Use of Mechanistic Evidence in Human Health Assessment

Vincent Cogliano
Director, EPA’s IRIS Program

National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington DC

This talk does not necessarily represent the views or policies of the U.S. Environmental Protection Agency
Conflict of Interest Statement

I have no conflicts of interests.
Overview

- Health assessments and systematic review
- Questions asked of mechanistic evidence
- Use of mechanistic evidence
  - In classification
  - Other uses
- Structured organization of mechanistic data
Health Assessments Are Extending the Boundary of Systematic Review

Multiple systematic reviews
- Does the agent cause cancer in humans?
- Does the agent cause cancer in animals?
- Does the agent cause neurotoxicity in humans?
- Does the agent cause neurotoxicity in animals?
  . . . etc

and more . . .
- Analysis of mechanistic evidence
- Exposure-response relationships for each health outcome

*Here we use processes that are structured and replicable*
Health Assessments Are Extending the Boundary of Systematic Review

Multiple systematic reviews
- Does the agent cause cancer in humans?
- Does the agent cause cancer in animals?
- Does the agent cause neurotoxicity in humans?
- Does the agent cause neurotoxicity in animals?
  . . . etc

and more . . .
- Analysis of mechanistic evidence
- Exposure-response relationships for each health outcome

Here we use processes that are structured and replicable

20% of effort and controversies
At IARC, Mechanistic Data Are Pivotal When Human Data Are Not Conclusive

<table>
<thead>
<tr>
<th>EVIDENCE IN HUMANS</th>
<th>Sufficient</th>
<th>Limited</th>
<th>Inadequate</th>
<th>ESLC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 2A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 strong evidence in exposed humans</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2A strong evidence ... mechanism also operates in humans</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Group 2B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 strong evidence ... mechanism does not operate in humans</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2A belongs to a mechanistic class where other members are classified in Groups 1 or 2A</td>
<td>Group 2B (exceptionally, Group 2A)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**EVIDENCE IN EXPERIMENTAL ANIMALS**

<table>
<thead>
<tr>
<th>Sufficient</th>
<th>Limited</th>
<th>Inadequate</th>
<th>ESLC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2A belongs to a mechanistic class</td>
<td>2B with supporting evidence from mechanistic and other relevant data</td>
<td>Group 3</td>
<td></td>
</tr>
</tbody>
</table>

**Group 3**

| 4 consistently and strongly supported by a broad range of mechanistic and other relevant data |

**Group 4**

Sufficient Limited Inadequate ESLC
The Same Is True at the EPA

EVIDENCE IN EXPERIMENTAL ANIMALS

- Sufficient
- Limited
- Inadequate
- ESLC

Carcinogenic to humans

Likely to be carcinogenic to humans

Likely

Suggestive evidence of carcinogenic potential

Inadequate information

Not likely to be carcinogenic to humans

EVIDENCE IN HUMANS

- Sufficient
- Limited
- Inadequate
- ESLC

Likely to be carcinogenic to humans

Likely to be carcinogenic to humans

Suggestive

Not likely

Not likely
Questions Asked of Mechanistic Evidence

IARC asks:

- Is there strong evidence of an operative carcinogenic mechanism(s)?
- Is the evidence from exposed humans, human in-vitro systems, or animals?
- Does the mechanism only operate in animals?
- Does the agent belong to a class of agents evaluated as Group 1 or Group 2A?

- http://monographs.iarc.fr/ENG/Preamble/instructions.php
Questions Asked of Mechanistic Evidence

The EPA asks:

- Is the hypothesized mode of action sufficiently supported in test animals?
- Is the hypothesized mode of action relevant to humans?
- Which populations or lifestages can be particularly susceptible to the hypothesized mode of action?

Uses of Mechanistic Evidence in IRIS Assessments

- **Hazard Identification**
  - Augment the human evidence by establishing the occurrence in humans of precursor events
  - Determine relevance of animal results to humans
  - Identify susceptible populations and lifestages

- **Dose-Response Assessment**
  - Suggestion of modeling approaches and parameters
  - Data for dose-response modeling
  - Data for relative potencies within a class of agents
Carcinogenic to humans (IARC 2006):

“Exceptionally, an agent may be placed in this category when evidence of carcinogenicity in humans is less than sufficient but there is sufficient evidence of carcinogenicity in experimental animals and strong evidence in exposed humans that the agent acts through a relevant mechanism of carcinogenicity.”

