CONTINUING EDUCATION IN TOXICOLOGIC PATHOLOGY
REPRODUCTIVE SYSTEM

Third Conference

ORGANIZED BY SOCIETY FOR TOXICOLOGIC PATHOLOGY IN INDIA (STPI)

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Reading the Rodent Bioassay: Problems, Pitfalls and Peer Review

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Pathology Rites-of-Passage

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Jane/John Pathologist, BS, DVM, PhD, Diplomate, ACVP, RCS

Carcinogenicity Studies

The **Ultimate** Toxicology Study

What is the Purpose of a Carcinogenicity Study?

Hint: They are called **Cancer** Studies

How Relevant is the Rodent Carcinogenicity Study?

The Two-year Rodent Bioassay is the "Gold Standard"

Positive Aspects of the Bioassay

- Yields positive results for known human carcinogens
- Standardized (informative databases)
- Trans-species carcinogens
- Appreciation of benefits of historical controls
- Reproducible

Limitations of the Bioassay

- Resource intensive
- Inherent insensitivity for detecting weak or moderate carcinogens
- Not ideal for determining if an agent has carcinogenic potential under actual human exposure conditions
- Historical inertia
- Debate regarding relevance
  - Rodent-specific mechanisms
  - High doses

- ICH/Alternative Models

- ICH/Alternative Models
- Proteonomics

- ICH/Alternative Models
- Proteonomics
- Metabonomics

Toxicological Sciences 2005
88(1):18-23

Prediction of 2-Year Carcinogenicity Study Results for Pharmaceutical Products: How Are We Doing?

Abigail Jacobs
Center for Drug Evaluation and Research, USFDA

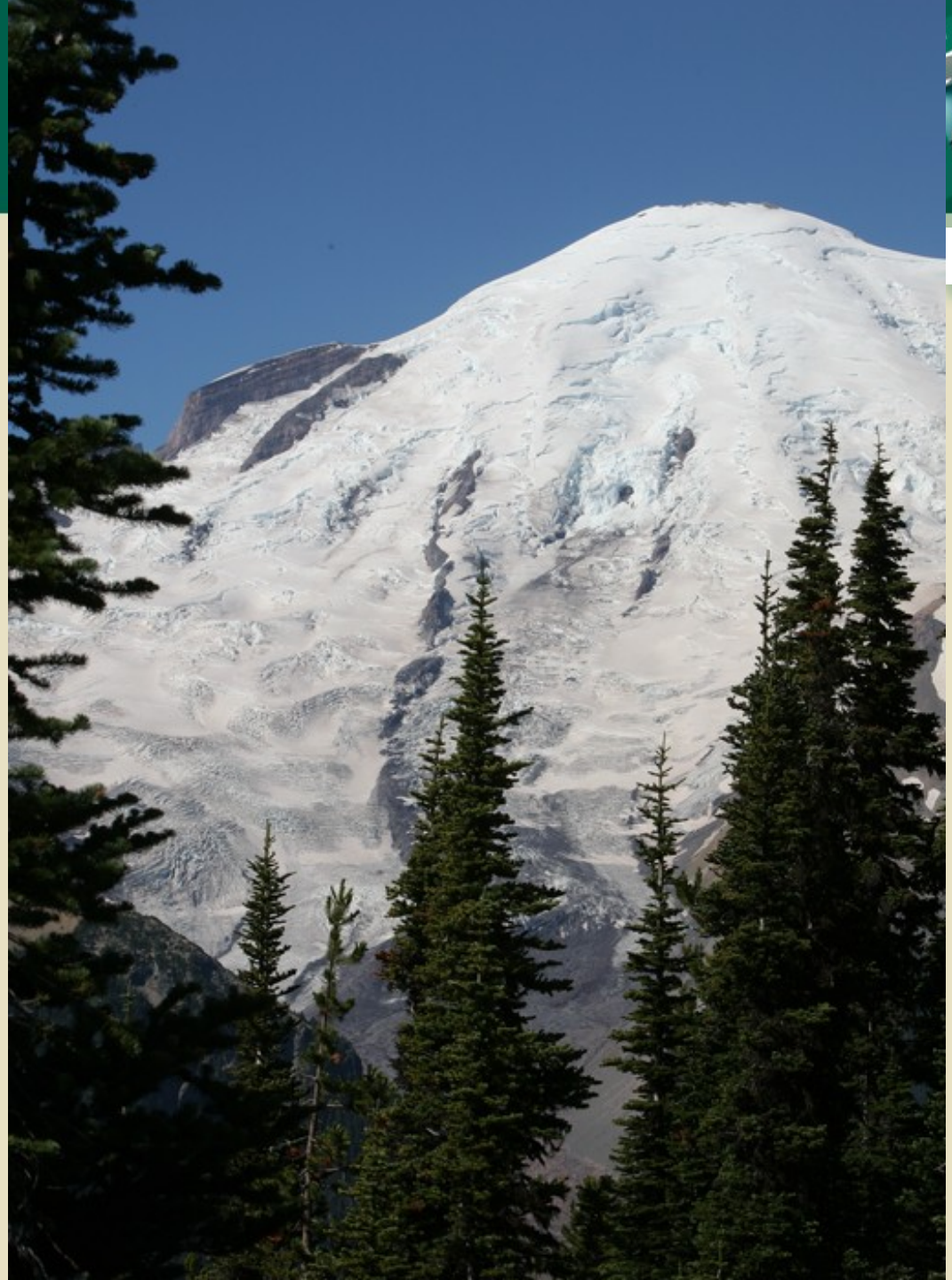
Some have proposed that 2-year carcinogenicity studies may not be necessary if the material is a direct-acting DNA mutagen, induces liver enzymes, causes hyperplasia or toxicity in particular organs, causes cell proliferation, is cytotoxic, causes hormonal perturbations, or if one has QSAR analyses or 'omics' information. Safety pharmacology data, pharmacologic activity, metabolism data, and results of 13-week dose ranging studies (with organ weight data, clinical chemistry data, hematologic data, clinical signs and histopathologic findings) were compared with results of 2-year carcinogenicity studies reviewed by the Center for Drug Evaluation and Research (CDER)/FDA. The experience with the ICH genetic toxicology battery and alternative carcinogenicity models was also reviewed.

