striking evidence for other species

ICH / S1B Testing for Carcinogenicity

- **Alternative models**
  - Initiation-promotion model for hepato-carcinogens
  - Transgenic mouse models
    (based on ILSI/HESI ACT experiences in the 1990ties)
    - e.g. p53+/- deficient model, Tg.AC model, TgHras2 model, XPA deficient model, Neonatal rodent tumorigenicity model
    - Selection based on
      - New information is not expected from 2y CS
      - Addressing existing concerns
      - Metabolism and systemic/local exposure
      - Experience with the model

ICH M3: Carcinogenicity Studies

- Completion NOT needed in advance of conduct of clinical trials unless:
  - Cause for concern (ICH: S1A)
  - Unexpected findings may lead to (partial) clinical hold !
- For pharmaceuticals to treat certain serious (“emerging”) diseases, carcinogenicity testing, if needed, may be concluded post-approval

Dose-Range-Finding Studies for Carcinogenicity Testing

- Usually:
  - 3 months for long-term studies
  - 1 month for neonatal or transgenic mice

- Range of different dose levels, frequently no. of 5
- Focus on toxicity endpoints, determination of MTD*

- Profiling of AuC
  - e.g. 1 and 3 months for rats,
  - e.g. 1 and 4 weeks for alternatives
  - transgenic = wild type

*The MTD is defined by the National Toxicology Program (NTP) as ”that dose which when given for the duration of the chronic study as the highest dose, will not shorten the treated animal’s longivity from any toxic effects other than the induction of neoplasms”

S1B: STUDY DESIGN for Carcinogenicity Studies

1. Animals

The choice of species should be appropriate, based on consideration outlined in ICH-guideline S1B.

Carcinogenicity studies should commence as soon as possible after weaning, i.e. as soon as the animals are accustomed to their diet and surroundings.

Animals should be specific pathogen free, in good general health initially and this should be maintained throughout the study. High standards of animals husbandry are essential (close monitoring!).

Environmental factors, such as humidity and light/dark cycles, as well as diet, manner of feeding, and drinking water should be specified and documented.

Typical rodent strains used for lifetime bioassays

- Rat
  - **F344**: Favored by the NTP (US) and many chemical companies for many years, but high incidence of renal disease (CPN), Leydig cell tumors and Large Granular Cell Leukemia (LGL)
  - **Sprague-Dawley**: Favored by US and Japanese pharmaceutical companies, but recent years have indicated poor survival over 2 years, food restriction recommended
  - **Wistar**: Favored by many EU pharmaceutical companies, becoming strain of choice for pharmaceuticals, NTP used it, but recently switched back to SD
- Mouse
  - **B6C3F1:** Favored by the NTP (US), but relatively high liver tumor incidence (inbred strain)
  - **CD-1:** Strain of choice in pharmaceutical industry (outbred strain)

STUDY DESIGN for Carcinogenicity Studies - cont.

2. Test substance

The characteristics of the test substance, including impurity profile stability should be clearly characterized prior to the study and documented in the final report.

3. Dosing and dose levels

normally at three dose levels, consider ICH-guidelines S1C and S1 CR.

Special care should be taken to eliminate contamination with compound under study of the control group

Preferably at regular intervals samples of diet (including control diet) should be analyzed to check the concentration and homogenicity.

STUDY DESIGN for Carcinogenicity Studies - cont.

4. Duration of studies

Rat: 24 months

Mouse: minimally 18 months (FDA 24 months)

Hamster: 18 months, better 24 months

5. Number of animals

50 (60) / sex / group

One control group with vehicle (double controls possible/recommended)

If interim sacrifices planned: increase number of animals

Survival rates: 25 / sex / group at scheduled termination

STUDY DESIGN for Carcinogenicity Studies - cont.

- Route as in clinical conditions, i.e. in feed, drinking water, by gavage, by inhalation, dermally
- Routine Monitoring
- Body weights
- Food consumption
- [Water consumption (where applicable)]
- Overt signs of toxicity
- Palpable masses
- Ophthalmoscopy

Monitoring of biochemical and hematological parameters as well as urinalysis should be considered during the study and should be performed at study termination (e.g. blood smears for differentiation of leukemias or lymphomas)

STUDY DESIGN for Carcinogenicity Studies - cont.

6. Terminal Investigations

A full autopsy should be made on all animals dying during the study or killed in extremis. Euthanasia in extremis is preferred to reduce the suffering of the animal and to prevent autolysis.

At the conclusion of the study all surviving animals should be sacrificed and a full necropsy conducted on each animal.

Previously demonstrated toxic effects may indicate particular areas for investigation.

Necropsy and organ weights

VI. Necropsy and Microscopic Examination

A. Gross Necropsy

All test animals should be subjected to complete gross necropsy, including examination of external surfaces, orifices, cranial, thoracic and abdominal cavities, carcass, and all organs. The gross necropsy should be performed by, or under the direct supervision of, a qualified pathologist, preferably the pathologist who will later perform the microscopic examination.

B. Organ Weight

Organs that should be weighed include the adrenals, brain, epididymides, heart, kidneys, liver, spleen, testes, thyroid/parathyroid, thymus if present, ovaries and uterus. Before being weighed, organs should be carefully dissected and trimmed to remove fat and other contiguous tissue. Organs should be weighed immediately after dissection to minimize the effects of drying on organ weight.

C. Preparation of Tissues for Microscopic Examination

Generally, the following tissues should be fixed in 10 % buffered formalin (or another generally recognized fixative) and sections prepared and stained with hematoxylin and eosin (or another appropriate stain) in preparation for microscopic examination. Lungs should be inflated with fixative prior to immersion in fixative.

From US FDA „redbook“

STUDY DESIGN for Carcinogenicity Studies - cont.