Carcinogenic to humans (EPA 2005):

- Strong human evidence for cancer or precursors
- Extensive animal evidence
- Mode-of-action and key precursors have been identified in animals
- Strong evidence that precursors are anticipated to occur in humans and progress to tumors
Not classifiable as to its carcinogenicity to humans (IARC 2006):
“evidence of carcinogenicity is inadequate in humans but sufficient in experimental animals . . . when there is strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans”

Not likely to be carcinogenic to humans (EPA 2005):
“convincing and extensive experimental evidence showing that the only carcinogenic effects observed in animals are not relevant to humans”
Strong Mechanistic Evidence Can—by Itself—Identify a Possible Carcinogen

- “In the absence of data from conventional long-term bioassays or from assays with neoplasia as the end-point, consistently positive results in several models that address several stages in the multistage process of carcinogenesis should be considered in evaluating the degree of carcinogenicity in experimental animals.”

  - IARC Scientific Publication 147 (1999)

- “An agent may be classified in this category [possibly carcinogenic to humans] solely on the basis of strong evidence from mechanistic and other relevant data.”

  - Preamble to the IARC Monographs (2006)
Using Mechanistic Evidence To Identify Human Carcinogens

Case Study from IRIS: Benzo[a]pyrene (under review)

- Extensive demonstration of carcinogenicity in multiple animal species exposed via multiple routes of administration
- Increased cancer risks, particularly in lung and skin, in humans exposed to different PAH mixtures containing benzo[a]pyrene
- Strong support linking benzo[a]pyrene to DNA-reactive metabolites and key mutational events in genes that can lead to tumor development
- Specific DNA adducts and characteristic mutations in oncogenes and tumor-suppressor genes observed in humans exposed to PAH mixtures

Draft conclusion: Benzo[a]pyrene is carcinogenic to humans.

- http://www.epa.gov/iris/
Using Mechanistic Evidence To Identify Human Carcinogens

Case Study from IRIS: Ethylene Oxide (under review)

- Strong human evidence of lymphohematopoietic cancers and breast cancer in exposed workers
- Extensive animal evidence of carcinogenicity: lymphohematopoietic cancers in rats and mice; mammary carcinomas in mice
- Clear evidence of genotoxicity and support for a mutagenic mode-of-action
- Strong evidence that key events occur in humans and progress to tumors, including evidence of chromosome damage in exposed humans

Draft conclusion: Ethylene oxide is carcinogenic to humans.

- http://www.epa.gov/iris/
## IARC Group 1 Classifications Based on Genetic Toxicity in Exposed Humans

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mechanistic Rationale</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylene oxide</td>
<td>Genotoxic, cytogenetic effects in human lymphocytes</td>
<td>1994</td>
</tr>
<tr>
<td>NNN and NNK</td>
<td>Uptake, metabolism, DNA and haemoglobin adducts in smokeless tobacco users</td>
<td>2007</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>Specific diolepoxide-induced DNA adducts, KRAS mutations in exposed humans</td>
<td>2010</td>
</tr>
<tr>
<td>MOCA</td>
<td>DNA adducts and micronuclei in urothelial cells of exposed workers</td>
<td>2010</td>
</tr>
<tr>
<td>Aristolochic acid</td>
<td>A:T → T:A transversions in TP53 of patients with nephropathy or urothelial tumours</td>
<td>2011</td>
</tr>
<tr>
<td>Etoposide</td>
<td>Translocations on MLL gene</td>
<td>2011</td>
</tr>
<tr>
<td>Ultraviolet radiation</td>
<td>C → T transition in human TP53 in solar keratosis and skin tumours</td>
<td>2011</td>
</tr>
</tbody>
</table>
IARC Group 1 Classifications Based on Mechanistic Evidence

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mechanistic Rationale</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,7,8-TCDD</td>
<td>Ah receptor binding, subsequent effects</td>
<td>1997</td>
</tr>
<tr>
<td>2,3,4,7,8-Pentachlorodibenzofuran</td>
<td>Same Ah receptor pathway as 2,3,7,8-TCDD</td>
<td>2011</td>
</tr>
<tr>
<td>12 dioxin-like PCB congeners</td>
<td>Extensive evidence of an Ah-receptor-mediated mechanism of carcinogenesis that is identical to that of 2,3,7,8-TCDD</td>
<td>2015</td>
</tr>
</tbody>
</table>
# IARC Group 1 Classifications Based on Other Relevant Data

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mechanistic Rationale</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutron radiation</td>
<td>Similar but more severe than gamma-rays</td>
<td>2001</td>
</tr>
<tr>
<td>Areca nut</td>
<td>Primary ingredient in all betel quid preparations</td>
<td>2004</td>
</tr>
<tr>
<td>Ethanol in alcoholic beverages</td>
<td>Primary ingredient in all alcoholic beverages</td>
<td>2010</td>
</tr>
<tr>
<td>Acetaldehyde associated with alcoholic beverages</td>
<td>Higher risks for cancers of the oesophagus and upper aerodigestive tract in ALDH-deficient populations</td>
<td>2011</td>
</tr>
</tbody>
</table>