Prediction of 2-Year Carcinogenicity Study Results for Pharmaceutical Products: How Are We Doing? (Continued)

It appears that the information available from short-term studies is not currently sufficient to accurately and reliably predict the outcome of long-term carcinogenicity studies.

Metaphors for Reading a Carcinogenicity Study

The Very Tall Mountain Metaphor



The Russian Novel Metaphor

Russian Translations

A Carcinogenicity Study is A Matter of Simple Arithmetic

Typical Recent Carcinogenicity Studies

	Control	Treated	Number of Animals/Group	Total No. Animals
Typical A	1	3	60	480
Typical B	2	3	65	650

Some Simple Study Arithmetic

- A “typical” rat has 60 tissues

- Typical Study A has 480 rats x 60 tissues/rat = 28,800 tissues/study
- Typical Study A has 650 rats x 60 tissues/rat = 39,000 tissues/study

How Long Will It Take to Read?

Tissues/Day	No. of Rats/ Day	No. of Weeks (480 rats)	No. of Weeks (650 rats)
300	5	19.2	26
480	8	12	16.25
600	10	9.6	13
900	15	6.4	8.66

Interpretation of Neoplastic Findings

Interpretation of Neoplastic Findings

- May have a small increase of tumors after treatment with a non-genotoxic compound
- Genotoxic compounds will generally not make it as far as a carcinogenicity study

Statistical Analysis

- PETO tests incorporating Cause of Death
- Common vs Uncommon tumors
- Combining Tumors

FDA Statistical p-value Cut Offs

	Tests for Positive Trend	Control-High Pairwise Comparisons
Two 2-year Studies	Common 0.005 Rare 0.025	Common 0.01 Rare 0.05
2-year Study + Alternative Model	Common 0.01 Rare 0.05	Not specified

The FDA uses these cut-offs to reduce false positive results.
Other countries do not formally recognize these recommendations.

Combining Neoplasms

- Progression from benign to malignant
- Hyperplasia as supporting evidence
- Same histomorphogenic type at different sites
- Different morphologic classification when histomorphogenesis is comparable

McConnell EE, Solleveld HA, Swenberg JA and Boorman GA
Guidelines for Combining Neoplasms for Evaluation of Rodent Carcinogenesis Studies.
JNCI (1986) 76:283-289

Interpretation of Findings

Importance of Findings in a Carcinogenicity Study

- Neoplastic findings are of primary importance
- Significance of non-neoplastic findings needs to be put in proper perspective

Interpretation of Findings

- Historical Controls
- Mechanistic Studies
- Literature Review

Historical Controls

- Concurrent controls from recent studies in same laboratory
- Older studies from same laboratory
- Published historical controls

Mechanistic Studies

- Effect may be exaggerated physiologic response
- Response may not be relevant to humans (elevated TSH in rats)

Literature Review

- Common lesions in aging rodents
- Published mechanistic explanations

Lumping vs. Splitting

Lumping vs. Splitting

- Splitting is useful
  - for subtle end-points
  - in short-term studies
- Lumping is useful
  - For complex entities
  - In longer studies

Diagnostic Drift

Diagnostic Drift

- The tendency for histopathologic diagnoses to change over time.
- Diagnostic drift may occur in a single group, across several groups in a single study, or when several studies are compared

Diagnostic Drift (continued)

- By definition, diagnostic drift cannot be appreciated by observing a single event
- Requires numerous data points separated by time

Possible Causes of Diagnostic Drift

- Thresholds for lesions
- Underdiagnosis of controls
- Overdiagnosis of controls
- Recording of “normal” changes

Thresholds

- Some pathologists utilize thresholds – any change whose severity or incidence is “below the threshold” are not recorded
- Thresholds are extremely difficult to apply consistently
- Lesions are either present or not – thresholds may actually not exist

Underdiagnosis of Controls

- Control animals are NOT animals without lesions
- Many control animals have a variable incidence of background lesions
- A treatment-related effect may often be noted as an increase in the incidence or severity of these changes