Histopathology from carcinogenicity studies:

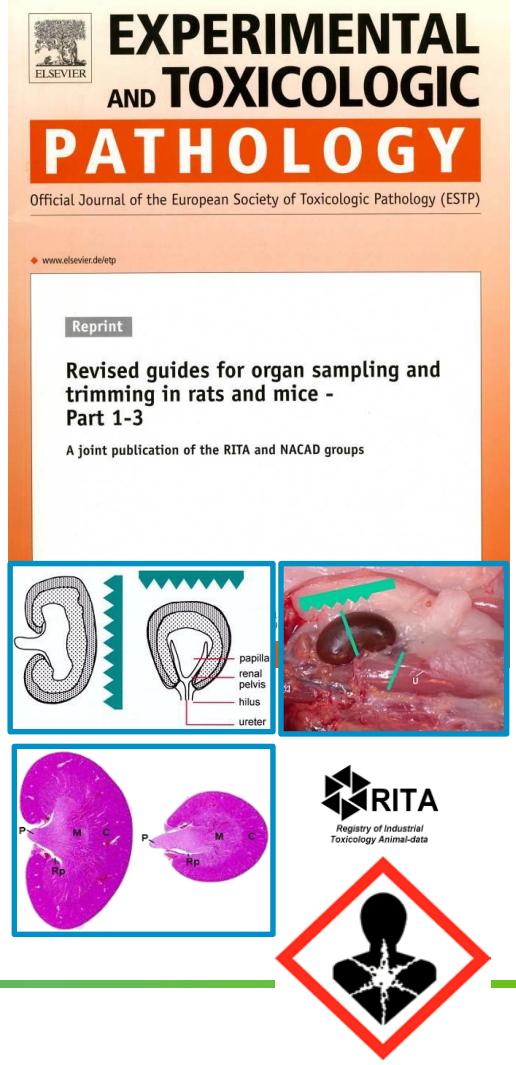
Listed tissues from **all** treated and control animals killed during or at termination of the study should be examined microscopically.

In addition, tissues from any animal in any group in which macroscopic lesions including tissue masses are found at autopsy should also be examined microscopically.

Tissue processing should allow standard and special stains, in particular stains allowing immunohistochemical determination of tumor origin.

Conditions of tissue trimming (number of sections, section size, presence of critical organ and tissue features, etc.) should be carefully considered.

Since 2004 available in English, French, German with additional features: www.item.fraunhofer.de/reni/trimming



Organs to be fixed and evaluated histopathologically

- Adrenals
- Aorta
- Bone (femur)
- Bone marrow (sternum)
- Brain (at least 3 different levels)
- Cecum
- Colon
- Corpus and cervix uteri
- Duodenum
- Epididymides
- Esophagus
- Eyes
- Gall bladder (if present)
- Harderian gland
- Heart
- Ileum
- Jejunum
- Kidneys
- Liver
- Lung (with main-stem bronchi)
- Lymph nodes (1 related to route of administration and 1 from a distant location)
- Mammary glands
- Nasal turbinates
- Ovaries and fallopian tubes
- Pancreas
- Pituitary
- Prostate
- Rectum
- Salivary gland
- Sciatic nerve
- Seminal vesicle (if present)
- Skeletal muscle
- Skin
- Spinal cord (3 locations: cervical, mid-thoracic, and lumbar)
- Spleen
- Stomach
- Testes
- Thymus (if present)
- Thyroid/parathyroid
- Trachea
- Urinary bladder
- Vagina
- Zymbal's gland
- All tissues showing abnormality

= 44 (bilateral) Organs + Gross findings, etc.

= approx. 24.000 Slides

> **120.000 Diagnoses**

Suitable computer system recommended!

How to Evaluate a Carcinogenicity Study: **Preparation**

When does the evaluation start ?

The Study Pathologist should be involved already in the preparation of the study protocol (nothing is worse than forgotten organs).

Ideally, the Study Pathologist should have (profound) knowledge of the test article from earlier studies (i.e. 4-wk, 13-wk and 26-wk for pharmaceuticals) and be familiar with the observed findings including their terminology.

The Study Pathologist should be informed during the course of the study about mortalities and gross findings to eventually integrate additional methods during terminal necropsy (monitoring).

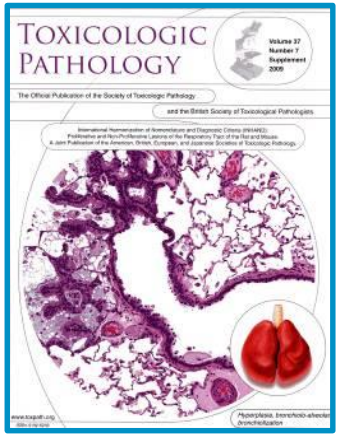
Feel and act responsible.
At least 400 animals have given their life !

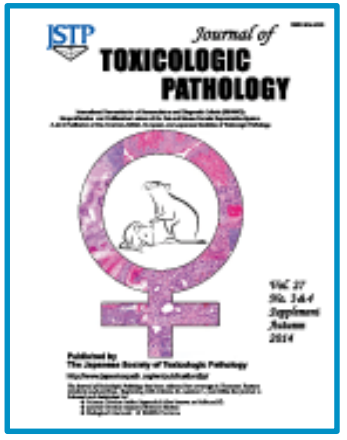
How to Evaluate a Carcinogenicity Study: **Preparation**

After terminal sacrifice, the monitoring Study Pathologist should carefully assess the Gross Findings to identify eventual targets that might be processed in advance. Don't leave it to the Study Director or Project Manager.

Before starting with the evaluation, study protocol and all amendments should be available and read.

Based on your pathology system, build up a suitable evaluation table. It helps if the order is arranged in the way the organs are processed. Neoplastic and preneoplastic findings might be placed on top of the finding list, followed by the most frequent findings.





How to Evaluate a Carcinogenicity Study: **Evaluation**

In principle, the evaluation can be performed ***by organ*** or ***by animal***. However, the latter is recommended as a first step to gain a holistic view on the situation and to determine a cause of death (demise). If a tumor was observed in the respective animal, it has to be decided whether it was an *incidental* or a *fatal* event. This is necessary for the statistical evaluation.

Reading across the organ is useful to assess potentially treatment-related effects (e.g. alterations in the size of adrenal zones) or to compare patterns of lesions (e.g. of pituitary tumors).

However, advanced reading of decedents bears some risk, e.g. in terms of continuity of terminology or grading scores.

How to Read a Carcinogenicity Study: **Evaluation** ctd.