Mechanistic Data Are Used to Discount Animal Results—but There’s a Trap

- Suppose two modes-of-action operate to cause tumors at a site either MOA1 or MOA2 \( \Rightarrow \) Tumor
- Suppose only one mode-of-action is investigated and we ask whether MOA1 \( \Rightarrow \) Tumor
- What happens when we examine the “modified Hill criteria” for MOA1:
  - Strength and consistency?
  - Dose-response?
  - Temporal relationship?
  - Biological plausibility and coherence?
- This could fool us into thinking that MOA1 is the cause of the tumors
- If we conclude that MOA1 is not relevant to humans, then we might dismiss the tumor response prematurely!
Some Questions That Avoid the Correlation-Implies-Causation Trap

- Are there strong data to establish each mechanism?
  - Are there consistent results in different experimental systems?
  - Is the overall database coherent?
- Has each mechanism been challenged experimentally?
  - Do studies demonstrate that suppression of key mechanistic processes leads to suppression of tumour development?
- Are there alternatives:
  - Could multiple mechanisms be involved?
  - Could different mechanisms operate in different dose ranges, in humans and experimental animals, or in a susceptible group?

Uneven support for different mechanisms may reflect disproportionate resources focused on one mechanistic hypothesis.
There’s so much more that we can do with mechanistic evidence . . .
Using Mechanistic Evidence:
1. Resolve Difficult-to-Interpret Results

Case Study from IARC: Ingested Nitrate and Nitrite

- The epidemiologic studies are perplexing
  - Nitrate in food: no observed cancer risk, especially among people with the highest nitrate intake
  - Nitrate in drinking water: not informative, but studies were few and concentrations were low
  - Nitrite in food: consistent, positive associations with stomach cancer; the strongest associations were in people with high nitrite and low vitamin C intake
- Ingested nitrate (NO$_3^-$) is reduced to nitrite (NO$_2^-$) by oral bacteria
  - This raises even more questions, as there should be little difference between nitrate in food and nitrite in food
Using Mechanistic Evidence: 1. Resolve Difficult-to-Interpret Results

- Mechanistic data further describe the endogenous nitrate cycle
  - Ingested nitrate ($\text{NO}_3^-$) is reduced to nitrite ($\text{NO}_2^-$) by oral bacteria
  - Nitrite reacts with amines and amides in the stomach to form $N$-nitroso compounds
  - These reactions can be inhibited by the presence of vitamin C or other antioxidants
- Studies in experimental animals support these statements

Conclusion

“Ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Group 2A).”

Using Mechanistic Evidence: 2. Specify the Agent Being Evaluated

Case Study from IARC: Shift-work that involves circadian disruption

- Human evidence is *limited*, based on increased risks of breast cancer in nurses and flight attendants.
- Animal studies show carcinogenic effects from exposure to constant light, to dim light at night, from simulated jet lag, from reduced melatonin levels, or from pineal gland removal.
- Mechanistic studies show that exposure to light at night suppresses melatonin production, deregulates circadian genes and promotes tumour development and immunodeficiency.

Conclusion

“Shift-work that involves circadian disruption is *probably carcinogenic to humans* (Group 2A)”

Using Mechanistic Evidence:
3. Facilitate Preventive Actions

Case Study from IARC: Aristolochic acid

- Unintentional exposure to plants of the genus *Aristolochia* caused clusters of renal nephropathy and renal cancer
  - Weight-loss pills in Belgium
  - Corn fields in the Balkans infiltrated with *Aristolochia* plants
- IARC (2002) classified *Aristolochia* plants in Group 1 and aristolochic acid (found in these plants) in Group 2A
- Subsequent research found aristolochic-acid-specific DNA adducts and A:T-->T:A transversions in *TP53* genes

Conclusion

“Aristolochic acid is carcinogenic to humans (Group 1)”
Using Mechanistic Evidence:
4. Identify Susceptible Groups

Case Study from IARC: Acetaldehyde and alcoholic beverages

- The major alcohol-metabolizing enzymes are
  - ADH, which oxidizes alcohol to acetaldehyde
  - ALDH, which detoxifies acetaldehyde to acetate
- A variant allele (ALDH2*2) is highly prevalent in some Asian groups
- Compared with carriers of the genotype that encodes the active enzyme (ALDH2*1/*1), heterozygous carriers (ALDH2*1/*2) have 10% of ALDH activity and 12 times the risk of esophageal cancer
- Homozygous carriers of the variant (ALDH2*2/*2) generally abstain

Conclusion

“Acetaldehyde associated with alcoholic beverages is (Group 1).”
Using Mechanistic Evidence: 5. Looking Forward . . .