Overdiagnosis of Controls

- Background lesions occur in controls
- May be exacerbated in treated animals
- Pathologist may look harder at controls for these changes:
  - To ensure that subtle differences are delineated
  - To prevent spurious findings from achieving significance

Recording of “Normal” Changes

- Minimal changes - part of normal life processes
- Some pathologists record these changes; others don’t
- Thyroid – ultimobranchial cysts
- Thymus – physiological involution
- Lymph nodes - plasmacytosis

Implications of Modifiers

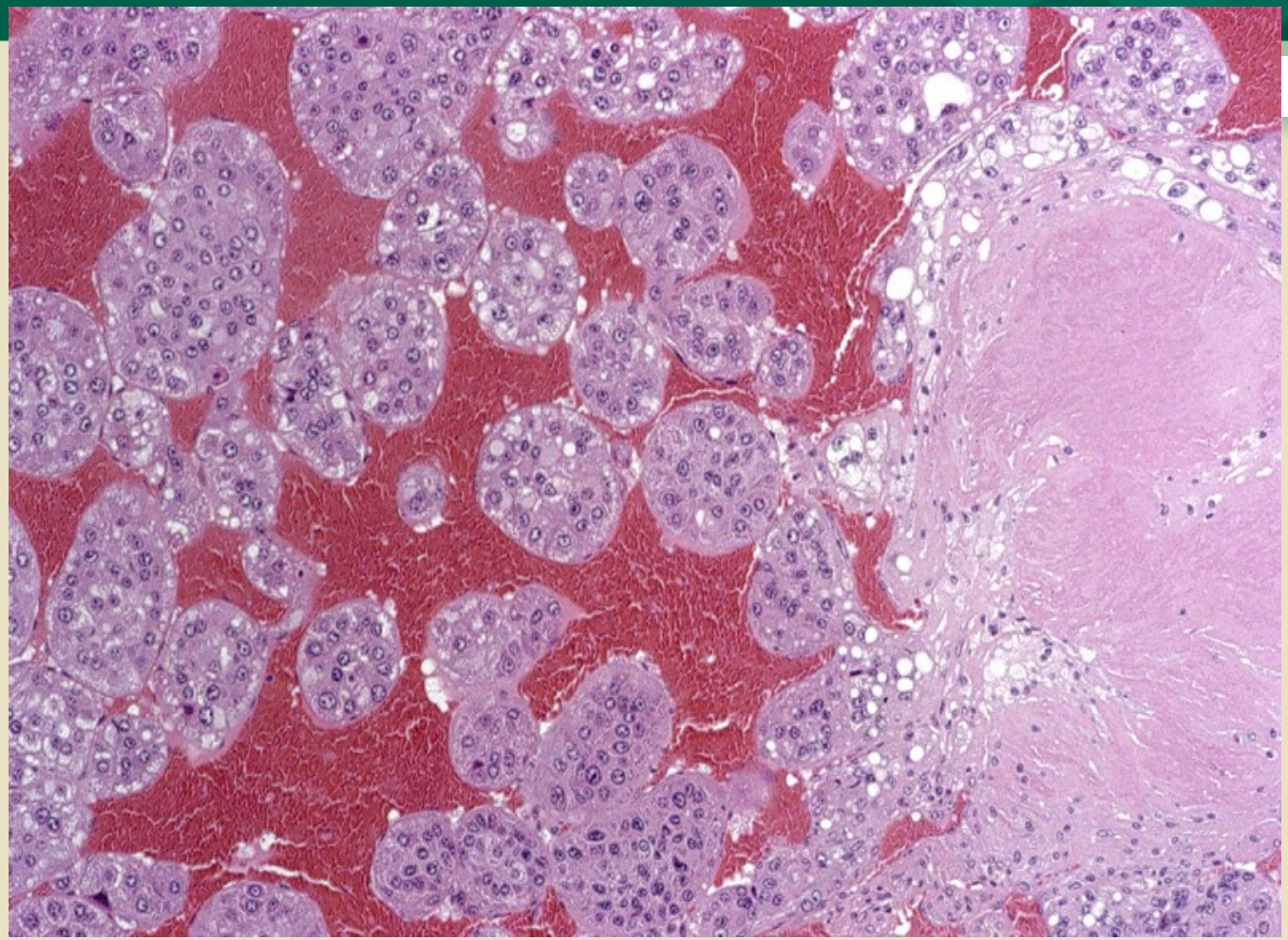
- Distribution Modifiers
  - Focal versus Multifocal versus Diffuse
- Severity Modifiers
  - Four grade scale versus Five
  - Numbers versus words
  - Percentage of organ effected
  - Qualitative differences of severity