From the Histo-Lab, slides are provided in different ways:

The “classic” arrangement on trays:

Advantage:
Good overview on all lesions of the entire animal

Disadvantage:
Space consuming, cross-reading is arbitrary

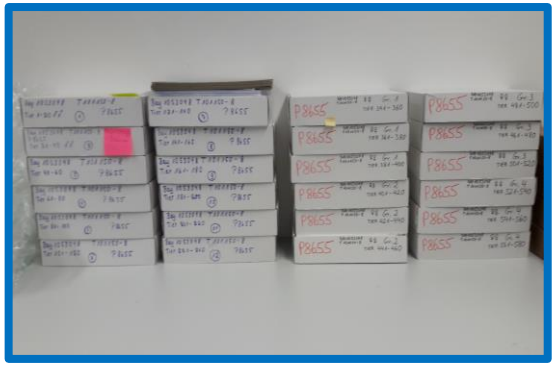
How to Evaluate a Carcinogenicity Study: **Evaluation**

Arrangement in envelops that are placed in cartridge “shoeboxes”:

Advantage:
Relatively safe to avoid damage

Disadvantage:
Work-intense, space consuming, cross-reading partly possible though kind of arbitrary

How to Evaluate a Carcinogenicity Study: **Evaluation**



Arrangement in cartridge boxes with grids:

Advantage:
All slides in place; space-saving, cross reading relatively easy

Disadvantage: If this box drops to the floor …

How to Evaluate a Carcinogenicity Study:

Decedent animals:

To save time, decedent animals are frequently processed and evaluated in advance.

Don’t wait until the gross observations pop up on your screen - read their description first. This gives you a first impression what has happened to the animal.

Consider the **AGE** of the animal at death: Decedents from the beginning of a study need a different terminology for certain findings. *Chronic progressive nephropathy* or *rodent progressive cardiomyopathy* are no useful terms for animals dying in week 4…

Try to correlate all gross observations to microscopical findings. An emaciated animal will most likely have a glycogen loss in the liver. Determination of the cause of death is frequently difficult, especially if animals die after blood sampling or other procedures the night after.

Autolysis, especially in mice, may interfere with proper diagnosis. However, try to evaluate as much as possible. A high number of non-readable organs may compromise the study.

How to Evaluate a Carcinogenicity Study:

Decedent animals:

Try to mix animals from different groups to avoid any shift in diagnosing.

After finishing the decedents, assess their findings in the incidence tables.

Animals from Terminal Sacrifce:

Evaluation of terminally killed animals should be performed also intermittently:

After 5 - 10 animals/group the investigated dose group should be changed:
e.g. Control (1) – HD – LD – (Control 2) – IMD

This holds true also for the **Peer Review:**

ICH/1SB: Peer-review of slides is required for all identified target organs and for at least 10% of all tumors. A complete review of 10% of the animals in each groups should also be performed. If more than one pathologist is involved more extensive peer review is needed to assure consistency. The peer review should be documented in raw data and in the study report.

How to Evaluate a Carcinogenicity Study: The Peer Review

Reasons to perform a Peer-Review:

- Ensure, that data meet the requirements of regulatory agencies
- Increase the accuracy of the data
- Increase the confidence in data
- Confirmation of target organs
- Confirmation of the NOEL / NOAEL / Carcinogenic Potential

A Peer-Review is NOT:

- A re-evaluation of a study
- A generation of a second data-set
- A blinded re-examination
- A „performance-review“ of the Study Pathologist

Sorry, this is a topic lasting an extra hour …

How to Evaluate a Carcinogenicity Study:

What should be recorded?

Age-related findings (broad knowledge of advantage)

- Threshold needed
  (e.g. CPN, RCMP, hepatocellular changes, adrenal cortical alterations)

Preneoplastic and Hyperplastic Lesions

- Grading / vs. non-grading (A borderline case cannot be grade 2 / slight)
- Differentiate between focal and diffuse lesions
  (Leydig cells, pituitary, adrenal medulla, thyroid, ovary, stomach, etc.)

Neoplastic Lesions

- Classify as benign, malignant, incidental or related to death finding (statistics)
- Classify also behavior: invading or metastasizing (or primary site unknown)

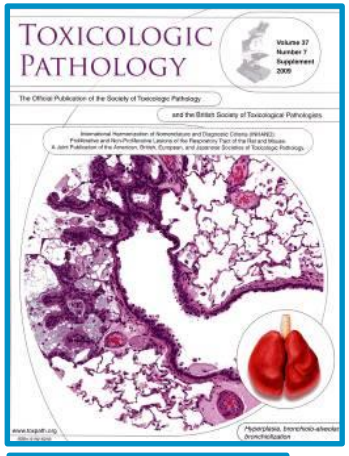
Target-related findings

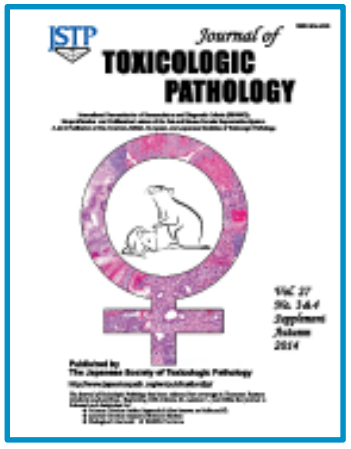
- Case by case

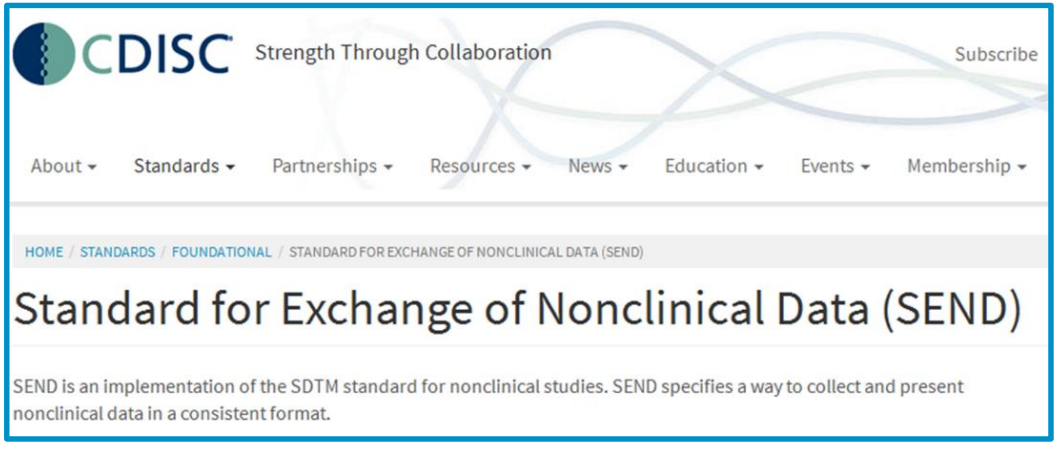
ICH/1SB:How to Evaluate a Carcinogenicity Study: Reporting

Pre-neoplastic and neoplastic lesions should be described in conventional histopathological terms according to well-defined classifications (e.g. ILSI, NTP, IARC, *go*RENI, INHAND) and other recent texts on rodent pathology. The method **must be referenced** in the report. Deviations must be explained.