Transcriptomics microarray analysis

1. Identify studies
2. Analyze raw data
3. Assess data quality
4. Identify genes that are reproducibly active across studies

Mechanistic Studies Usually Dominate the Database

- **Cancer in humans**: 10s of studies, max
- **Cancer in animals**: 10s of studies, max
- **Mechanistic data**: 100s to >1000 studies
Systematic Review of Mechanistic Studies

1. Identify all pertinent studies through well-documented literature searches
2. Develop an organized inventory of studies to facilitate subsequent analyses
3. Evaluate key groups of studies using uniform criteria

*The objective is to analyze the numerous mechanistic database efficiently*
# 10 Key Characteristics of Human Carcinogens

<table>
<thead>
<tr>
<th>Key characteristic:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Electrophilic or ability to undergo metabolic activation</td>
</tr>
<tr>
<td>2. Genotoxic</td>
</tr>
<tr>
<td>3. Alters DNA repair or causes genomic instability</td>
</tr>
<tr>
<td>4. Epigenetic Alterations</td>
</tr>
<tr>
<td>5. Oxidative Stressor</td>
</tr>
<tr>
<td>6. Induces chronic inflammation</td>
</tr>
<tr>
<td>7. Immunosuppressant</td>
</tr>
<tr>
<td>8. Modulates receptor-mediated effects</td>
</tr>
<tr>
<td>9. Immortalization</td>
</tr>
<tr>
<td>10. Alters cell proliferation, cell death, or nutrient supply</td>
</tr>
</tbody>
</table>

IARC Monographs
Volume 100

http://monographs.iarc.fr/ENG/Preamble/instructions.php
# 10 Key Characteristics of Human Carcinogens

<table>
<thead>
<tr>
<th>Key characteristic</th>
<th>Examples of pertinent evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Electrophilic or ability to undergo metabolic activation</td>
<td>Parent compound or metabolite with an electrophilic structure (e.g. epoxide, quinone, etc.), formation of DNA and protein adducts</td>
</tr>
<tr>
<td>2. Genotoxic</td>
<td>DNA damage (DNA strand breaks, DNA-protein crosslinks, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g. chromosome aberrations, micronucleus formation)</td>
</tr>
<tr>
<td>3. Alters DNA repair or causes genomic instability</td>
<td>Alterations of DNA replication or repair (e.g. topoisomerase II, base-excision or double-strand break repair)</td>
</tr>
<tr>
<td>4. Epigenetic Alterations</td>
<td>DNA methylation, histone modification, microRNAs</td>
</tr>
<tr>
<td>5. Oxidative Stressor</td>
<td>Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g. DNA, lipids)</td>
</tr>
<tr>
<td>6. Induces chronic inflammation</td>
<td>Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production</td>
</tr>
<tr>
<td>7. Immunosuppressant</td>
<td>Decreased immuno-surveillance, immune system dysfunction</td>
</tr>
<tr>
<td>8. Modulates receptor-mediated effects</td>
<td>Receptor in/activation (e.g. ER, PPAR, AhR) or modulation of exogenous ligands (including hormones)</td>
</tr>
<tr>
<td>9. Immortalization</td>
<td>Inhibition of senescence, cell transformation</td>
</tr>
<tr>
<td>10. Alters cell proliferation, cell death, or nutrient supply</td>
<td>Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell-cycle control, angiogenesis</td>
</tr>
</tbody>
</table>
Using the 10 Key Characteristics to Identify Pathways to Carcinogenesis

1. Electrophilic or can be metabolically activated
2. Genotoxic
3. Alters DNA repair or causes genomic instability
4. Induces epigenetic alterations
5. Induces oxidative stress
6. Induces chronic inflammation
7. Immuno-suppressive
8. Modulates receptor-mediated effects
9. Causes immortalization
10. Alters cell proliferation, cell death, or nutrient supply

Mutagenesis

Neoplasia
Using the 10 Key Characteristics to Identify Pathways to Carcinogenesis

1. Electrophilic or can be metabolically activated
2. Genotoxic
3. Alters DNA repair or causes genomic instability
4. Induces epigenetic alterations
5. Induces oxidative stress
6. Induces chronic inflammation
7. Immuno-suppressive
8. Modulates receptor-mediated effects
9. Causes immortalization
10. Alters cell proliferation, cell death, or nutrient supply

Mutagenesis

Neoplasia
Some Observations about Recent Mechanistic Findings

- Many agents operate through multiple mechanisms—do not stop when one mechanism is described
- The genotoxic/non-genotoxic dichotomy is overly simplistic and may lead to false conclusions; there can be interactions between genotoxic and non-genotoxic mechanisms
- Some mechanisms other than mutagenicity (for example, receptor mechanisms or hormone disruption) may be active at very low doses
- Some populations can be highly susceptible to particular mechanistic events

Mechanistic data can be used for more than classification or discounting animal studies