More Possible Causes of Diagnostic Drift

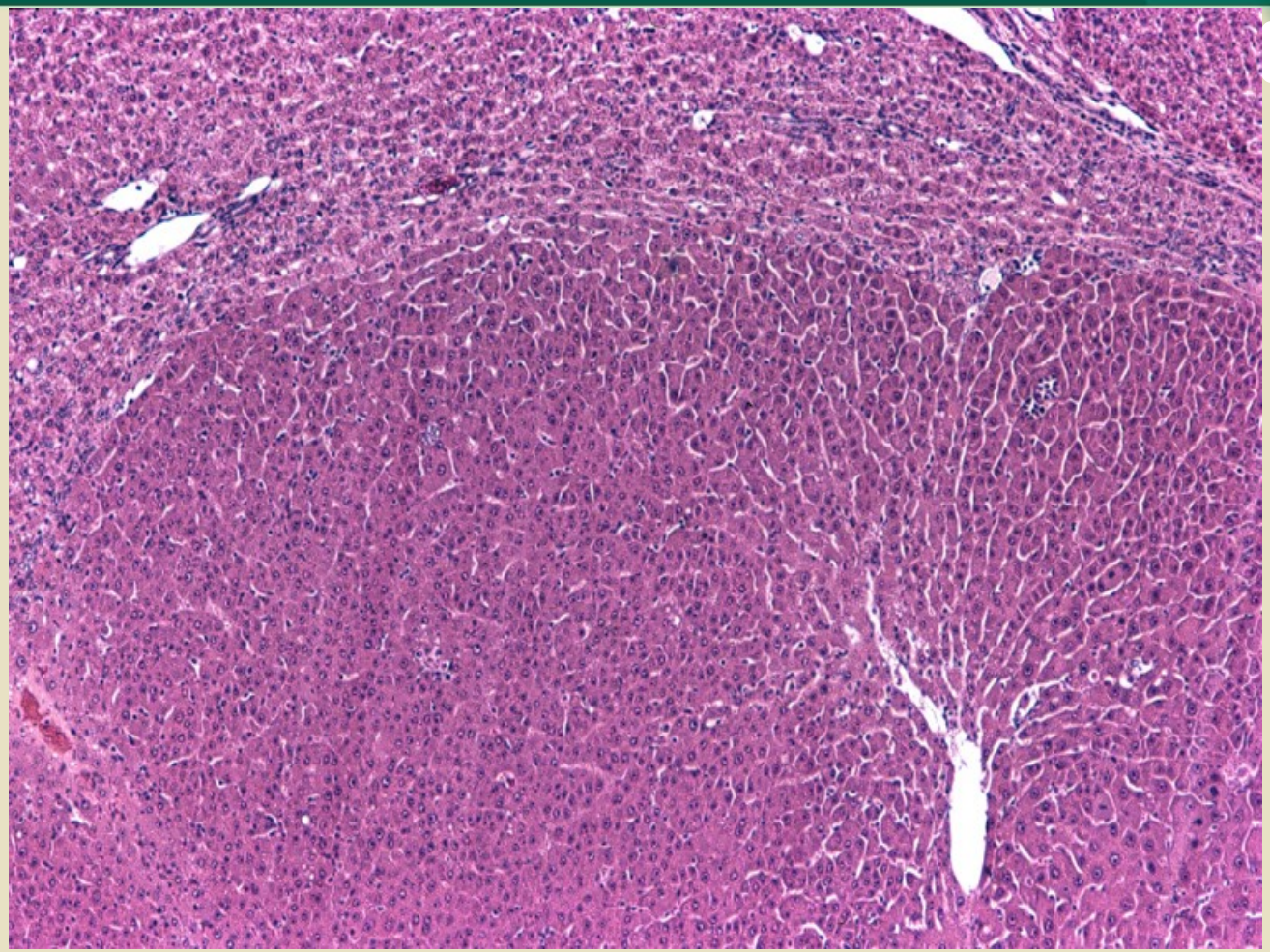
- Borderline lesions
- Unusual lesions
- Drift over time
- Operator bias