Consider **SEND ! (**https://www.cdisc.org/standards/foundational/send)







goRENI
Registry Nomenclature Information System

www.goreni.org

ICH/1SB:How to Evaluate a Carcinogenicity Study: Other points to consider

- **Presentation of the data:** Tables and Individual Data Sheets
- **Analysis of the Data:**

The form of the analysis and the tests of statistical significance used should be appropriate to the type of data and to the basic experimental design. The statistical procedures used should be clearly stated.

The following types of responses can be assessed:

- occurrences of neoplastic lesions (and non-neoplastic lesions, if related)
- number of animals at risk and examined
- the incidence of combined tumors of common histiogenic origin,
- the incidence of tumors judged to be malignant
- the sum of benign and malignant tumors in the same tissue when applicable
- the latent period to tumor appearance (using actuarial approaches)

Statistics should be integrated into Pathology Program, e.g. Peto, poly-3, poly-k

FDA will perform their own statistics
(demand of XPT File for Tumor Data) !

ICH/1SB: How to Evaluate a Carcinogenicity Study: Historical Control Data

The concurrent control group should always be the primary reference with respect to treatment-related tumorigenicity. (double controls helpful !?)

If historical control data are used, they must be derived primarily from the same strain and testing facility. Data should have been obtained from several studies during the last 5 years prior to the study, taking into account genetic drift.

Data from literature might be added if thought to be informative.

charles river

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National Toxicology Program
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What We Study | How We Work | Data & Resources | Publications | Who

Home » Data & Resources » Historical Controls

Data & Resources

Historical Controls

RITA
Registry of Industrial Toxicology Animal-data

Alternative carcinogenicity bioassays suggested for drug testing

Neonatal mouse (Pietra et al, 1959)

Transgenic approaches:

- Oncogene-transgenic models:
  - TG.AC (v-ras) transgenic mice (Leder et al, 1990)
  - TG rasH2 transgenic mice (Saitoh et al, 1990)
- Tumor suppressor gene models:
  - TSG-p53 knock-out mice (Donehower et al, 1992)
- DNA repair deficient models:
  - XPA knock-out mice (De Vries et al, 1995; Nakane et al, 1995**)**

➡ **Multinational effort by the ILSI/HESI ACT*: 1996 - 2001**

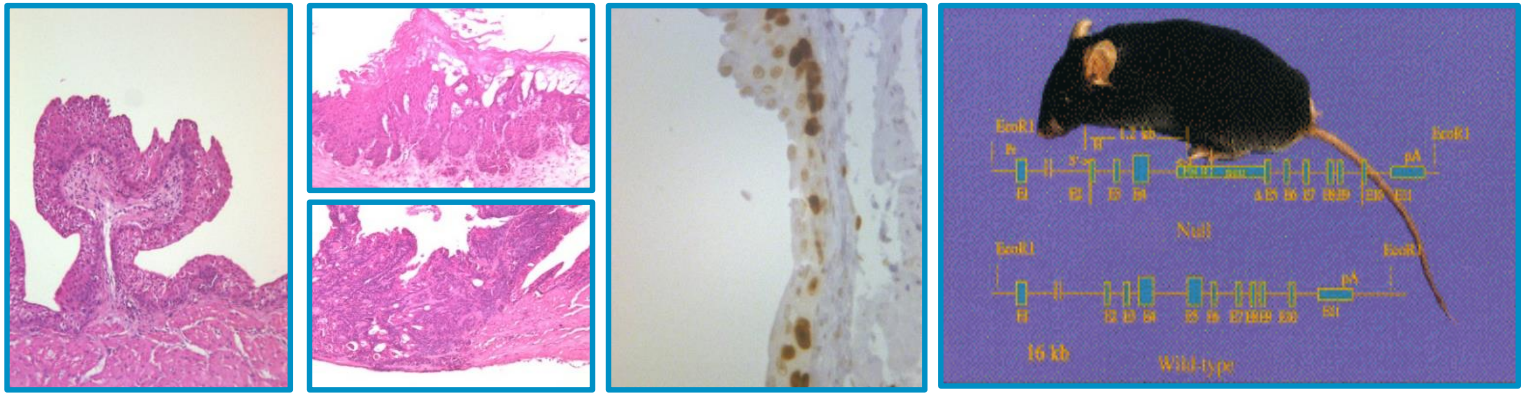
*International Health and Life Sciences Institute/Health and Environmental Sciences Institute - Alternatives to Carcinogenicity Testing

Alternative carcinogenicity bioassays suggested for drug testing

p53: “the Guardian of the Genome”

p53 gene regulates cell proliferation, it plays a role in preventing cells that have experienced DNA damage from entering into the cell cycle

- Outcome from ILSI/HESI: 41/46 compounds with expected results
- 26 wk period seems adequate (better 39 weeks?)
- MTD was achieved in both sexes
- Positive control p-Cresidine was positive in 16/17 cases
- Benzene is not a reliable positive control
- Spontaneous tumor rate low in heterozygous mice, mainly sarcoma, lymphoma and leukemia
  However: difficulty with animals chipped with transponders !
- Some cases demonstrated loss of heterozygosity or inactivation of the wild-type
- Costs about 1/3 of a conventional bioassay

