Can Mode of Action Predict Mixture Toxicity for Risk Assessment?

Borgert et al. 2004. TAAP Vol *201*(2): 85-96.

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Definitions & Concepts

- **Mechanism of action:** a molecular sequence of events from absorption of an effective dose to production of a specific biological response.
- **Mode of action:** a set of physiological, biochemical and behavioral signs characterizing a specific biological effect.
  - Mechanism includes mode, but not necessarily vice versa.

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Focus on Mechanistic Data

- How are concepts of "mode of action" and "mechanism of action" used in regulatory guidance on risk assessment?
- What data is needed to fulfill regulatory guidance on "mode" and "mechanism" of action?
- Is this sufficient mechanistic information to predict mixture toxicity?

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Mode / Mechanism of Action in Risk Assessment

- USEPA Cancer Assessment Guidelines
- USEPA Mixture Risk Assessment Guidance
- USEPA Guidance on Identifying Pesticides with Common Mechanisms of Toxicity
- ATSDR Guidance on Interaction Profiles

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Mode of Action Used To:

- Establish that a threshold exists for the carcinogenic effects of a particular chemical.
- Establish that two chemicals have a similar mechanism (i.e., mode) of toxicity.
  - Predict the dose-response characteristics of chemicals in mixtures.

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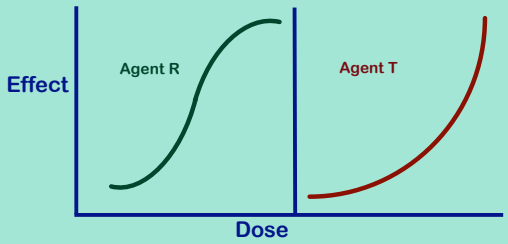
Predictions



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Interactions

Synergism

More response from a given dose / lower dose required to achieve a given level of response . . . *than expected.*

Antagonism

Less response from a given dose / higher dose required to achieve a given level of response . . . *than expected.*

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What is "Expected"?
2 Classical Models for Non-Interaction

Loewe Additivity
[Dose Additivity]

- No self-interaction
- Agents act as simple dilutions (potency, DRC)
- Sum doses & potencies of each agent
- $D_a/D_A + D_b/D_B = 1$

Bliss Independence
[Response Additivity]

- Statistical independence
- Relative effect of A not influenced by B
- Sum effects of each agent
- $E_{A+B} = E_A + E_B - (E_A \times E_B)$

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Simplifications for Mixture Risk Assessment

- Chemicals do not interact (synergize or antagonize) in producing effects.
- Chemicals with similar mechanistic features (Modes) exhibit dose additivity for a specific effect (Loewe: single drug = no interaction)
- Chemicals with dissimilar mechanistic features (Modes) exhibit response additivity for a specific effect (Bliss: A acts as if B is not present)

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What is "Non-Interaction"

- Many possible models for non-interaction
  - Quantitative, *Not* Qualitative (Math, *Not* Biology)
  - Dependent on Doses *And* Ratios
- No strict correlation with pharmacodymic or pharmacokinetic behavior
  - So why dose-addition & response-addition?
- Choice based on convention and empiricism

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Convention

Berenbaum MC, 1981. Criteria for analyzing interactions between biologically active agents. Advances in Cancer Research 35: 269-335.

Now, a combination that must, by definition, always show zero interaction *between agents* is the spurious "combination" of an agent with itself, in any arrangement of doses. This must hold, irrespective of the nature of the dose-response curve of the agent or the type of effect measured. Whether the agent shows self-interaction or not, it is axiomatic that a combination of particular doses of one and the same agent must have the same effects as the sum of those doses, because the "combination" and the sum identical. (p288)

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Similar Modes of Action Produce Dose Additivity (?)

- Pozzani et al. 1959 } vapors additive within 2 Std.Dev.
- Smyth et al. 1969 } 27 chems. additive within 5-fold
- Ikeda, 1988 } Lit. survey - additivity prevalent

- Feron et al. 1995
- Jonker et al. 1996
- Groten et al. 2000

} Dose-dependence of additivity; irritants and nephrotoxicants

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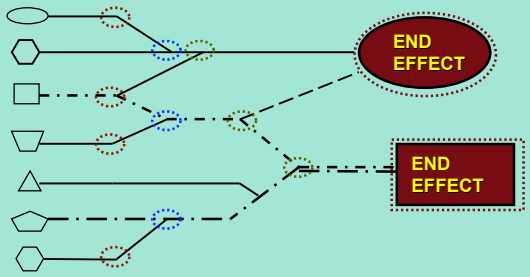
Similar Modes of Action Produce Dose Additivity (?)

- Hermans et al. 1984, 1985, 1988. } Non-polar narcosis
- McCarty and McKay, 1993. } 0.3 - 0.02 of specific NOAEL does not produce additivity by same mechanism.
- Freidig et al. 1999. } Multiple mechanisms of action requires separate evaluations for each effect.