Borderline Lesions

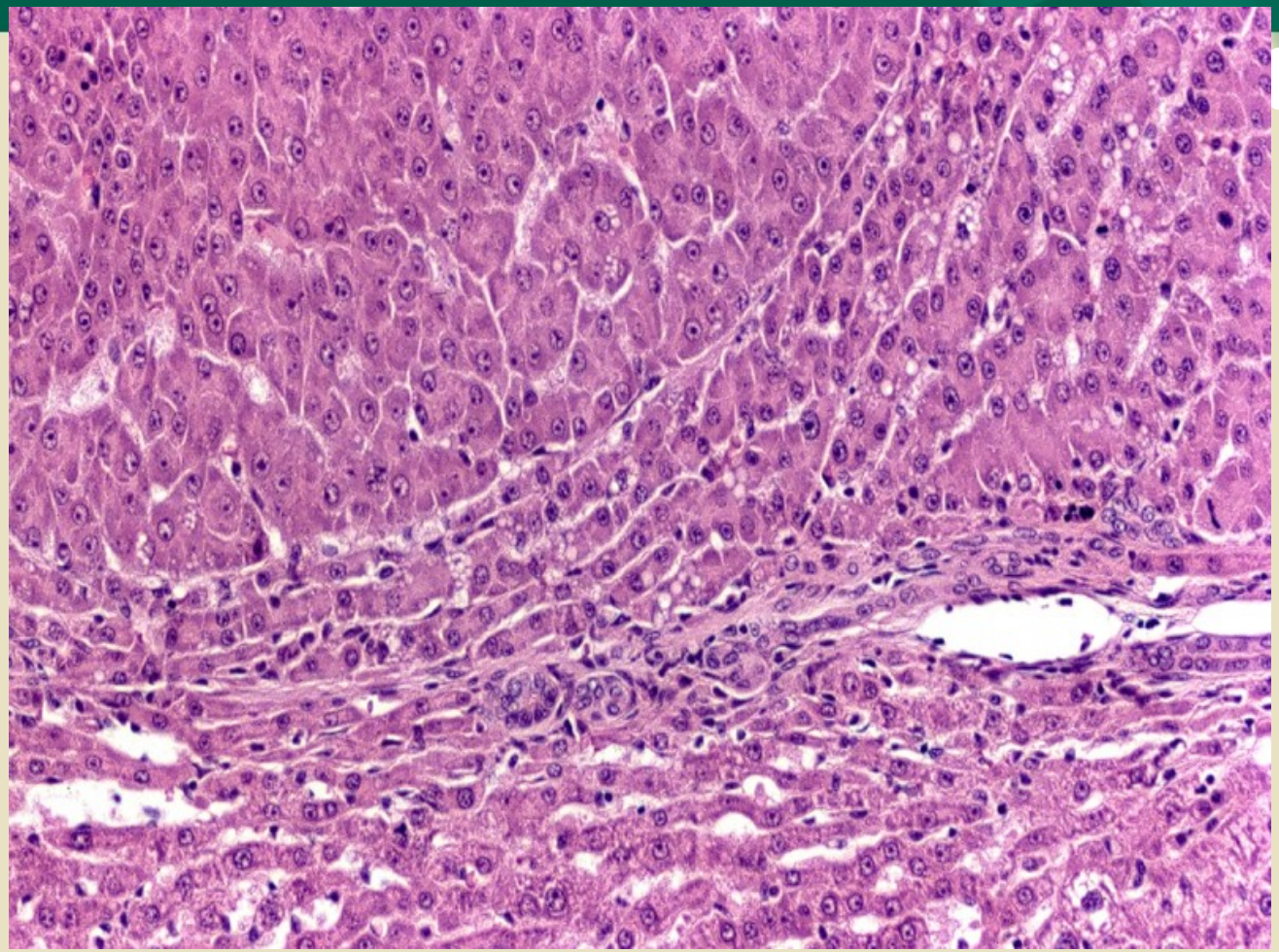
- Some changes are “textbook” clear
- Other changes are ambiguous; three pathologists may give three opinions
- Reports require a definitive diagnosis
- Difficult to maintain consistency
- May require a panel of experts - PWG



Liver – Hepatocelluar Carcinoma



Liver – Hepatocellular Adenoma



Liver – Hepatocellular Carcinoma

Effect of Unusual Lesions

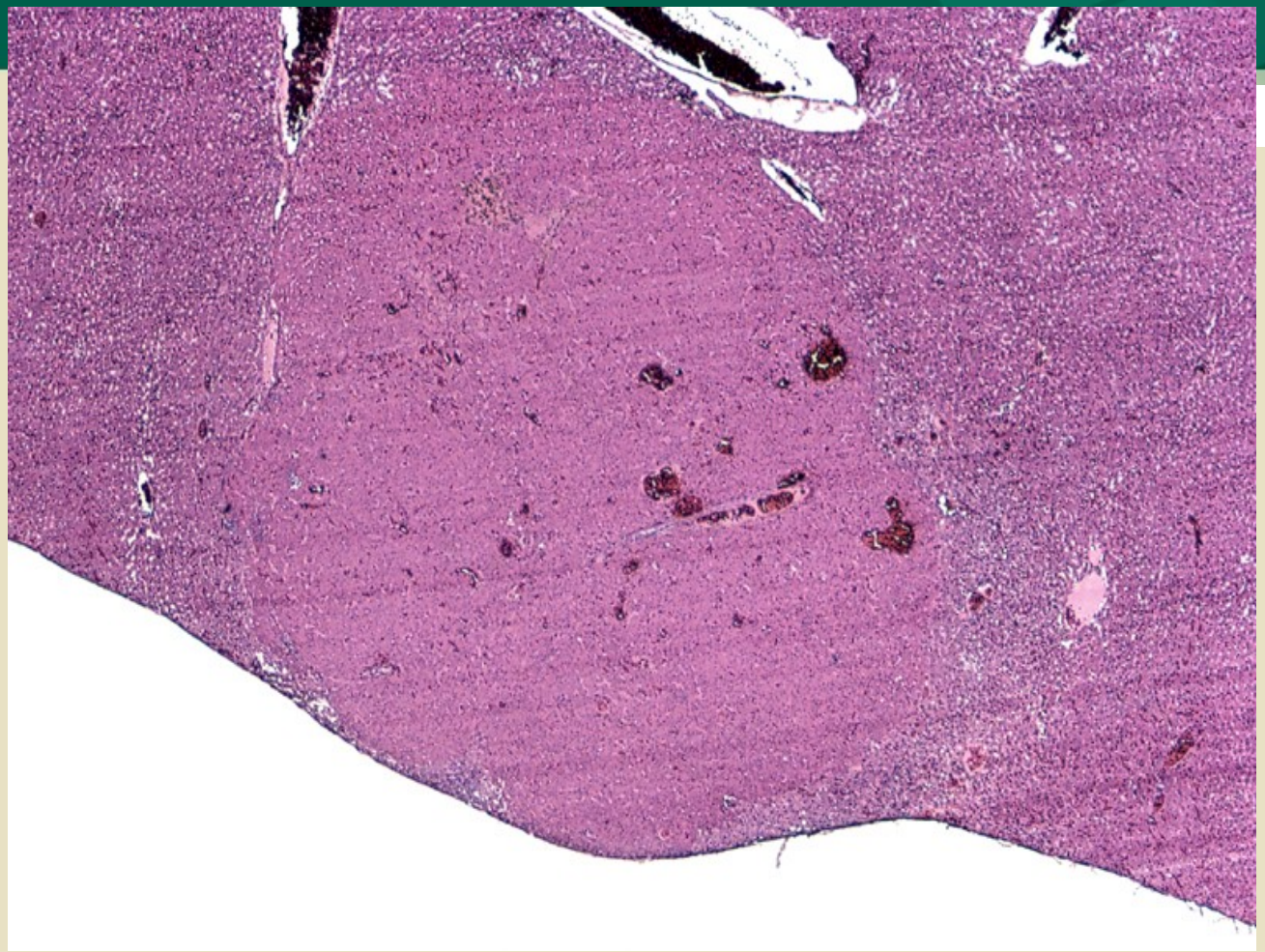
- Unique or uncommon lesions often scrutinized closely
- May have no threshold for these changes
- Difficult to maintain consistency