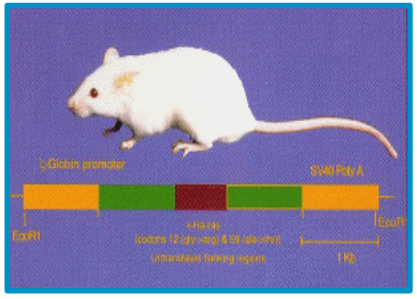


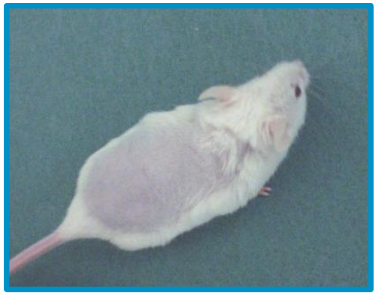
Alternative carcinogenicity bioassays suggested for drug testing

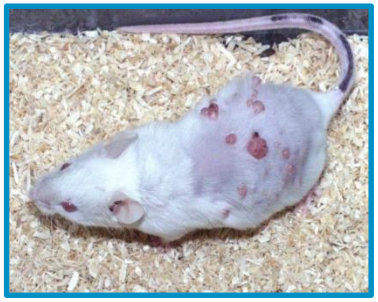
TgAC

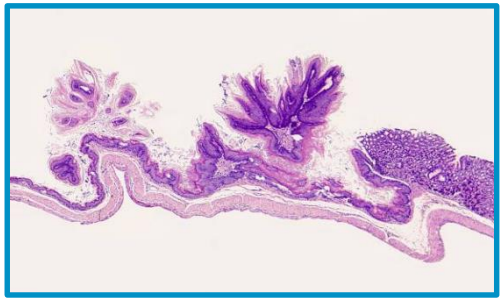
TgAC responds to:

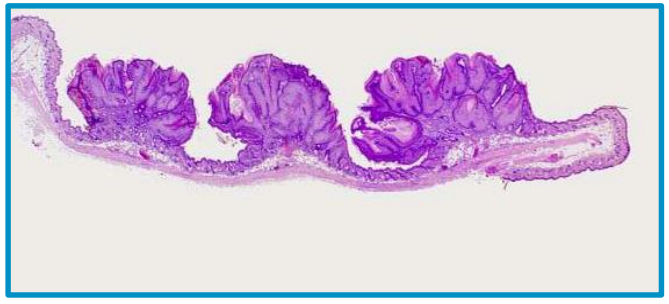
- specific chemicals
- known human geno-toxic and nongenotoxic carcinogens
- topical and oral exposure











TgAC Mode in ILSI/HESI ACT: Positive outcome in 23 / 28 studies

What was learned from the TgAC model? Lots of unexpected results due to an extensive histopathology which was initially not planned !

Alternative carcinogenicity bioassays suggested for drug testing

Tg.rasH2

The Tg.rasH2 model was accepted by regulatory agencies worldwide for 26-week carcinogenicity assays as an alternative to the standard 2-year assays in conventional mice in 2003.
Currently considered No. 1 choice of TG models

- Low incidence of spontaneous liver tumors*
- Respononse to GT and NGT Carcinogens
- Considered neither insensitive nor prone to false positve results

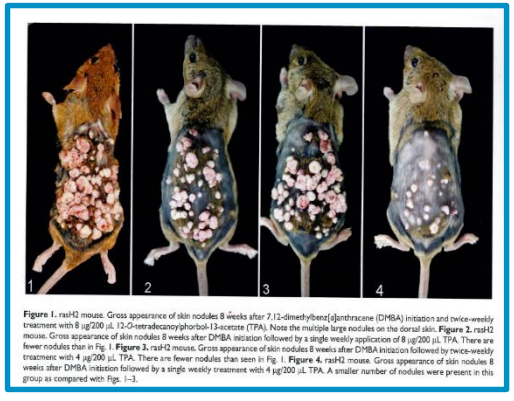


Figure 1. rasH2 mouse. Gross appearance of skin nodules 8 weeks after 7,12-dimethylbenz[a]anthracene (DMBA) initiation and twice-weekly treatment with 8 µg/200 µL 12-O-tetradecanoylphorbol-13-acetate (TPA). Note the multiple large nodules on the dorsal skin. **Figure 2.** rasH2 mouse. Gross appearance of skin nodules 8 weeks after DMBA initiation followed by a single weekly application of 8 µg/200 µL TPA. There are fewer nodules than in Fig. 1. **Figure 3.** rasH2 mouse. Gross appearance of skin nodules 8 weeks after DMBA initiation followed by twice-weekly treatment with 4 µg/200 µL TPA. There are fewer nodules than seen in Fig. 1. **Figure 4.** rasH2 mouse. Gross appearance of skin nodules 8 weeks after DMBA initiation followed by a single weekly treatment with 4 µg/200 µL TPA. A smaller number of nodules were present in this group as compared with Figs. 1–3.

It allows differentiation into Squamous Cell Hyperplasia, Papilloma, Keratoacanthoma, Squamous Cell Carcinoma

*But relatively high incidences in:

Lung:	B/A Adenoma/Carcinoma (10%)
Spleen:	Hemangiosarcoma (4%)
Adrenal:	Subcapsular Cell Hyperplasia (80 – 100%)

