Predicting Interactive Toxicity from MOA
(p'Dynamics + p'Kinetics)

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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.*

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

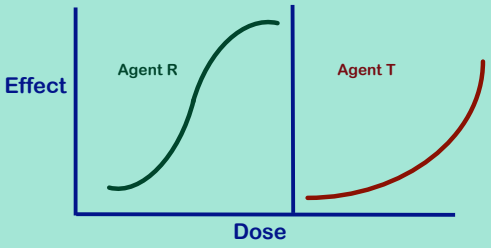
Loewe Additivity
[Dose Additivity]

- 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
- $E_{A+B} = E_A + E_B - (E_A \times E_B)$

Predictions



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
- Choice based on convention and empiricism
- No models for predicting "interaction" based on DRC

Simplifications for 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)

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 to predict mixture toxicity?
- How can omics technology address the questions about mechanism needed for risk assessment?

Modes of Toxicity

Casarett & Doull	Hayes	Sullivan&Krieger	Rand
Receptor-ligand		Receptor-med	Dioxin
+ membranes	Membrane	Lipid perox	Narcosis
Bind macromol	Carcinogen	Covalent bind	
Cell energy	Organelle		Uncoupler
Cell death		Necrosis	
Respirat	Inhalation		Respiratory
Mutations (n/l)	Genotox		
	Enzymes		AchE inhib
		Inflammation	Irritants
Immune	Immunotox	Immuno	
Nervous	Neurotoxic		Seizures
Repro	M Repro	Devel & Repro	
	F Repro		
	Endocrine		
Skin	Dermatotox		
Liver	Hepatotox		
Kidney	Nephrotox		
Eye	Eye		
Calcium			
Cardiovas			
Blood		Lethal synth. Pharmacol.	

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.

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

Mode / Mechanism of Action in Risk Assessment

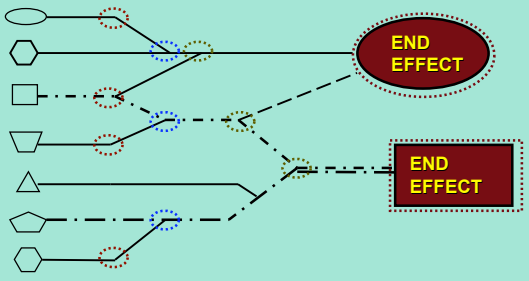
Borgert et al. 2004. TAAP Vol *201*(2): 85-96.

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

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?



Can Chemicals Be Categorized by Mode of Action ?

"It is obvious from the above survey that classification of the mechanism of action of a chemical to one specific process or site of action is difficult and not mutually exclusive. . . . Although "target molecules" have been identified for many toxic substances, the specific mechanism of and site(s) of action are poorly understood for the majority of hazardous chemicals."

[Casarett & Doull's, 1991, page 30]

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

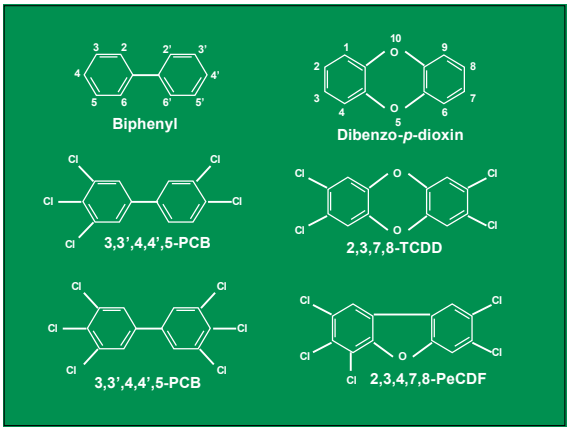
Similar Modes of Action Produce Dose Additivity (?)

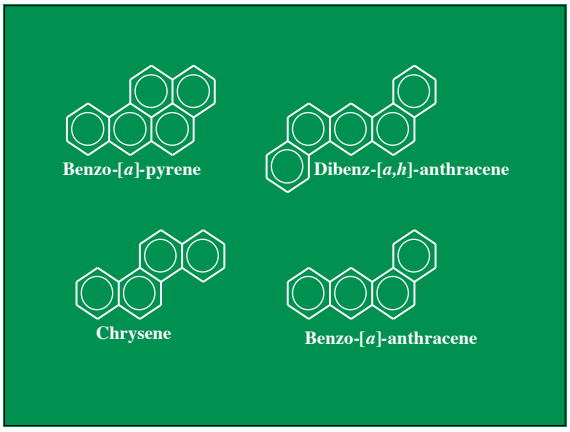
- Hermans et al. 1984, 1985, 1988. } Non-polar narcosis
- McCarty and McKay, 1993. } 0.3 - 0.02 of specific NOAEL does not produce additivity by same mechanism.
- Freidig et al. 1999. } Multiple mechanisms of action requires separate evaluations for each effect.

