Dose-Time-Response Implications of the Approach to Saturation for Metabolism- and Receptor-Mediated End-Effects

Dale Hattis

Clark University

Themes

- Causal Modeling is Key to Good Analysis of Dose Response Relationships
- We do have Some First-Principles Understanding of the Likely Mathematical Forms Governing Reactions Mediated by Saturation Effects and Co-factor Depletion
- Sensible Projections of Risk Depend on Interdisciplinary, Not Just Multidisciplinary Collaboration Between Biologists and Exposure Assessors

Outline

- Without Saturation Effects—Why do we expect basic linearity in reactions with macromolecules?
- Basic Michaelis-Menten Math for Enzyme, Transporter, or Receptor Saturation
- What Happens at Low Doses? (Far below levels needed for appreciable saturation)
- What Happens at Very High Doses?
- Basic Math where Nonlinearity Results from Cofactor Depletion (e.g., Glutathione) or Cell Death
- The Way Forward—Build Exposure Databases and Analyze them to Reduce Uncertainties in Exposure Patterns to Observable Variability on Different Biologically Relevant Time Scales

Without Saturation Effects

- Reaction rates depend on the number of collisions between reacting molecules that exceed a particular activation energy
- With constant temperature, and a constant concentration of reaction sites on macromolecules, the number of collisions with small molecules depends directly on the concentration of the small molecule reactants
- Thus low-dose linear response of reaction rates to internal concentrations must be expected.

Why an Approach To Saturation at High Concentrations?

- Limited number of binding/reaction sites on macromolecules responsible for metabolism, transport, and hormone-like signaling.
- Sometimes high reaction rates can deplete key enzyme cofactors such as glutathione.

Basic Michaelis-Menten Math for Saturating Effects

$$\text{Reaction rate} = \frac{V_{max}\,[S]}{K + [S]}$$

- Where Vmax is the maximum rate of reaction at the limit of high substrate concentration
- [S] is the substrate concentration (small molecule)
- K is the substrate concentration at which the reaction proceeds at half its maximum rate

What Happens at Low Doses?
([S] much less than K)

$$\text{Reaction rate} = \frac{V_{max}\,[S]}{K + \cancel{[S]}}$$

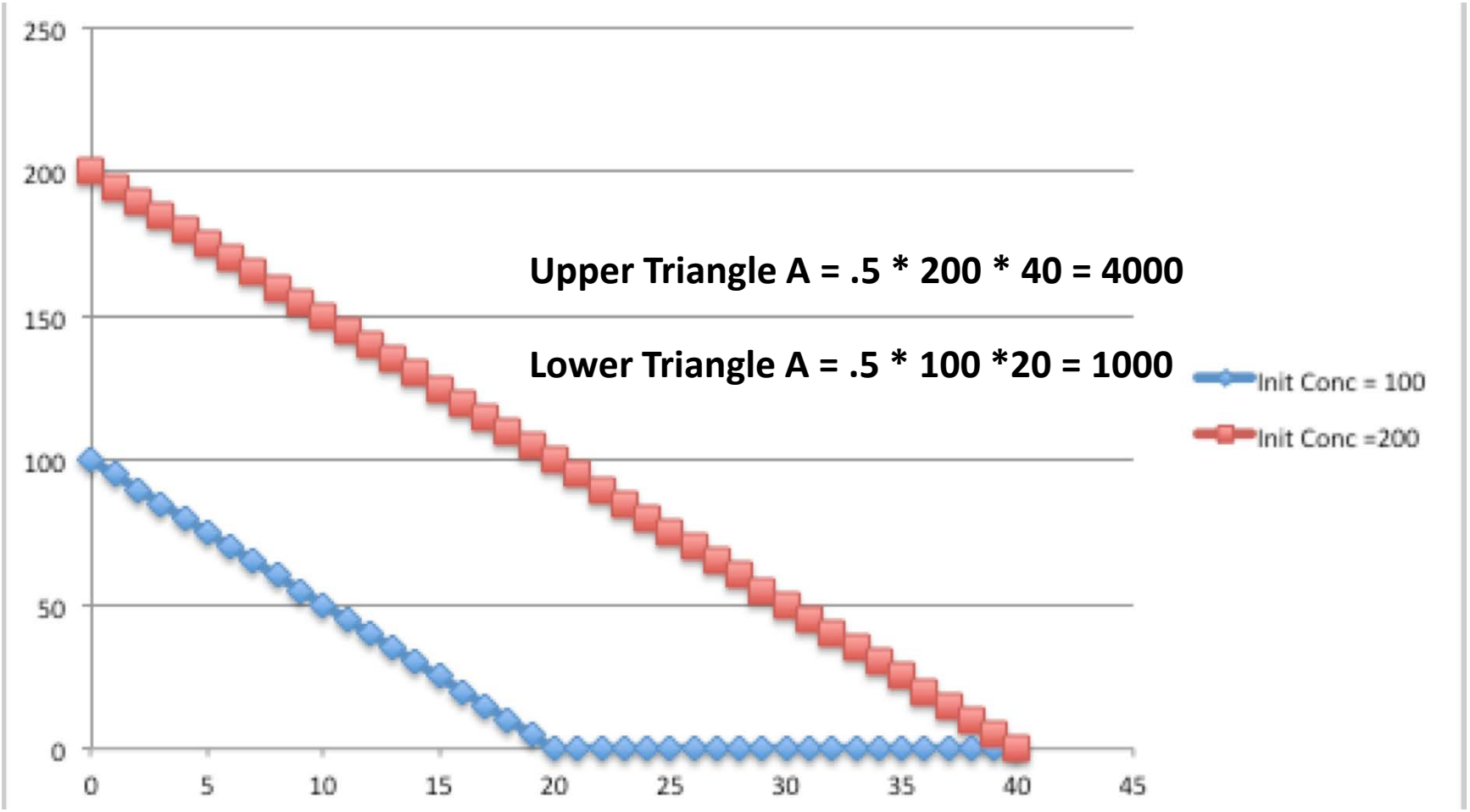
- Reaction proceeds linearly with a rate constant equal to Vmax/K
- With no continuing supply of S, S concentration declines exponentially with a half life of ln(2)*K/Vmax and Area Under the Curve proportional to the initial [S]