Kawabe et al. 2013, Vet. Pathol 50, 903 – 908

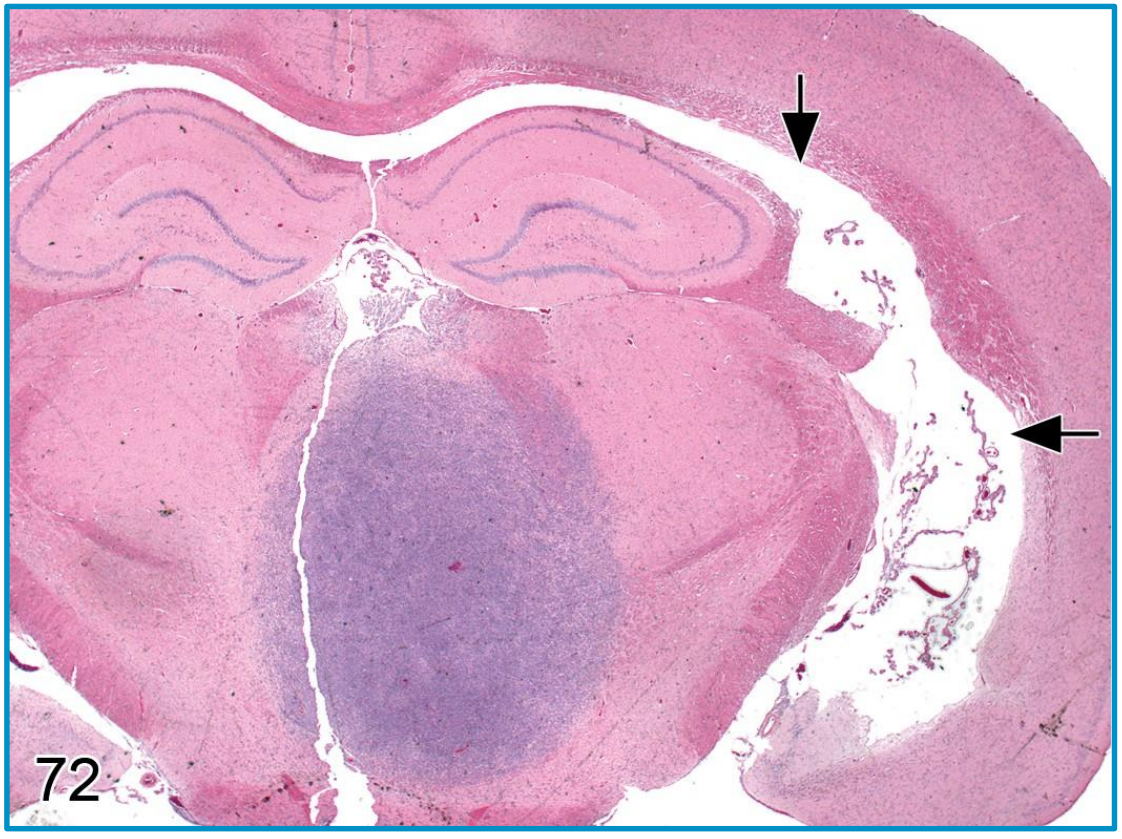
Accuracy of transgenic models vs. IARC classification of 99 compounds

IARC Classification	Trp53+/-	Tg.AC	RasH2	Overall
Group 1 (HC)	**83% (10/12)**	**89% (8/9)**	**57% (4/7)**	**79% (22/28)**
Group 2A (probably HC)	**62% (5/8)**	**50% (2/4)**	**100% (9/9)**	**76% (16/21)**
Group 2B (possibly HC)	**55% (6/11)**	**64% (7/11)**	**69% (9/13)**	**63% (22/35)**
Group 3 (not classifiable)	**0% (0/13)**	**21% (3/14)**	**36% (5/14)**	**20% (8/41)**
Not classified	**7% (1/15)**	**29% (7/24)**	**0% (0/8)**	**17% (8/47)**

From: CJ Portier, NIEHS, NTP

Interpretion of Long Term Ca Studies:

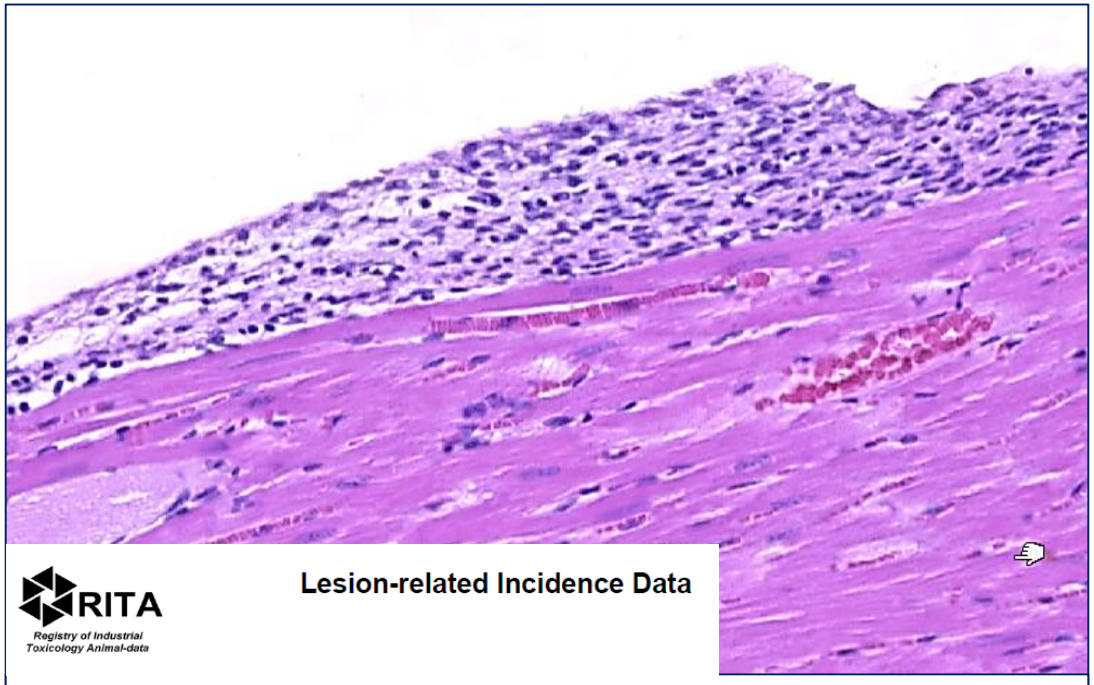
NTP Radiofrequency Radiation studies in rats* and mice



Malignant Glioma

*NTP Technical Report 595 on the Toxicology and Carcinogenesis Studies In Hsd:Sprague Dawley SD Rats Exposed to Whole-body Radio Frequency Radiation at a Frequency (900 Mhz) and Modulations (GSM and CDMA) Used by Cell Phones (2018)