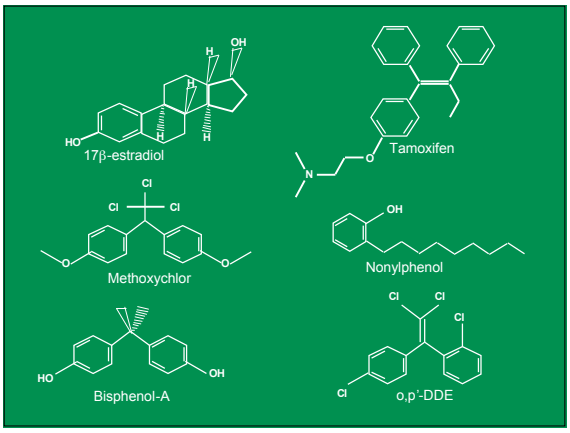
Assumptions of TEF Approach

- 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







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

Omics Technologies
Promises

- Identify DNA, RNA, protein, or metabolic profiles associated with toxic responses
- More sensitive biomarkers of exposure
- More precise biomarkers of effect
- Identification of pre-toxicological changes

Omics Technologies
Promises (cont'd)

- Identify molecular mechanistic steps
- Elucidate complete toxicologic pathways
- Categorize chemicals by polyomic profiles
- Identify fundamentally similar modes of toxic action

Mode and Mechanism in Understanding Omics for Risk Assessment

With respect to risk assessment, it is important to distinguish "mechanism of action" from "mode of action." Transcript profiling can certainly aid in the latter, but the former is absolutely dependent on "one gene at a time" biochemical toxicology and molecular biology to determine the role of transcriptional responses in altering phenotype.

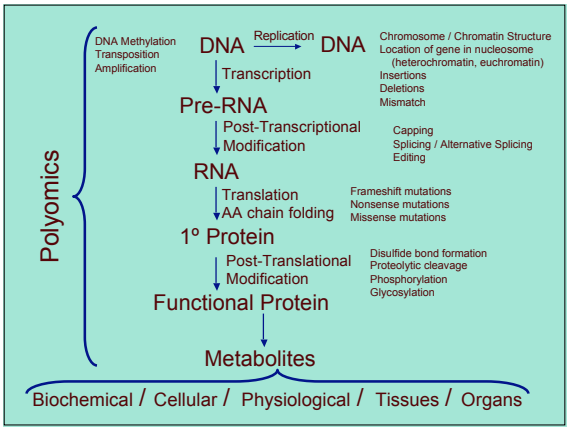
Morgan et al. 2002. HERA 8(6): 1339-1353.

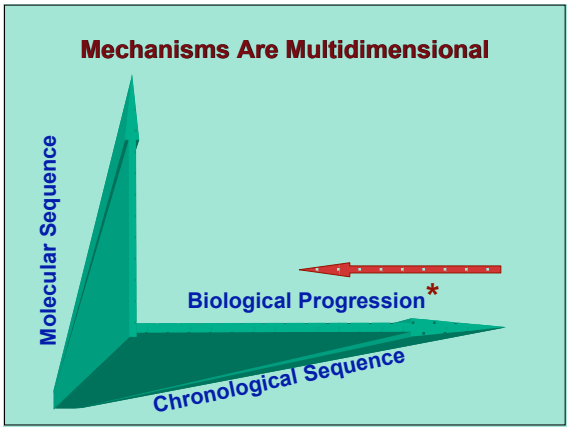
How do the new possibilities promised by omics technology meet the needs of risk assessment?

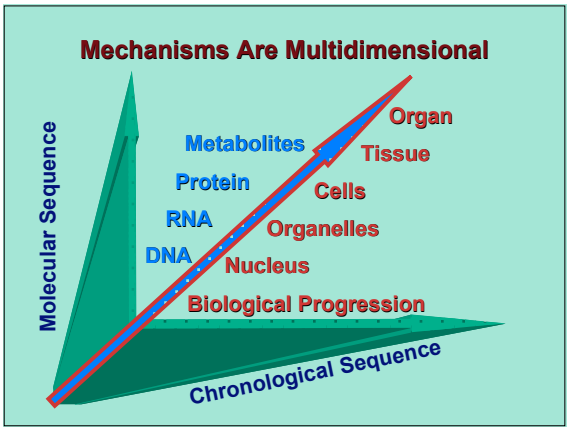
Example of Mis-Match Thinking:

"Toxicogenomics is the study of the structure and transcriptional output of the entire genome as it relates and responds to adverse xenobiotic exposure."

