Key Events Dose-Response Analysis.
Part 1: Conceptual Framework and Application to Chemicals
SOT RASS Teleconference
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Alan Boobis (Imperial College London)
George Daston (Procter & Gamble)
Steve Olin (ILSI Research Foundation)
Dose-Response and Thresholds

- Recognition of the centrality of dose-response concept in life sciences
- **QUESTION**: Can our increasing understanding of modes of action provide insights for characterizing dose-response relationships at low doses (including thresholds)?
- Not only for chemicals but also for other bioactive agents.

*ILSI Research Foundation*
ILSI RF Threshold Working Group

- Characterizing fundamental biology of human health effects for chemicals, pathogens, allergens, nutrients
- Implications for dose-response, practical thresholds, public health standards
- Fostering cross-disciplinary discussion
- → Key Events Dose-Response Framework
• **Chemical Group:** Alan Boobis (Imperial College London), George Daston (Procter & Gamble), and Julian Preston (EPA).

• **Nutrient Group:** Sanford Miller (U Maryland), Joseph Rodrigicks (ENVIRON), Ian Munro (CANTOX), A. Catharine Ross (Pennsylvania State), Robert Russell (Tufts), and Elizabeth Yetley (retired NIH).

• **Allergen Group:** Steven Gendel (FDA CFSAN), Geert Houben (TNO), and Steve Taylor (U Nebraska).

• **Pathogen Group:** Bob Buchanan (U Maryland), Arie Havelaar (RIVM), Mary Alice Smith (U Georgia), and Richard Whiting (Exponent).
Work Products and Next Steps

• 5 papers – *Crit Rev Food Sci Nutr*, 49 (8), Sept 2009 (Overview, Chemicals, Nutrients, Pathogens, Allergens) – open access.

• **Next Steps**
  • Encourage the development of additional case studies illustrating and evaluating the utility of the Framework
  • Organize small meetings and workshops to work through specific examples
  • Explore application and integration of the Framework into MOA analysis for risk assessment

• CONTACT: Steve Olin (solin@ilsi.org)
The KEDRF: Overview and case study – chloroform

Alan R Boobis
a.boobis@imperial.ac.uk
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- ILSI RF: Stephen Olin and Elizabeth Julien
- All others in ILSI RF Threshold Working Group
Derivation of reference values (RfDs, etc) for effects exhibiting non-linear dose-response relationships

- Reference Dose (a reference value (RV)): “An estimate, with uncertainty spanning perhaps an order of magnitude, of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime”

- The RV approach is based on the premise that most toxicological endpoints have a true biological threshold, although this is not identifiable with accuracy
Traditional approach to hazard characterization

- Identify point of departure (NOAEL/BMDL), from epidemiological or experimental evidence, to serve as reference point, and apply default uncertainty factors.
- The POD is not a no-effect level and derivation of “acceptable” doses requires assumptions about thresholds and variability in those thresholds.
- In studies of inherently limited power, it is implicit that there is uncertainty as to the magnitude of the response, if any, at the POD.
- Are the assumptions in risk assessment conservative overall?
Derivation of reference values

Toxic effect

Test species

RV

UF

NOAEL

Sensitive human

Average human

Dose

Response

0 0.1 1 10 100 1000

0 10 100 1000

Derivation of reference values

Toxic effect

Test species

RV

UF

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Sensitive human

Average human

Dose

Response

0 0.1 1 10 100 1000

0 10 100 1000
Key Events Dose-Response Framework

- ILSI Research Foundation established a tripartite, multidisciplinary activity to develop an integrated framework to incorporate advances in scientific knowledge to support sound scientific decisions.

- Based on mode of action concept, with focus on understanding the fundamental biology and dose-response (including possible thresholds) at each key event, to inform hazard characterization and risk assessment.

Mode of action and key events

EXTERNAL DOSE (exposure)

KEY EVENT (absorption)

KEY EVENT (target tissue exposure to ultimate toxic species)

KEY EVENT[S] (biological perturbation[s])

KEY EVENT[S] (pathological change[s])

Toxicological effect of concern
Factors operating at the level of a key event

- Effects on shape of dose-response curve
- Consequences for biological threshold
Address each key event systematically

- Is a minimum dose level required in order for this key event to occur? What data would be needed to demonstrate this?
- What response mechanisms (e.g. homeostasis, repair) are involved? At what dose would these be overwhelmed?
- What modifying factors (e.g. lifestage, disease state, nutritional status) can potentially reduce the effectiveness of response mechanisms? What factors can increase the effectiveness of response mechanisms?
- Do such modifying factors change the dose level at which response mechanisms become overwhelmed? What data would be needed to demonstrate this?
- Is any one key event rate limiting, driving the shape of the overall dose-response curve?
Chloroform as a case study to investigate the KEDRF