Proliferative Lesions of the Heart: Hyperplasia, Schwann Cell



RITA
Registry of Industrial Toxicology Animal-data

Lesion-related Incidence Data

Heart
Hyperplasia, schwann cell, subendocardium

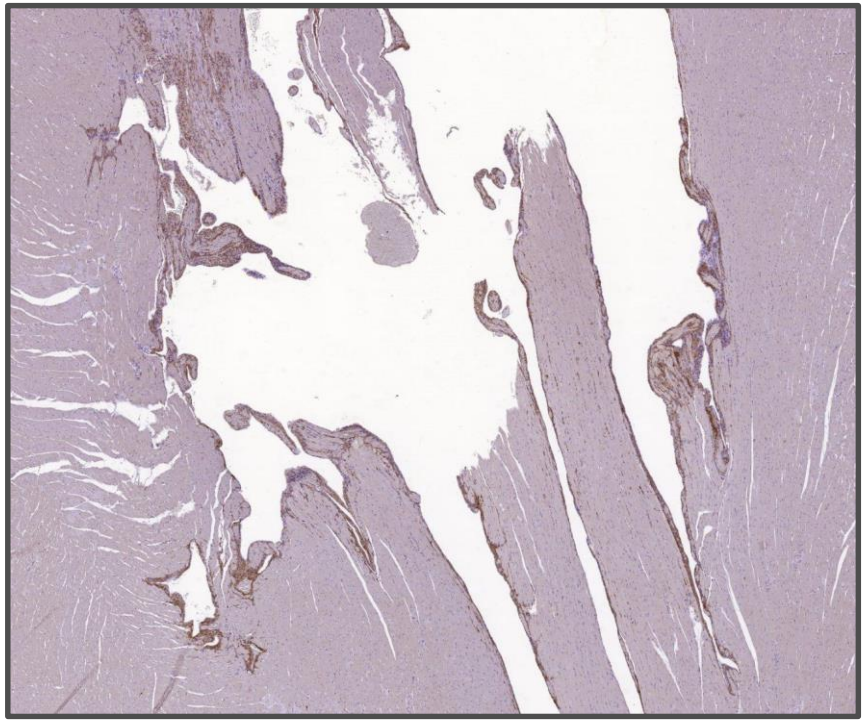
Summary	Males					Females				
Strain	total exam.	with lesion	%	% range min	% range max	total exam.	with lesion	%	% range min	% range max
F344	100	0	0.0			100	0	0.0		
SPRD	3089	7	0.2	0.0	2.9	2991	4	0.1	0.0	2.0
WIST	6811	50	0.7	0.0	6.7	6665	21	0.3	0.0	3.1
All strains	10000	57	0.6	0.0	6.7	9756	25	0.3	0.0	3.1

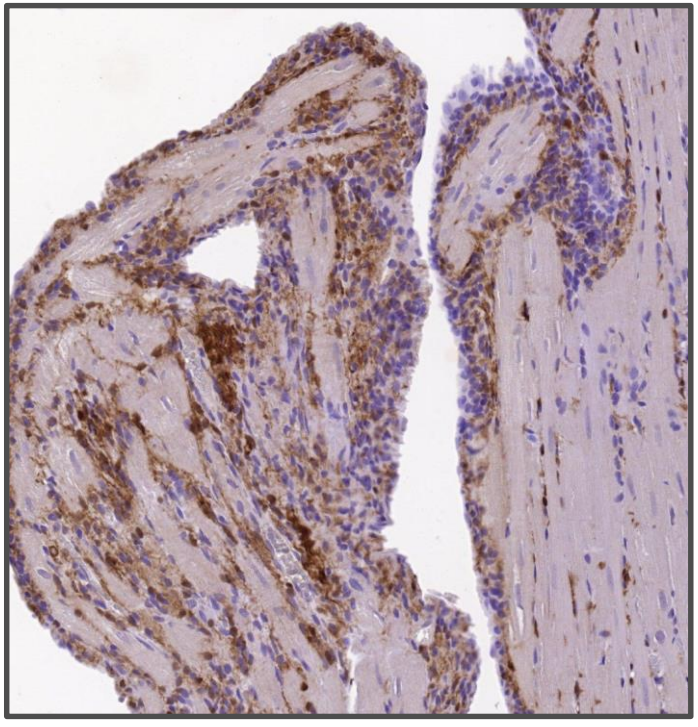
The lesion is composed of a thin (<20 cells), hypercellular layer of mesenchymal ovoid cells beneath an intact endocardium and distinct from the underlying myocardium; myocardial infiltration is minimal.

The left ventricular endocardium is most often affected

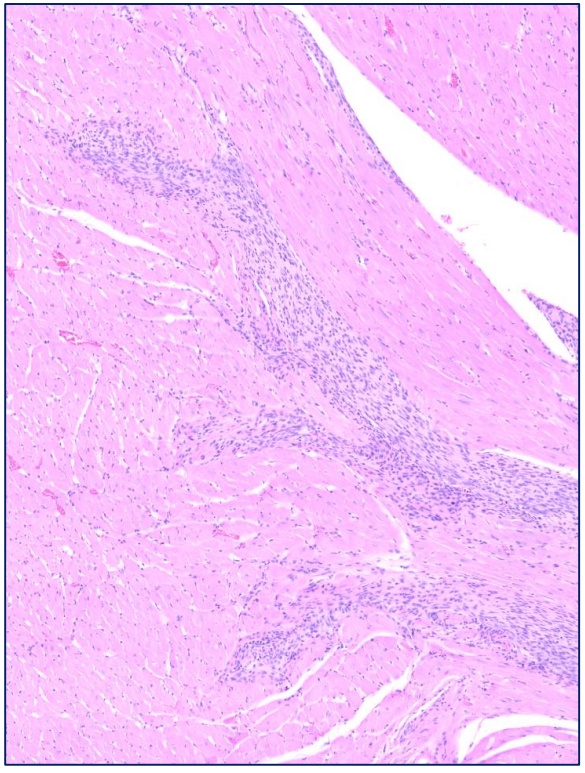
Proliferative Lesions of the Heart: Hyperplasia, Schwann Cell