Drift Over Time

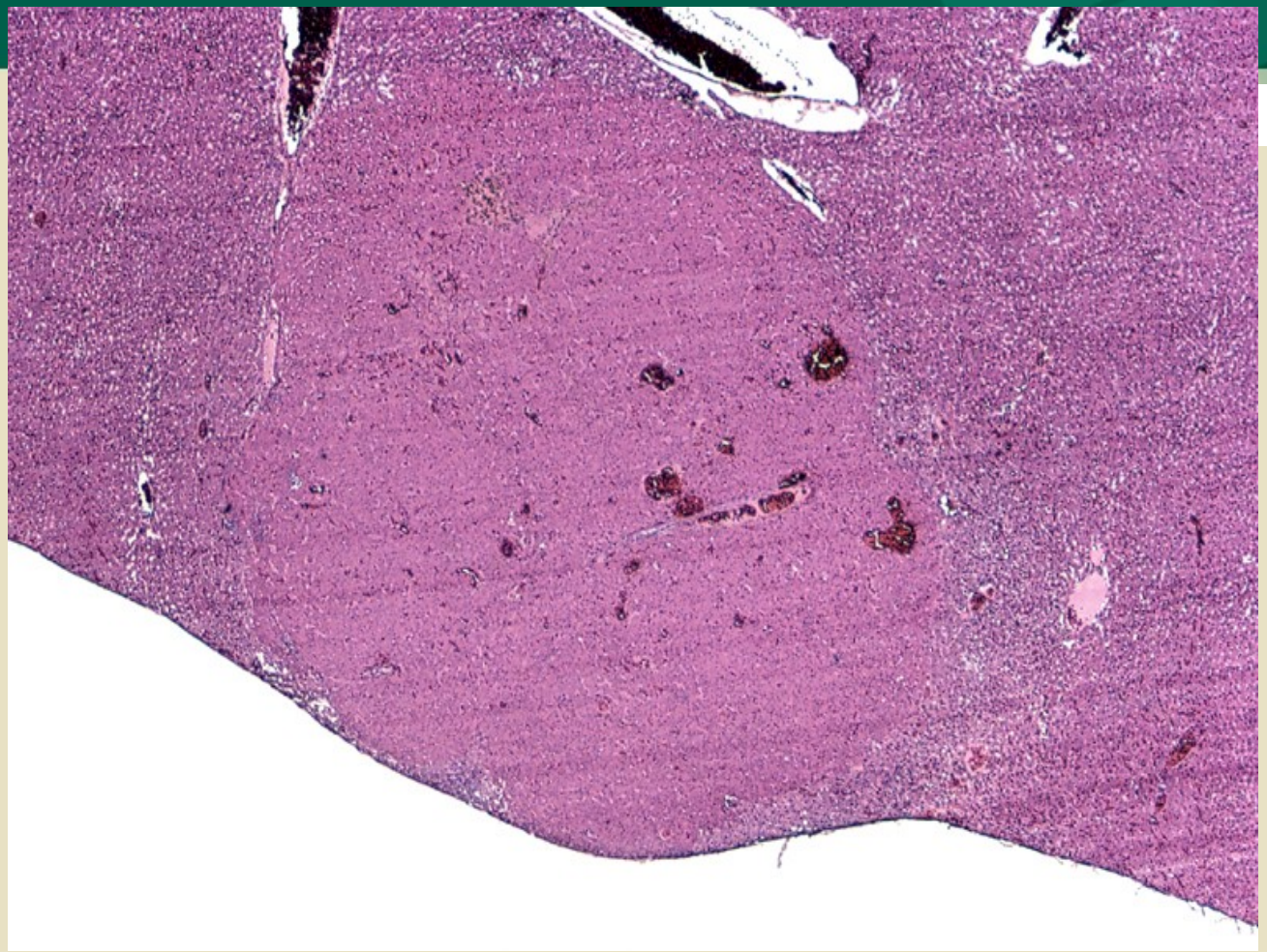
- Professional drift – changing criteria for a given lesion
- Personal drift – Increased familiarity with a given lesion with greater exposure

