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Cumulative Risk: The Argument Against *Estrogen Equivalents*

Conducting and Assessing the Results of Endocrine Screening

ISRTP Workshop, Lister Hill Auditorium

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Convention

Relative potency approaches, including dioxin TEFs, are simply dose addition applied to a specified endpoint, such as Ah receptor binding. The approach assumes that the endpoint - e.g., Ah receptor binding - is a common mechanistic step in the production of all toxic effects of the chemicals to which the approach is applied.

Extension

An estrogen equivalents (EE) approach would apply dose addition to some endpoint assumed to be a surrogate for estrogen-mediated adverse effects. Direct mixture testing to confirm dose addition for all potential environmental estrogens would never occur.

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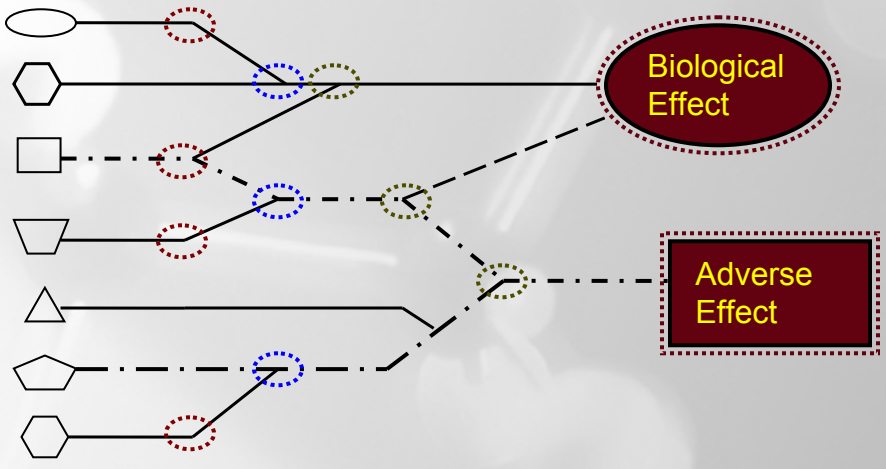
Outline

I. Impracticality
II. Irrelevance
III. Unusability
IV. Nonsensicality

Mode of Action Classification Criteria: Borgert 2007. *TAAP 223:* 114-120. I.

	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?



Joint Toxic Action of Chlorinated Pesticides in Bass Gonads

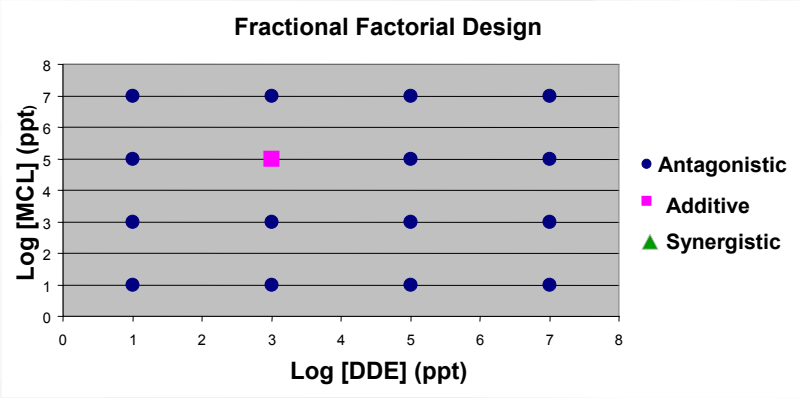
Methoxychlor (MCL) and *p*,*p*'-DDE are structurally similar, putative endocrine disrupting chemicals, exhibiting estrogenic and/or anti-androgenic effects.

DDE and MCL would be categorized as having a common mode of action according to recent regulatory criteria.

Tested the effects of combinations of DDE and MCL on steroid hormone synthesis in bass ovarian explant cultures. Altered steroid hormone levels in female bass were alleged to cause reproductive decline in Florida bass populations.

Borgert et al. 2004. *Environ. Toxicol. & Chem. 23*(8): 1947–1956.

Interactive Effect of DDE and MXCL on Testosterone Production in Bass Ovarian Cultures



Borgert et al. 2004 *Environ. Toxicol. & Chem. 23*(8): 1947–1956.

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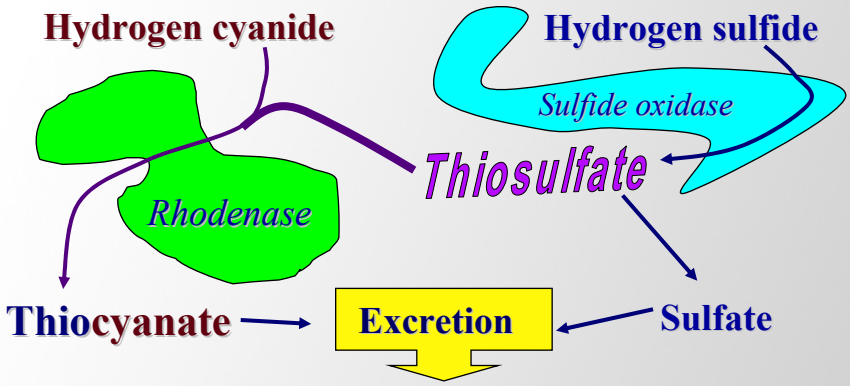
Dose-Dependent Transitions in Mechanism and Interaction

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



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

Charles et al. 2007. *Toxicol. Appl. Pharmacol. 218: 280-288*

Question- Are mixtures of estrogenic synthetic chemicals (SC) dose additive in combination with phytoestrogen (PE) mixtures?