- Chloroform is hepatocarcinogenic
- Mode of action (MOA) involves hepatotoxicity
- Risk assessment is based on evidence that cancer arises only after a certain degree of liver damage is produced and maintained
- Analyze MOA for mechanistic evidence of thresholds
Chloroform

Sustained cytotoxicity

Regenerative cell proliferation

Tumor development

Key events

Oxidative CYP2E1 Metabolism

Phosgene
Key events for chloroform carcinogenicity:

1. **EXTERNAL DOSE** (exposure to CHCl₃)
2. **KEY EVENT (absorption)**
3. **KEY EVENT (oxidative metabolism to phosgene and target tissue exposure)**
4. **KEY EVENT (sustained cytotoxicity)**
   - **TOXICOLOGICAL EFFECT** (hepatic necrosis)
   - **KEY EVENT (regenerative cell proliferation)**
5. **CARCINOGENIC EFFECT** (hepatocarcinogenesis)
   - **Homeostatic compensation, adaptation, repair**
   - **Adaptation, repair**

**Reduced absorption**

**Reduced metabolism or target tissue exposure to phosgene**
Cytotoxicity

Homeostatic ‘steady state’

Cellular adaptations

Reversible cell injury

Irreversible cell injury

Cell death
There is evidence that several of the key events in chloroform carcinogenesis exhibit a threshold. It is not possible to determine which of these is the critical determinant (if any), from available data. For this, studies in which each event was studied in isolation would be necessary. Information from all biologically relevant models should be used in such analyses. This is of particular value for more distal key events, which will be less compound specific.
Individual vs. population thresholds

- Thresholds will vary among individuals.
- Once the determining key events are understood, research to study contributions to population variability (including identification of susceptible subpopulations) can be targeted on those events.
- The goal is to understand how various factors (age, gender, disease state, nutritional status, etc.) may quantitatively affect the doses at which those determining events occur.
- Some key events are likely to show absolute population bounds, thus determining population thresholds.
Conclusions

- Identify key events at the appropriate level of biological organization
  - Contribution of toxicogenomics
- Ensure that all important events are included, and that non-essential events are not included
- Understand interindividual variability in the rate determining molecular events involved, to enable a true population threshold(s) to be identified
Key Events Dose-Response Analysis: Carcinogens and Estrogens

George P. Daston
The Procter & Gamble Company

SOT RASS Teleconference, January 13, 2010
Testing the Threshold Assumption: New Research

- Research on the basis of cancer has led to the understanding that multiple cellular changes are needed to progress from normal to a cancerous state.
- Research in gene expression at a genomic level is helping to provide experimental data at the low end of the dose-response curve.
Evidence of Thresholds for Mutations - Mice

(Russell et al., 1982)
Evidence for Mutation Thresholds in Drosophila

(Zimmering et al., 1989)
Mechanisms of Carcinogenesis

Cancer is a multistep process based on the requirement of a cell to develop a set of 6 acquired characteristics leading to uncontrolled cell division and a survival advantage. These “steps” require genetic alterations (gene mutations and chromosome alterations). Specific phenotypes are selected based on growth advantage in a Darwinian selection model. The substrate is cells with an unstable genome.
initiating mutation

second mutation

~10^6 cells

FIRST CLONAL EXPANSION

third mutation

~10^6 cells

SECOND CLONAL EXPANSION

fourth mutation

~10^6 cells

THIRD CLONAL EXPANSION

e.t.

FOURTH CLONAL EXPANSION
Initiation

Repair
DNA Damage

Promotion

Apoptosis
Proliferation

Progression

Apoptosis
Proliferation

Normal Cell

Initiated Cell

Focal Lesion

Cancer
Multistage Model of Carcinogenesis

Normal Cell  \( \xrightarrow{\delta_1, \alpha_1} \)  Initiated Cell  \( \xrightarrow{\delta_2, \alpha_2} \)  Malignant Cell