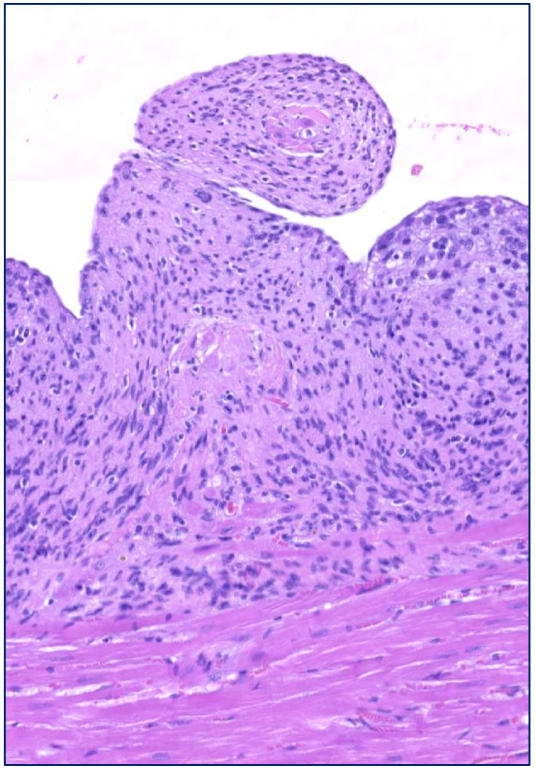
S100 - positive

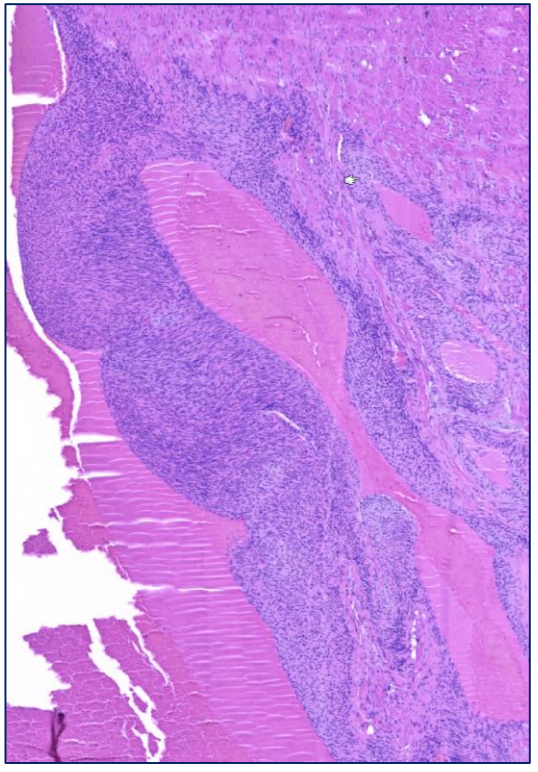




Proliferative Lesions of the Heart: Schwannoma, endocardial







Endocardial schwannomas tend to have more discrete boundaries with expansile growth but infiltration of subjacent myocardium is common as well.

Interpretation of Long Term Ca Studies:

NTP Radiofrequency Radiation studies in rats* and mice

The 10-year, $25 million toxicological studies are the most comprehensive assessments of health effects and exposure to radiofrequency radiation in SD rats and B6C3F1 mice to date.

Finding	Males GSM	Females GSM	Males CDMA	Females CDMA
Survival	**25/90, 45/90, 50/90, 60/90**	**48/90, 53/90, 48/90, 57/90**	**25/90, 43/90, 56/90, 43/90**	**48/90, 46/90, 50/90, 61/90**
Heart: Schwann Cell Hyperplasia	**0/90, 1/90, 0/90, 2/90**	**none**	**0/90, 0/90, 0/90, 3/90**	**none**
Heart: Schwannoma, malignant	**0/90, 2/90, 1/90, 5/90**	**0/90, 0/90, 2/90, 0/90**	**0/90, 2/90, 3/90, 6/90**	**0/90, 2/90, 0/90, 2/90**
Brain: Glial Cell Hyperplasia	**0/90, 2/90, 3/90, 1/90**	**none**	**0/90, 2/90, 0/90, 2/90**	**0/90, 0/90, 1/90, 1/90**
Brain: Glioma, malignant	**0/90, 3/90, 3/90, 2/90**	**none**	**0/90, 0/90, 0/90, 3/90**	**0/90, 3/90, 0/90, 0/90**

*NTP Technical Report 595 on the Toxicology and Carcinogenesis Studies In Hsd:Sprague Dawley SD Rats Exposed to Whole-body Radio Frequency Radiation at a Frequency (900 Mhz) and Modulations (GSM and CDMA) Used by Cell Phones (2018)

How to Read a Carcinogenicity Study: The Report Narrative

The Pathology Report will be (only) a Part (Addendum) of the Main Study Report. However, its narrative is the **most important piece** and should be readable also without the information of the main report, i.e.

- All important information should be given in the „Materials and Methods“ chapter.
- In the “Results” chapter, describe (pre-)neoplastic lesions in detail by organ (system) and provide illustrative tables about incidences and/or gradings.
- Describe non-neoplastic treatment-related findings in detail, especially if different from SEND terminology
- In the “Conclusions” chapter ´try to group data from organ weighing, gross observations and histopathology as a whole.
- Give reasonable explanation on the outcome of the study but avoid speculation.
- Confirm the correct transmission of the results into the main study report

Always consider that the reviewer has to understand the report. He/she will be most likely no pathologist !

Summary/Conclusion

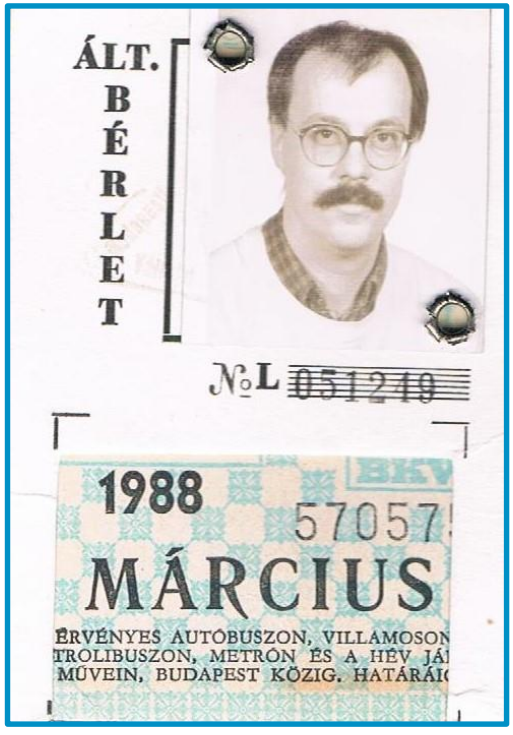
Genotoxic Compounds are covered by ICH.

Equivocal or Non-genotoxic compounds still require long term studies in rodents.

Sound basic knowledge is needed for an appropriate evaluation and interpretation of the data.

Be aware that the narrative in your report is essential for the outcome and thus, is the most important part of the study.

Only a good and trustful collaboration between the study director, the study pathologist, and the responsible sponsor monitor guarantee satisfying results.



Kösönöm: Let‘s target a good discussion: