Rodent Hepatic Carcinogens: Mode of Action: Framework (IPCS/ILSI)

1. Is the Weight of Evidence Sufficient to Establish a MoA in Animals?

2. Is the Animal MoA Plausible in Humans?

3. Taking Into Account Kinetic and Dynamic Factors, Is the Animal MoA Plausible in Humans?
1.) Is the Weight of Evidence Sufficient to Establish a MoA in Animals?
- Not Relevant in Humans
- Sufficient

2.) Is the Animal MoA Plausible in Humans?
- No
- Yes

3.) Taking Into Account Kinetic and Dynamic Factors, Is the Animal MoA Plausible in Humans?
- No, Not likely
- Yes
Mode of action

1. **Sequence of key events and processes**
   - starting with the interaction of the cell, proceeding through operational and anatomical changes resulting in cancer formation (USEPA 2005 cancer guidelines)

2. **Mode of action analysis** (Boobis et al 2008)
   - Strength, consistency, and specificity of the associations
   - Dose response relationships between key events and tumors
   - Temporal relationship between key events and tumors
   - Biological plausibility of key event
   - Alternatives modes of action considered
Mode Of Action And Human Relevance Of Concordance Table

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<thead>
<tr>
<th>Key Event</th>
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MultiStage Hepatic Carcinogenesis

Initiation          Promotion          Progression

Repair  Apoptosis  Apoptosis  Growth advantage and genetic instability
Damage   Proliferation  Proliferation

Normal Cell  Initiated Cell  Focal Lesion  Neoplasia

Normal  Baso Foci  Adenoma  Carcinoma
Modes Of Action (MOU) Of Hepatic Carcinogens

**DNA Reactive**

**Non DNA Reactive**

- Non Receptor Mediated
  - Cytotoxicity
  - Infectious
  - Iron / Copper overload
  - Other

- Receptor Mediated
  - PPAR alpha
  - CAR
  - PXR
  - AHR
  - Estrogen
  - Other
Mechanisms associated with hepatic carcinogenesis in the rodent Non DNA reactive

- Increase cell growth/proliferation
  - increased DNA synthesis
  - decreased apoptosis
- Selective clonal expansion of a preneoplastic population
- Modulation of intercellular communication
  - hormonal disruption/modification
  - inhibition of gap junctional intercellular communication
- Modulation of gene expression
  - hypomethylation
  - transcription factor activation
- Induction of oxidative stress
  - direct radical formation and interaction
  - decrease in antioxidants
  - inflammatory cell involvement
- Induction of specific CYPs
Mode of Action of Rodent Liver Carcinogens: 

**DNA reactive**

**Key Events**

1. Metabolic activation  
   - (If necessary)
2. DNA adduct formation
3. Mutation in gene(s) critical to carcinogenesis
4. Increased hepatocellular proliferation  
   - Cytotoxicity at high dose
5. Neoplasm formation

**Necessary data to support this MOA**

- DNA reactivity
- Increased hepatocyte proliferation  
  - measured by BrdU labeling index and/or cell number.
- Cytotoxicity (high doses)

Ex. 
- Aflatoxin B1
- DEN
- MNU.ENU
- 2- AAF
Concordance Table:
Mode Of Action And Human Relevance Of
DNA reactive

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<th>Key Event</th>
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<td>Metabolic activation (If necessary)</td>
<td>Yes</td>
<td>yes</td>
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<tr>
<td>DNA adduct formation</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Mutation in gene(s) critical to carcinogenesis</td>
<td>Yes</td>
<td>yes</td>
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<td>Increased hepatocellular proliferation</td>
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<td>yes</td>
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<td>Selective increase in focal liver lesion growth</td>
<td>Yes</td>
<td>?</td>
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<tr>
<td>Formation of neoplastic lesion</td>
<td>Yes</td>
<td>yes</td>
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Mode of Action of Rodent Liver Carcinogens:

**Non receptor mediated: Cytotoxic**

**Key Events**

1. Metabolic activation
   - (If necessary)
2. Cytotoxicity
   - (persistent)
3. Increased hepatocellular proliferation
4. Neoplasm formation

**Necessary data to support this MOA**

- Not DNA reactive
- Persistent toxicity
  - histopathology (necrosis) with or without serum enzyme changes
- Increased hepatocyte proliferation
  - measured by BrdU labeling index and/or cell number.
  - may need to collect data in a zonal way
- Reversibility
  - Upon treatment discontinued

Ex. Chloroform
## Concordance Table: Mode Of Action And Human Relevance Of Cytotoxicity

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<th>Key Event</th>
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<tr>
<td>Metabolic activation (If necessary)</td>
<td>Yes</td>
<td>yes</td>
</tr>
<tr>
<td>Cytotoxicity (persistent)</td>
<td>Yes</td>
<td>Yes (cirrhosis)</td>
</tr>
<tr>
<td>Increased hepatocellular proliferation</td>
<td>Yes</td>
<td>yes</td>
</tr>
<tr>
<td>Selective increase in focal liver lesion growth</td>
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<td>Formation of neoplastic lesion</td>
<td>Yes</td>
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Mode of Action of Rodent Liver Carcinogens: Non receptor mediated: **Metal Overload**

**Key Events**

1. Increased iron or copper accumulation in hepatocytes
2. Oxidative damage
3. Cytotoxicity
   - (persistent)
2. Increased hepatocellular proliferation
3. Neoplasm formation

**Necessary data to support this MOA**

- Demonstration of iron or copper
- Persistent toxicity
  - histopathology (necrosis) with or without serum enzyme changes
- Increased hepatocyte proliferation
  - measured by BrdU labeling index and/or cell number.

Examples. Iron Copper
## Concordance Table:
### Mode Of Action And Human Relevance Of Metal Overload

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<tr>
<td>Increase in metal accumulation</td>
<td>Yes</td>
<td>yes</td>
</tr>
<tr>
<td>Oxidative damage</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Cytotoxicity (persistent)</td>
<td>Yes</td>
<td>yes</td>
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<td>Increased hepatocellular proliferation</td>
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Mode of Action of Rodent Liver Carcinogens: **Receptor Mediated** GENERAL

**Key Events**

1. Metabolic activation  
   - (If necessary)
2. Activation of the receptor
3. Increased hepatocellular proliferation
4. Selective expansion of preneoplastic hepatocytes
5. Neoplasm formation

**Necessary data to support this MOA**

- Activation of the receptor
- Specific CYP enzyme induction
- Increased hepatocellular proliferation  
  - measured by BrdU labeling index and/or cell number.
  - Apoptosis
- Reversibility  
  - Upon treatment discontinued
Mode of Action of Rodent Liver Carcinogens: CAR Receptor mediated

**Key Events**

1. Metabolic activation
   - (If necessary)
2. Activation of the CAR receptor
3. Increased hepatocellular proliferation
4. Selective expansion of preneoplastic hepatocytes
5. Neoplasm formation

**Necessary data to support this MOA**

- Activation of the receptor
- Specific CYP enzyme induction (2B family)
  - hypertrophy
- Increased hepatocellular proliferation
- Reversibility
  - Upon treatment discontinued

![Phenobarbital](image-url)

- Sedatives
- Hypnotics, for the short-term treatment of insomnia
- Preanesthetics
- Long-term anticonvulsants for the treatment of generalized tonic-clonic and cortical-local seizures
Figure 16: Proposed Mechanism for the involvement of the Constitutive androstane receptor (CAR) in phenobarbital-induced gene expression changes. PP = protein phosphatase; CamK = CaM Kinase; RXR = retinoic acid receptor; PBREM = Phenobarbital response element. Following dephosphorylation of CAR by protein phosphatase, CAR crosses the cell membrane and becomes phosphorylated by CaM Kinase. CAR then forms a dimer with RXR and binds to PBREMs, resulting in increased gene expression.

Klaunig and Kamendulis, Casseret and Doull, 2008
Phenobarbital
B6C3F1 Mice: Hepatic Focal Lesion Percentage Volume

![Graph showing hepatic focal lesion percentage volume over different treatment durations and doses for Phenobarbital in B6C3F1 mice. The graph indicates the percentage volume of hepatic focal lesions for untreated mice and for mice treated with 10 mg PB/kg, 100 mg PB/kg, and 500 mg PB/kg over 7, 30, and 60 days.]
Phenobarbital
F344 Rats: Labeling Index in Hepatic Foci

Untreated
10 mg PB/kg
100 mg PB/kg
500 mg PB/kg

Treatment Duration
7 days 30 days 60 days

Labeling Index (%)
## Concordance Table:

**Mode Of Action And Human Relevance Of CAR Receptor mediated**

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<td>Activation of the CAR receptor</td>
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Peroxisome Proliferators (PPAR alpha activators)

- Response to peroxisome proliferators is mediated by PPAR alpha
- PPREs in PP responsive genes (ACO, CYPs)
- PPAR alpha null mouse is nonresponsive
  - Peroxisomes
  - DNA synthesis/apoptosis
  - Tumors?


A chemically diverse group of rodent carcinogens
- phthalate plasticizers: DEHP
- herbicides
- hypolipidaemic drugs: nafenopin, fenafibrate
- Organic solvents: TCE, PCE, TCA
Mode of Action of Rodent Liver Carcinogens:
**PPAR alpha Receptor mediated**

**Key Events**

1. Metabolic activation
   - (If necessary)
2. Activation of the PPAR alpha receptor
3. Increased hepatocellular proliferation /decrease apoptosis
4. Selective expansion of preneoplastic hepatocytes
5. Neoplasm formation

**Necessary data to support this MOA**

- Activation of the receptor
- Peroxisome proliferation
- Oxidative stress/damage
- Increased hepatocellular proliferation
- Reversibility
  - Upon treatment discontinued
Rodent liver

PPARα agonist

1

Non-peroxisome lipid gene expression

Cell cycle, growth and apoptosis gene expression

PPARα

2a 2b 2a

Peroxisome gene expression

Peroxisome proliferation

Oxidative stress

Apoptosis

Cell proliferation

GJIC

3a 3b

DNA damage

Preneoplastic foci

7 Clonal expansion

Tumors

NPC Kupffer

6

23
## Concordance Table:
### Mode Of Action And Human Relevance Of PPAR alpha Receptor mediated

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Modes Of Action (MOU) Of Hepatic Carcinogens

- **DNA Reactive**
- **Non DNA Reactive**
  - Non Receptor Mediated
    - Cytotoxicity
    - Infectious
    - Iron / Copper overload
    - Other
  - Receptor Mediated
    - PPAR alpha
    - CAR
    - PXR
    - AHR
    - Estrogen
    - Other
Liver carcinogens

1.) Is the Weight of Evidence Sufficient to Establish a MoA in Animals?

Not Relevant in Humans

PPAR alpha
CAR
PXR
AHR

Not Sufficient

Sufficient

2.) Is the Animal MoA Plausible in Humans?

No

Yes

3.) Taking Into Account Kinetic and Dynamic Factors, Is the Animal MoA Plausible in Humans?

No, Not likely

Yes

DNA reactive
Estrogen
Metal overloads
Infectious Cytotoxicity

Relevant or Unknown Human Relevance