Risk Assessment is the process of defining adverse effects and estimating the probability of their occurrence in a population, including levels of exposure at which the probability is *de minimus*.

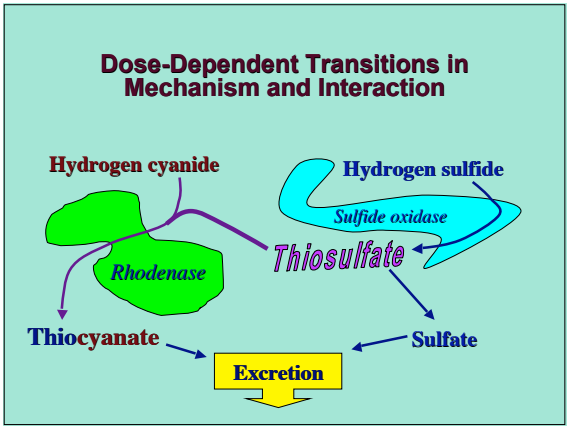






Levels of Biological Organization and Various Toxicity Metrics

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



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

Conclusions

1. A comprehensive conceptual framework is needed to integrate knowledge about modes of action and mechanisms of interaction with DRC models;
2. Differentiate between pre-toxicological, compensatory, and protective responses to chemicals;
3. Understanding causal links essential, but requires several levels of biological organization beyond molecular level;
4. Identify dose-dependent transitions in mechanisms that most impact risk estimation;
5. A combination of polyomic, molecular biological, *and traditional* approaches needed;
6. *And . . . .*

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.

Joint Toxic Action of Chlorinated Pesticides in Bass Gonads

- Methoxychlor (MCL) and *p,p'*-DDE are structurally similar, putative endocrine disrupting chemicals, exhibiting estrogenic and/or anti-androgenic effects.
- DDE and MCL would be categorized as having a common mode of action according to recent regulatory criteria.
- We tested the effects of combinations of DDE and MCL on steroid hormone synthesis in bass ovarian explant cultures.

Minced Gonad Assay

1 Gonads collected from female largemouth bass (2-3 yrs of age) during the peak reproductive season (February - April) to maximize steroidogenic activity and available gonadal mass.

2 Tissue was collected immediately upon sacrifice, minced and 100 mg added to each culture (MEM Eagle supplemented with 100 units/ml sodium penicillin G, 0.1 mg/ml streptomycin S and 0.1% BSA).

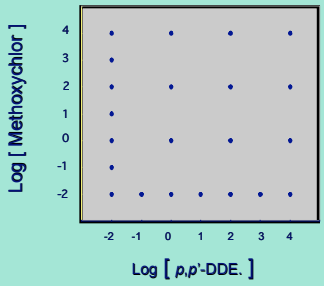
Minced Gonad Assay

3 Culture plates were incubated for 48 hours in an atmosphere of 4% CO2 at 26.5°C. Culture media was collected following incubation and stored frozen at -80°C until RIA analysis was performed.

4 The cultures were analyzed for 11-ketotestosterone and estradiol by validated RIA procedures.

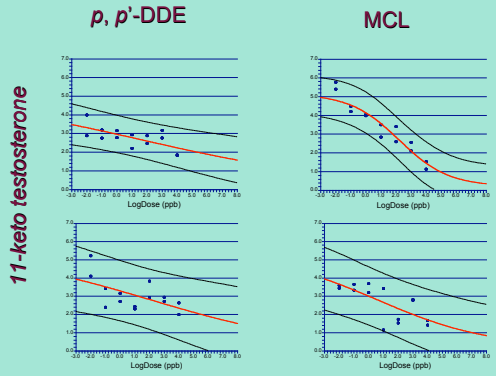
5 Each treatment group consisted of tissues from 3 females, two replicates from each bass in separate culture wells. 234 culture wells total.

Fractional Factorial Design



Dose-Response & Interaction Analysis

- The Hill Model was used to estimate DRCs.
- Dose additivity was used as the "no-interaction" (null) hypothesis.
- Additive Index values (Z*) were calculated for each combination at a significance level of 0.05.
- Variances for the predicted additive response were estimated by Monte Carlo simulation, assuming a normal distribution.
- Z* > 0 indicates antagonism and Z* < 0 indicates synergism.



