www.apt-pharmatox.com

APPLIED PHARMACOLOGY AND TOXICOLOGY, INC.

Consulting & Research Services

Criteria Requirements for Data-Driven Carcinogenicity Mode of Action (MoA) Determinations as Exemplified by Chloroform Carcinogenicity

Alliance for Risk Assessment

Beyond Science and Decisions: From Problem Formulation to Dose-Response Assessment: Summary of Case Study

May 22-24, 2012.

Austin, Texas

Christopher J. Borgert, Ph.D.

Problem Formulation

- Updated assessments continue to strengthen non-linear MoA-based approaches for assessing carcinogenesis.
- Biologically based models continue to refine extrapolations to human cancer risks.
- *Clear criteria have yet to be defined for determining when departure from a policy-based default model is justified and should be used so that the policy is consistent with the best available science.*
  - *Chloroform: data - rich; already assessed by threshold models*
- Without criteria, policy decisions regarding use of MoA-based approaches tend to rely on subjective, case-by-case judgments about what constitutes sufficiency of the data for any particular chemical.
- Clear criteria would enhance scientific integrity and transparency in cancer risk assessments and would facilitate consistency in policy-making.

Guiding Concerns

- Flexibility
  - Evaluation of MoAs that have been defined by approaches other than HRF
  - Evaluation using either Critical Reviews or Primary Data.
- *Compatible but not redundant with HRF*
  - HRF widely used and supported … but …
  - Redundancy would introduce a “test set / validation set” conundrum: self-fulfilling when HRF was used.
- Widely accepted, recognizable basis

Methodology

Critical reviews and consensus panel reports regarding chloroform's carcinogenic MoA were evaluated *according to a subset of components* from a recently published, hypothesis-driven weight of evidence framework (Borgert et al. 2011):

1. *Define specific hypotheses to be evaluated*
2. Systematically search, *review and select data relevant to each hypothesis*
3. *Evaluate* the primary, secondary, and *tertiary validity of each study*
4. Weight endpoints quantitatively if possible; if not, qualitatively
5. Weight responses quantitatively
6. Combine weightings for endpoints and responses to derive overall WoE score
7. Develop narrative for overal WoE determination

Data Validity

- 1˚ Validity (Minimal Epistemic Status – Gori's Three Tenets)
- 2˚ Validity (Reliability and Transparency; All the data)
- 3˚ Validity (Relevance and Probative Design; Predictivity)

Minimum Requirements of Scientific Evidence Are Few *but* Firm

1. measurements have been authentically identified within an explicit margin of error that is testable and reproducibly small enough to avoid ambiguity;
2. extraneous factors that could affect the measurements and conclusions have been measured and adequately controlled;
3. measurements and conclusions have been consistently replicated by independent investigators.

Gori GB. 2002. Considerations on guidelines of epidemiologic practice. *Ann Epidemiol. 12(2):* 73-8.

Gori GB. 2010. Regulating unknown risks. *Regulation (*Spring Issue)*:16-21.*

Klimisch Codes: Klimisch et al., 1997

- "test species, test substances (purity, origin), number of animals evaluated, scope of investigations per animal (e.g., clinical chemistry, organ weights, hematology, histopathology), description of changes or lesions observed, control and historical control groups, test conditions, route of administration, dose schedule and dose concentration (including analytical verification)"
- diets, composition of water bottles and cage materials, bedding, stressors such as handling and manipulation, and any other factors that could affect hormonal systems, as well as details on the mathematical and statistical algorithms used to analyze the data
- GLP; Guideline Studies;
- Published studies
- **All Data Should Be Reported**

- Counterfactual Study Design:

  Counterfactual experiments test whether the effect of interest still occurs when a putative causal step is prevented under conditions that would otherwise produce the effect of interest.

  - Sonich-Mullen et al. 2001 - IPCS Framework

- Predictive Value

  1. Positive: Fraction of positive tests that correctly indicate the condition.
  2. Negative: fraction of negative tests that correctly indicate absence of the condition.
  3. Actual incidence of the condition

Causality in Pharmacology, Toxicology and Epidemiology: *The Counterfactual*

- Demonstrating that a specific relationship exists between elements of the physical world – e.g., causality – requires more than the mere temporal coincidence; it requires the *counterfactual.*
- David Hume, who wrote that a cause is ". . . an object followed by another . . .where, if the first object had not been, the second never had existed."
- Does R still occur when Q, and only Q, is eliminated or blocked?
- Beyond capability of most epidemiology studies;
- Implicit in pharmacology and clinical medicine.
- Hill's Aspects must be evaluated using data that comport with Gori's three tenets.

Methodology

Each Review / Consensus Panel Report Was Evaluated According To the Following:

a) Support for the particular key event (hypothesis) in the MoA.

b) Evaluation or discussion of data quality supporting (or refuting) the key event.

c) Evaluation or discussion of counterfactual concepts in experimental design and interpretation for data supporting the key event.

d) Evaluation or discussion of alternative hypotheses or data interpretations regarding the key event.

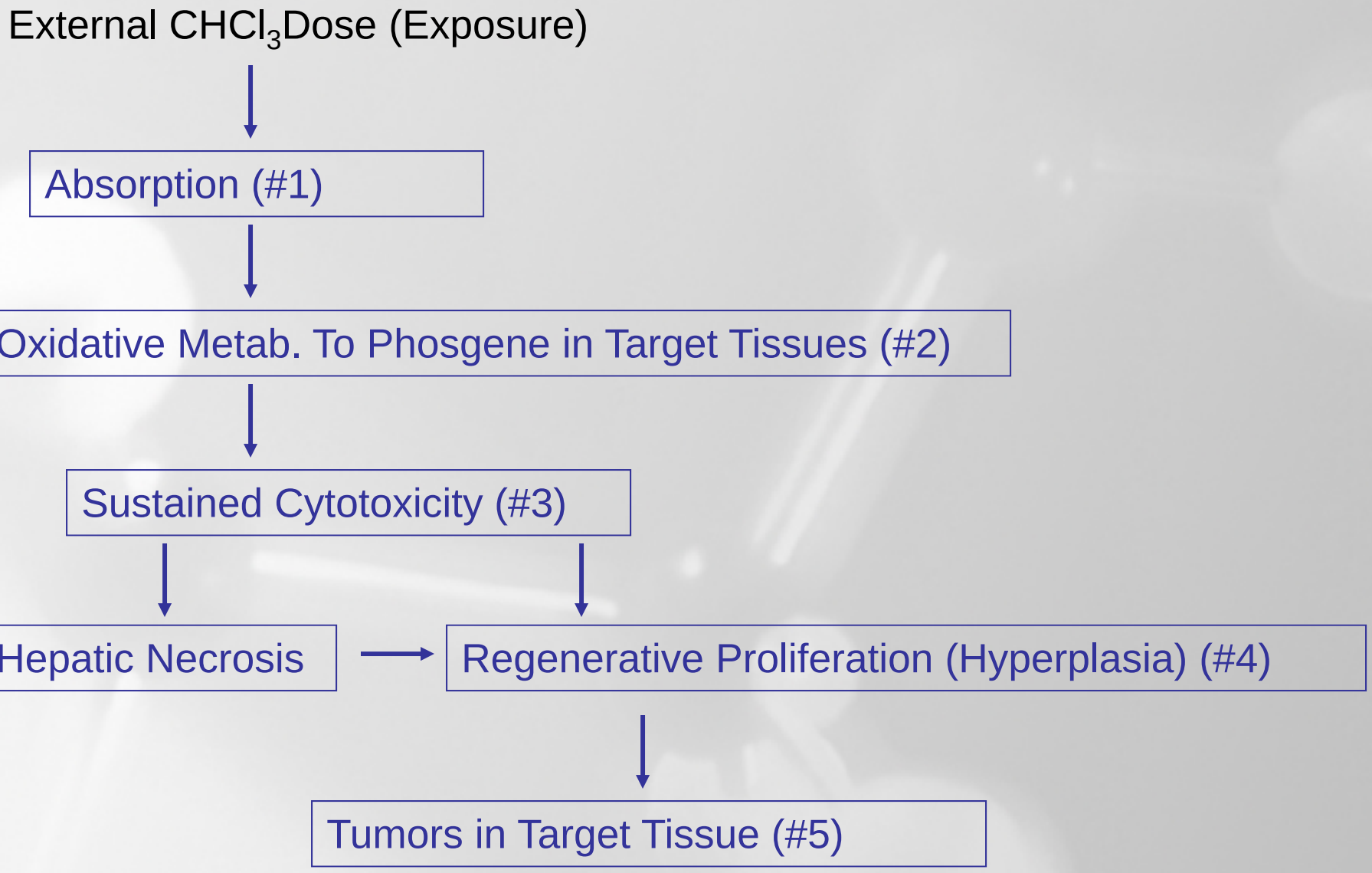
Methodology

- These components allow the various critical reviews and consensus conclusions to be evaluated according to a consistent metric.