Professional Drift

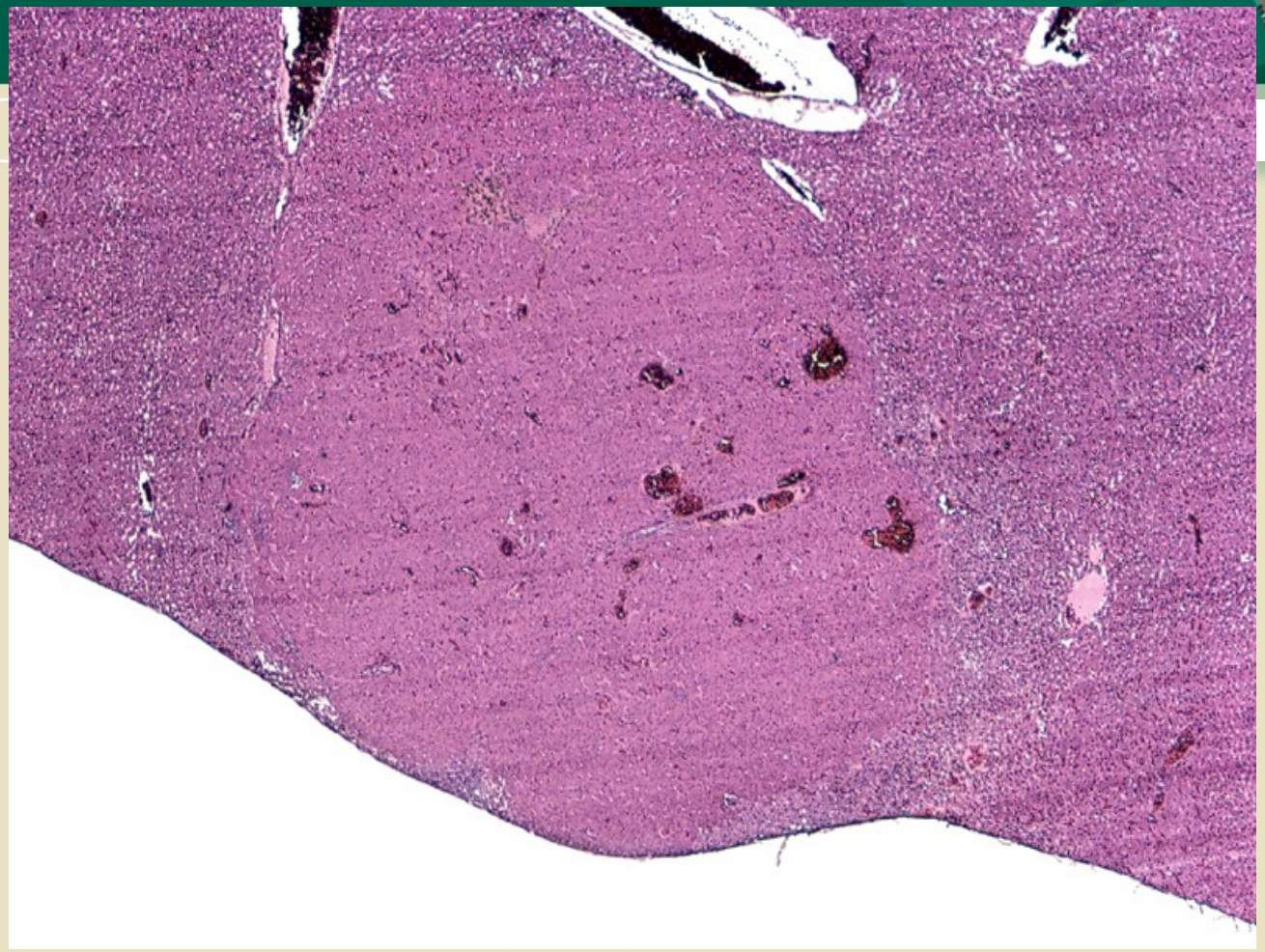
- The lesions stay the same, but the names change
- As we learn more about the biology of certain lesions, the nomenclature is changed to reflect this new knowledge



Hepatoma - 1970



Neoplastic Nodule – 1975



Hepatocellular Adenoma - 1990

Personal Drift

- Inexperienced pathologists tend to overdiagnose neoplastic changes
- Thousands of tissues later, the number of tumors diagnosed decreases
- Result of increased familiarity with spectrum of hyperplasia and neoplasia in laboratory animals, increased confidence