What Happens at Very High Doses? ([S] much greater than K)

$$\text{Reaction rate} = \frac{V_{max}[S]}{\cancel{K} + [S]}$$

- [S] in the denominator cancels [S] in the numerator and therefore reaction rate approaches the constant Vmax independent of [S].
- With no continuing supply of S, S concentration declines linearly
- A plot of [S] vs time resembles a triangle with the Area Under the Curve proportional to the square of the initial concentration--$[S]^2$

Full Saturation of an Elimination Process—Effect on the Area Under the Curve of Toxicant Delivery



Modeling Needs where Nonlinearity Results from Cofactor Depletion (e.g., Glutathione) or Cell Death

- For depletion of a cofactor, the key task is to model the dynamics of generation and loss with and without exposures to the toxicant on biologically relevant time scales.
- For cell killing, the key issues are
  - Differential internal doses and sensitivities of the cells subject to the killing (generally modeled by “probit”-type dose response relationships assuming lognormal distributions of individual cellular thresholds for response)
  - The dynamics of stimulation of cell division in precursor cells to replace cells lost from the toxicant.

Estimated Normal Human Glutathione Stocks, Generation and Loss Rates in Different Compartments

	GSH stock (mmoles)	Fract normal turnover per hour	Total GSH Flux Expected (mmole per hour)
Lung	0.476	1.2	0.57
Liver	10.55	0.037	0.39
Muscle group	26.1	0.021	0.54
Vessle-Rich Group (other than Liver)	6.53	0.18	1.18

Recent Assessment Task

- Model the possible extent of toxicokinetic interactions among three soil fumigants via depletion of glutathione (depletion of glutathione by one agent is expected to prolong the internal availability of other fumigants, leading to expected enhancement of sensitivity to adverse effects from DNA reaction, generation of reactive oxygen species).

Expected Percentage Reduction in Lung Glutathione Assuming all Chloropicrin Reacts Locally

Time after start of exposure (hrs)	ppm chloropicrin exposure			
	0.5	1	2	4
	% reduction in lung GSH			
1	7.5%	15%	30%	60%
2	10%	19%	39%	77%
4	10%	21%	42%	84%
8	11%	21%	42%	85%

Sad story—Exposure assessor only supplied expected air concentrations in 168 hour (1 week) aggregates. However possible variability in air dispersion within that period could yield large increase in expected maximal GSH reduction effects at specific places. If delivered over a single 8 hour period the aggregate exposure rate and maximal GSH depression could have been larger by up to 168/8 = 21 fold.

Take Home

- Time aggregates for expressing exposure need to be chosen to allow the data to relate to the dynamics of the biological processes being modeled. If aggregation times are too long there can be dilution of the real effective exposures and underestimation of peak effects.
- In general, good risk assessments need interdisciplinary, not just multidisciplinary collaboration.

The Way Forward Where Relevant Data are Not Readily Available

- Model uncertainties in the time pattern of exposures by making analogies to the observable variability among putatively analogous cases.
  - Build data bases of exposure variability using different time scales for aggregation.
  - Treat data-poor cases as random draws from the data base with reasonable similarity to the case to be assessed.