Experiment- Six SCs were combined at equimolar ratios and tested as a mixture at 5-6 dose levels in combination with a mixture of six PEs, also at 5-6 dose levels. Estrogenicity measured by an in vitro ER transactivation assay and an immature rat uterotrophic assay.

Determined dose of SC mixture necessary to produce an estrogenic response greater than PEs alone.

Charles et al. 2007. *Toxicol. Appl. Pharmacol. 218: 280-288*

Results- No increase in response due to the addition of SC mixture at 0.02μM and 0.2μM.

Very slight increase with addition of SC mixture at 1.0μM and 2.0μM.

Clearly significant increase (p=0.006) at 3.0μM

Conclusion- **SC mixture increased estrogenic response over PE background only when each chemical in the mixture was ≥ 0.5x its individual NOEL in the estrogenic assay.**

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Relative Estrogenic Potency

Borgert et al. 2003. *Environ. Health Persp. 111(8):*1020-1036

o,p-DDT	p,p-DDT	o,p-DDE	p,p-DDE	MXCL
9.00E-03	5.68E-04	7.74E-04	0.00E-00	
1.12E-04	9.97E-05	1.03E-04	0.00E-00	
0.00E-00	0.00E-00	0.00E-00	0.00E-00	
		7.00E-06	4.20E-06	
			3.60E-05	
2.00E-04		ND		1.00E-03
4.00E-01			9.00E-01	8.80E-01
5.90E-06				3.10E-06
4.00E-01	9.00E-01			

Relative Estrogenic Potency

Borgert et al. 2003. *Environ. Health Persp. 111(8):*1020-1036

Methoxychlor	Bisphenol A
1.00E-03	1.00E-04
	3.70E-05
ND	1.30E-04
3.10E-06	1.30E-05
8.80E-01	
	8.00E-05
	4.40E-04
	1.30E-03
9.50E-03	2.10E-03
1.36E-04 – 3.3E-06	1.20E-05 – 5.00E-06

APT

DDE estimates range 2–3 orders of magnitude in various ER transcription activation assays, but it is unclear whether this chemical is more potent as an estrogen or an anti-androgen. Thus, it is difficult to unambiguously categorize the hormonal activity or estimate the hormonal potency of o,p-DDE based on the available data.

In practice, however, potency measurements among these assays can vary widely, making a determination of estrogen equivalence confusing and uncertain. Even assays that target the same biologic level of hormone action may produce disparate results.

Borgert et al. 2003. *Environ. Health Persp. 111(8):*1020-1036

Not only can potency measurements vary among estrogenicity assays, but hormone receptor specificity may also be unclear for some chemicals. Estrogenic and antiestrogenic effects have been reported for various chlorinated hydrocarbons (Safe 1995).

DDT isomers and metabolites show activity in estrogenicity assays but also interact with the androgen receptor in human hepatoma cells transfected with a human androgen receptor-reporter gene construct (Maness et al. 1998). o,p -DDE, the most potent estrogen agonist among the metabolites, antagonized androgen stimulated transcription at concentrations similar to those shown to have estrogen agonist activity.

Borgert et al. 2003. *Environ. Health Persp. 111(8):*1020-1036

Discrepancies in potency estimates are also apparent in measurements of transcription activation by phytoestrogens.

Potency estimates relative to estradiol exhibit marked discrepancies between assay systems and, in select cases, within assay systems.

When binding assays, in vitro functional assays, and in vivo end points are compared, discrepancies are evident for other phytoestrogens (e.g., coumestrol) and mycoestrogens (α-zearalenone) as well as for genistein (Whitten and Patisaul 2001).

Borgert et al. 2003. *Environ. Health Persp. 111(8):*1020-1036

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*EEs for Natural Ligands

Transcriptional activation of human ERα - pBD-GAL4 reporter construct in yeast YRG2

Estradiol 17-β (E2)	1.00
Estriol	6.67E-02
2-OH-E2	2.00E-02
Testosterone	**2.00E-05**
Progesterone	0.00

*calculated from figure 2b

Chen et al., 2004. J.Biol.Chem. 279(2):33855-33864

Summary

I. Estrogen Equivalents are *impractical* because we don't know the rules for applying them to the right chemicals;

II. Estrogen Equivalents are *irrelevant* for the dose range of interest and may not address adverse effects;

III. Estrogen Equivalents are *unusable* because there is such wide disparity between relative potency estimates;

IV. Estrogen Equivalents are *nonsensical* - *testosterone* is NOT estrogenic.

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The Theory

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 are identical. (p288)