\( \mu_1 \)  \( \mu_2 \)
A

<table>
<thead>
<tr>
<th>Component</th>
<th>Acquired Capability</th>
<th>Example of Mechanism</th>
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<tbody>
<tr>
<td></td>
<td>Self-sufficiency in growth signals</td>
<td>Activate H-Ras oncogene</td>
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<tr>
<td></td>
<td>Insensitivity to anti-growth signals</td>
<td>Lose retinoblastoma suppressor</td>
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<tr>
<td></td>
<td>Evading apoptosis</td>
<td>Produce IGF survival factors</td>
</tr>
<tr>
<td></td>
<td>Limitless replicative potential</td>
<td>Turn on telomerase</td>
</tr>
<tr>
<td></td>
<td>Sustained angiogenesis</td>
<td>Produce VEGF inducer</td>
</tr>
<tr>
<td></td>
<td>Tissue invasion &amp; metastasis</td>
<td>Inactivate E-cadherin</td>
</tr>
</tbody>
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B
Key Events for Tumor Development: DNA-reactive MoA

- Exposure of target cells to ultimate DNA-reactive and mutagenic species
- Reaction with DNA in target cells to produce DNA damage
- (For Non DNA-reactive MoA DNA damage produced by other processes but remaining key events the same)
- Replication or repair errors from damaged template
- Mutations in critical genes in replicating target cell
- Enhanced cell proliferation
- Additional mutations induced from DNA damage and replication
- Clonal expansion of mutant cells
- Preneoplastic lesions and neoplasms develop
- Malignant behavior
The use of mechanistic data to define mode-of-action for cancer risk assessment is described in the current U.S. EPA’s *Guidelines for Carcinogen Risk Assessment (2005)*. This provides a framework for considering approaches to extrapolations from high to low doses and from biomarkers to tumors.
Key Events and MoA (I)

• To produce a gene mutation or a chromosome structural alteration it is necessary to have some form of DNA damage (e.g., a DNA strand break, an adduct) and to have DNA and cell replication. Enhanced DNA damage and/or enhanced cell proliferation will increase the probability of producing a genetic alteration.
Key Events and MoA (II)

- The great majority of carcinogens that induce DNA damage will also induce cell killing that can lead to regenerative proliferation as noted by the key events for a DNA-reactive MoA. Thus, unique MoAs are quite unlikely; “combined” MoAs will be common. Thus, incorporating MoA into the tumor dose-response characterization requires consideration of key events with different dose-response curve shapes.
Key Events and MoA (III)

• In the absence of sufficient data, EPA assumes a linear low dose response for tumors.
• However, based on key events and MoA, it is very likely that other forms of dose-response are feasible. This view is enhanced by the fact that cancer is a complex process that is unlikely to be defined mathematically by a single mechanism.
Genomics to Address Thresholds

- Intermediate step between initial molecular interaction and frank effect
- High degree of sensitivity
- We have used this to demonstrate a lack of a low-dose effect of estrogens on rat testicular development
Estrogen: Mechanism of Action

Estrogen-responsive genes

Specific mRNAs (Up- or Down-regulated)

Transporters
Extracellular matrix
Receptors
Enzymes

Cellular Response

Organ Response
Key Events for an Estrogen-Mediated Developmental Effect

• Binding of ligand to receptor
• Translocation to DNA and initiation of gene transcription
• Cell and tissue responses
  – Proliferation
  – Changes in differentiation
  – Physiological changes
  – Angiogenesis
Fluid imbibition

Cell proliferation

Epithelium remodeling

Regression to basal level

Time (h)

1 2 8 24 48 72 96

Transcription factors, cell signaling, vascular permeability, growth factors

mRNA and protein synthesis

Cell growth, differentiation, suppression apoptosis,

Cell cycle regulators

DNA replication and cell division

Tissue remodeling and cytoarchitecture

Immune response

Adapted from Fertuck et al. (2003); Moggs et al. (2004); and Naciff et al. (2007)
Low Dose Issue

- Is there a different transcript profile at low doses than at high doses?

- What is the threshold for gene expression changes induced by exposure to estrogens?
Dose-Response of Gene Expression Changes Induced by EE, Ges or BPA in Testis/Epididymis of the Developing Rat
Dose-response: Fetal Testis
Population Threshold

- **Population Threshold:** a dose below which an effect will very likely *not* occur in a population
- Must consider the mechanisms underlying individual thresholds as well as the variability of the population, and background exposures
Individuals may have differing susceptibilities.
Conclusions

• New information about the mechanism of toxicity or disease production can be used to inform about the nature of the dose-response at low exposure levels.
• Detailed understanding of the steps from exposure through pathogenesis can be displayed in a key events framework to facilitate the development of models describing the quantitative behavior of critical steps in pathogenesis
Conclusions 2

• New technologies will make it possible to empirically test for biological responses at low doses and may be useful in setting pragmatic thresholds

• Better information about the nature of biological responses at low exposure levels will make risk assessment less uncertain