- Various elements of the approach are compatible with, but not redundant to, HRF considerations:
  - Hypothesis Testing & Alternative Hypotheses
  - Data Quality
  - Counterfactual Studies

From an analysis of components a) - d) for chloroform, criteria were derived to allow data-driven carcinogenicity risk determinations in place of default carcinogenicity assumptions whenever the data for a specific chemical are similarly compelling.

MoA: CHCl3 Carcinogenesis



Methodology

The five key events in chloroform's MoA are:

1. Absorption and distribution to target tissue(s).

2. Oxidative metabolism of chloroform by the P450 enzyme CYP2E1 to highly reactive phosgene.

3. Sustained cytotoxicity to target cells, hepatocytes and / or renal proximal tubular epithelial cells.

4. Regenerative cell proliferation in liver and / or kidney.

5. Threshold development of tumors in liver and / or kidney.

Results

- All four weight of evidence components have been addressed for all five key events in chloroform's carcinogenic MoA, including a threshold for tumor development in rodent liver and kidney.
  - Although some key events were unaddressed in some evaluations, none made countervailing conclusions regarding the five key events.

Results

- Animal tumors have been assessed from predominantly oral exposure, with some data from inhalation routes.
  - Only pharmacokinetic assessments are available from dermal exposure.
  - The physical-chemical properties, pharmacokinetic behavior, role of metabolism in toxicity and tumor development, and experimental data from dermal exposure, albeit limited, would predict no deviation from this MoA irrespective of the route of exposure.

Results

Dermal & Inhalation Exposure

- Chloroform is a small, lipophilic molecule well absorbed through biological membranes.
- Chloroform carcinogenicity is dependent on tissue CYP2E1.
- A single gene for CYP2E1 is common to all tissues, including skin and lung.
- Dermal absoprtion may contribute 1-28% of total daily chloroform dose.
- *"CYP2E1 substrates was not significantly affected by accounting for extrahepatic metabolism. These findings imply that liver-only metabolism may be a reasonable simplification for PBPK modeling of lipophilic VOCs, and the PBPK predictions using this assumption can be applied confidently."* Yoon et al. 2007

Results

- Three of the five key events in chloroform's carcinogenic MoA appear to exhibit a biological threshold.
  - Cytotoxicity*
  - Regenerative proliferation
  - Tumor development

- Counterfactual evidence* is compelling for three key events, two of which demonstrate a biological threshold.
  - Oxidative metabolism by CYP2E1
    - Gene knockout mouse study
  - Regenerative proliferation
    - Not observed with drinking water exposure at doses higher than those that induce regenerative proliferation by gavage
  - Tumor development
    - Not observed with drinking water exposure at doses higher than those that produce tumors.

Results

- Data quality issues were evaluated for most key events, including for principal alternative hypotheses that would predict a non-threshold MoA.
  - Inconsistent results; non-standard tests
- Alternative hypotheses and interpretations for each key event have been considered.
  - DNA reactivity [ICPEMC: International Commission for Protection against Environmental Mutagens and Carcinogens].
    - Maximum absolute score = 100, with negative values indicating lack of support and positive values the converse for genotoxicity.
    - The highest score obtained was + 49.7 and lowest -27.7 from the evaluation of more than 100 chemicals.
    - Forty studies on chloroform yielded a quantitative net score of -14.3, indicating strong weight of evidence support for a non-genotoxic MoA.
  - Reductive Metabolism via CYP2E1: high dose only; would exhibity a threshold

CRITERIA

1. Defined key events are consistently (not necessarily unanimously) supported among objective analyses;

2. Issues regarding data quality should not weaken support for the key events; i.e., data supporting the proposed key event should be of equal or higher quality than contradictory data;

3. At least one of the key events should be counterfactually demonstrated to exhibit a biological threshold;

4. The MoA should not differ by routes of exposure possible in the human population (if a chemical's MoA does differ by route of exposure, the MoA evaluation should reflect those differences);

5. Alternative MoA hypotheses that would dictate a linear biological model of tumor development should be consistently ruled out or considered unlikely among objective analyses.

Strengths / Weaknesses

- Simple, Broadly Applicable
  - Reliable foundation
    - Chloroform MoA well researched & defined
    - Criteria based on fundamentals of scientific method
- Too stringent ?
  - Chloroform uniquely well characterized ?
  - Fit other MoAs ?
- Data requirements excessive ?
  - Counterfactual demonstration of threshold ?
- Other Limitations ?