Results

DOSES (ppb) and Score		
DDE	MCL	Z*
0.01	0.01	2.562
0.01	1	8.297
0.01	100	11.050
0.01	10000	29.263
1	0.01	5.172
1	1	8.145
1	100	1.497
1	10000	12.158
100	0.01	10.256
100	1	25.624
100	100	12.013
100	10000	5.845
10000	0.01	9.231
10000	1	6.833
10000	100	6.580
10000	10000	6.360

Conclusions

⇒ 15 out of 16 dose combinations indicate antagonism between DDE and DDL. i.e., |Z*|>1.96 and Z*>0.

⇒ Only one dose combination (DDE=1, MCL=100) indicates additivity.

⇒ *p*,*p*'-DDE and MCL may *NOT* inhibit testosterone synthesis in bass ovaries by a common mode of action.

Key Questions

- ***Can chemicals be categorized by mode of action?*** ***NO***
- ***Does general mechanistic information predict joint toxic action?*** ***NO***
- ***What information is required to predict the correct dose response model for chemical mixtures?***

Osteolathyrism

- Developmental failure of collagen and elastin polymerization
- Inhibition of lysyl oxidase activity

Semicarbazide, β–Aminopropionitrile } Posterior lesions of axial skeleton in *Xenopus*

Penicillamine } Anterior spinal cord lesions in *Xenopus*

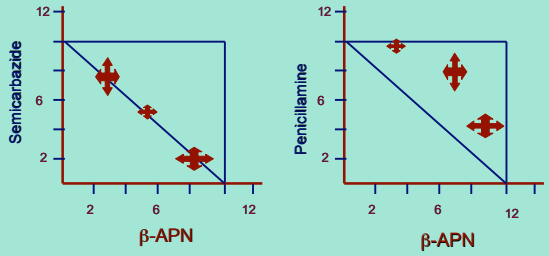
Joint toxic action of binary mixtures of Osteolathyrogens at malformation-inducing concentrations for *Xenopus* embryos.

Dawson DA, Wilke TS. 1991.
Journal of Applied Toxicology 116: 415-421

- Does the different specific site of action for Penicillamine signify a different mechanism of action from β-aminopropionitrile and semicarbazide?
- Determined EC_{50} values for various combinations of the agents in FETAX.
- Plotted results by isobologram.

Joint Toxic Action of Osteolathyrogens

Dawson DA, Wilke TS. 1991. Journal of Applied Toxicology 116: 415-421



Joint Toxic Action of Osteolathyrogens *Conclusions*

- Semicarbazide and β-aminopropionitrile have very similar mechanisms of action.
- Semicarbazide and Penicillamine have dissimilar mechanisms of action.
- ***"The mixture testing approach has potential value in determining compounds that act similarly."***

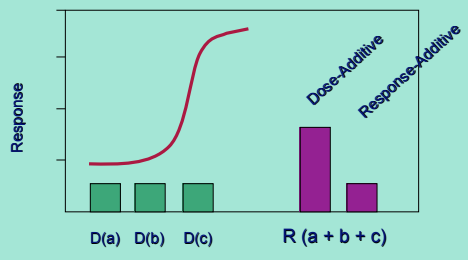
Dawson DA, Wilke TS. 1991. Journal of Applied Toxicology 116: 415-421

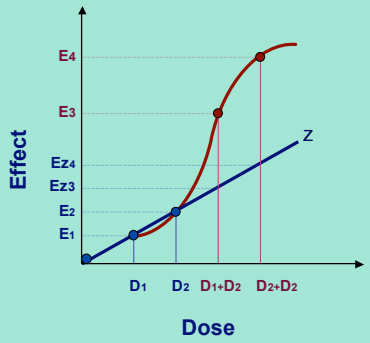
Establishing Common Mode

- targets: molecular, cellular, physiological, organ
- toxic intermediate
- causality between steps
- pharmacokinetics
- detoxification pathways
- parallel DRCs
- dose additivity in mixture

Common Mode of Action

Impact of the No-Interaction Model on Risk Assessment





Mode is *NOT* Mechanism

Mechanism:

. . . the molecular sequence of events that lead from the absorption of an effective dose of a toxicant to the production of a specific biological response in the target organ or system.

(Butterworth et al., 1995; Dellarco and Wiltse, 1998; EPA 1999 cancer risk assessment guidelines; Schlosser and Bogdanffy, 1999; EPA 2000 dioxin reassessment).

Mode is *NOT* Mechanism

Mode:

- ". . . a common set of physiological and behavioral signs that characterize a type of adverse biological response." Rand et al. 1995.
- " . . . a class or category of mechanisms that share general features critical to the production of toxicity." Shlosser and Bogdanffy, 1999.

Key Questions

- ***How can chemicals be categorized by mode of action?***
  - ***What constitutes a mode or mechanism?***
- Does general mechanistic information predict joint toxic action?
- What information is required to predict the correct dose response model for chemical mixtures?

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