Mode of Action Classification Criteria

	EPA CanR	ILSI CumR	EPA Mix	ATSDR	TEF Safe, 1998
Molecular target	x	x	x	x	x
Cellular target	x		x		x
Physiological target	x				
Target organ	x	x	x	x	x
Toxic intermediates	x	x			x
Causality of steps	x				
Pharmacokinetics	x				x
Detox. pathways	x				x
Parallel DRCs	x		x		x
Dose Addition					

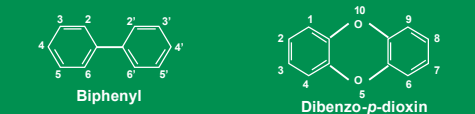
Where Does Mode End and Mechanism Begin?



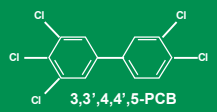
Assumptions of TEF Approach

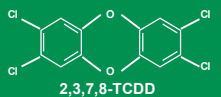
- Chemical congeners;
- Same molecular targets;
- Same biochemical pathways;
- Similar pharmacokinetic characteristics;
- Similar detoxification/elimination pathways;
- Identical tissue- and organ-level toxic manifestations;
- Parallel dose-toxicity curves;
- Non-interaction (dose additivity in mixtures);
- Simplifies mixture assessments.

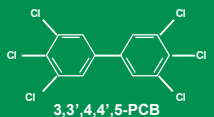
Safe, 1998

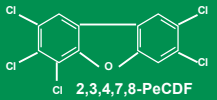

Biphenyl

Dibenzo-*p*-dioxin

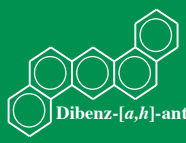

3,3',4,4',5-PCB


2,3,7,8-TCDD


3,3',4,4',5-PCB

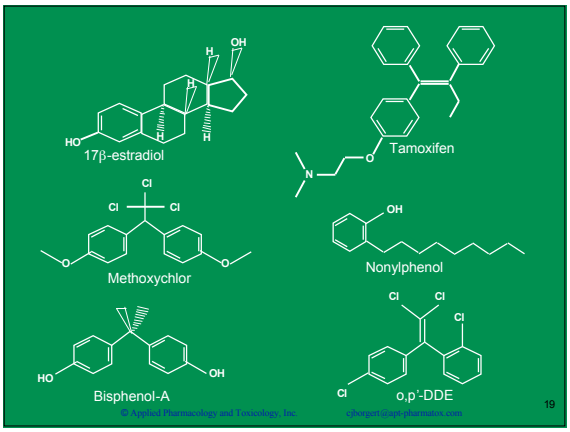

2,3,4,7,8-PeCDF


Benzo-[*a*]-pyrene


Dibenz-[*a*,*h*]-anthracene


Chrysene


Benzo-[*a*]-anthracene



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Common "Mechanism" Criteria

ILSI Panel (Mileson et al., 1998)

Ethanol / Methanol

	Repro	Devel	CNS	Hepato	Optic
critical effect	√	√	√	√	X
molecular target	√	√	√	√	X
target tissue	√	√	√	√	X
biochemical mechan.	√	√	√	√	X
toxic intermediate	√	√	√	√	X

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Levels of Biological Organization and Various Toxicity Metrics

	Dose	Biological Effect	Interaction	Adverse Effect
• Ecosystem	?			
• Community	?			
• Population	?			X
• Individual	X			
• Organ/Tissue	?	X		
• Cell	?	X		
• Molecule	?		X	

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Examples of Mechanisms Which Could Produce Dose-Dependent Transitions

Slikker et al. 2004. TAAP Vol *201*(3): 203-225.

- Absorption / Distribution / Excretion
- Metabolic handling
- Efficiency
  - DNA repair
  - Cell killing
  - Rate of cell replication
- Detoxifying enzyme systems
  - Modifying factors
- Co-substrate depletion
- Chemical transformation / activation
- Altered homeostasis
  - Essential nutrients
  - Hormones
- Repair mechanisms
- Blood flow and diffusion limitation

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Dose-Dependent Transitions in Mechanism and Interaction

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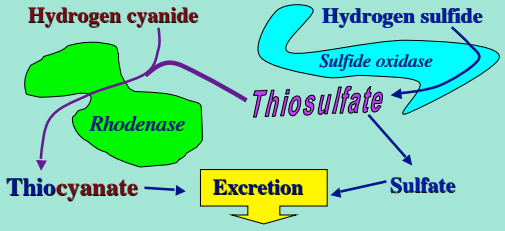

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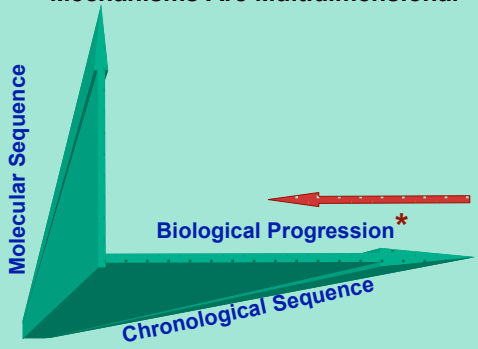
Mechanisms Are Multidimensional



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Ashby J. 2000. The Gerhard Zbinden memorial lecture. Are environmental chemicals affecting the integrity or expression of the human genome? *Toxicol Lett 112-113: 3-8.*

Toxicology is entering a new phase wherein powerful model systems will become available to predict toxicity and to study mechanisms of action. For these new techniques to achieve their potential, it will be necessary for toxicologists to pose precise questions, and to design experiments to answer those questions unequivocally..

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