Operator Bias

- I missed that lesion on the last study I read
- The phone rang while I was reading that animal
- I saw the change, but it was below my threshold
- I wasn’t sure what to call it, so I picked the closest term in our lexicon
- I think it’s an artifact/function of cut

How to Control Diagnostic Drift

CONSISTENCY

CONSISTENCY

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Pathology Peer Review

REASONS FOR PATHOLOGY PEER REVIEW

- Ensure data meets requirements of regulatory agencies
- Increase accuracy of data
- Increase confidence in data
- Confirm target organs
- Confirm no effect level (NOEL)

REASONS FOR PATHOLOGY PEER REVIEW

- Ensure consistency of diagnoses within the study
- Intraorganizational harmonization of nomenclature and diagnostic criteria
- Continuing education

Is Formal Peer Review Required by Regulatory Agencies?

- Sometimes Yes and
- Sometimes No

PESTICIDE REGULATION (PR) 94-5 NOTICE TO REGISTRANTS OF PESTICIDE PRODUCTS

1994 - Background

- The Office of Pesticide Programs receives requests for reconsideration of Peer Review Decisions based on reevaluations of the pathology readings.
- A voluntary activity on the part of the registrants.
- The Agency is asked to disregard the original readings and base its evaluation on the most recent ones.

PESTICIDE REGULATION (PR) 94-5 NOTICE TO REGISTRANTS OF PESTICIDE PRODUCTS

1994 - Background

- The Agency may have two (or at times even more) pathological diagnoses for the same study.
- The Agency is instituting a procedural requirement for any voluntary submissions of revised pathology diagnoses.

PESTICIDE REGULATION (PR) 94-5 (continued)

Policy and Rationale

- The Agency believes that a procedure for obtaining consensus in pathology re-reads will improve the quality of decision-making in classifying pesticide chemicals having carcinogenic potential.
- Unless re-reads have been conducted using a peer review procedure, the Agency will base its evaluations upon the original readings.
- For any target tissue being reevaluated, all slides containing that tissue in all dose groups, as well as controls, must be re-read by the peer refview pathologist.

Peer Review and Regulatory Agencies

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

Peer Review and Regulatory Agencies

6. REPORTING ON CARCINOGENICITY STUDIES

6.1 General principles

Pre-neoplastic and neoplastic lesions should be described in conventional histopathological terms according to commonly used classifications (e.g. ILSI, STP, IARC, RENI and other recent texts on rodent pathology). Deviations from standard diagnoses should be explained in the report.

Ideally, one pathologist should be responsible for the histological evaluation. If several pathologists are involved, slides from all treatment groups must be distributed evenly among them. Peer-review of slides is required for all identified target organs and for at least 10% of all tumours. A complete review of 10% of the animals in each group 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. Board certification or equivalent should qualify pathologists.

Peer Review and Regulatory Agencies

Unclassified

ENV/JM/MONO(2002)19

Organisation de Coopération et de Développement Economiques
Organisation for Economic Co-operation and Development

04-Sep-2002

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

Cancels & replaces the same document of 28 August 2002

ENV/JM/MONO(2002)19
Unclassified

OECD Environment, Health and Safety Publications.
Series on Testing and Assessment No. 35 and Series on Pesticides No. 14

GUIDANCE NOTES FOR ANALYSIS AND EVALUATION OF CHRONIC TOXICITY AND CARCINOGENICITY STUDIES

Peer Review and Regulatory Agencies

While it is highly important that the study pathologist uses standardised diagnostic criteria, the reliability of diagnosis is greatly enhanced by comprehensive quality assurance and peer review. Some laboratories, such as the US National Toxicology Program, institute these procedures to ensure diagnostic criteria are applied consistently. This subject is discussed further by Ward *et al.* (1995), Boorman *et al.* (1985 & 1986) and Hardisty & Boorman (1986).

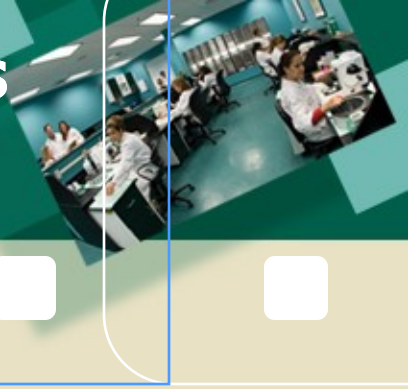
Pathology Peer Review

- Performed by a second pathologist
- Routinely performed by many companies
- May also be done to address specific issues
- Involves a subset of tissues from initial evaluation

PROSPECTIVE PEER REVIEW

- Frequently used to finalize study data
- Evaluation of a pathologist's original findings by an informed reviewer
- General procedures included in Study Protocol
- Data in final report documents results of prospective peer review

CHRONIC TOXICITY/ONCOGENICITY IN RATS OR MICE



Technical Approach

- Review of all tissues from 10% of control and 10% of high-dose male and female animals selected randomly.
- Review of all reported proliferative lesions.
- Review of potential target organs from all animals in all groups for specific toxicologic endpoints to verify the probable “no observed effect level.”
- Resolution of all differences of opinion with the study pathologist.