Meta-Hormesis and Arsenic

Tony Cox

tony@cox-associates.com
tcoxdenver@aol.com

Outline

- Inconsistent shapes proposed for arsenic-lung cancer dose-response curves
  - The “scandal of epidemiology”?
- Can mechanistic knowledge help?
  - Too many fragmented mechanisms, ambiguous effects
  - Can knowledge of lung cancer biology provide a framework to constrain possible quantitative relations?
- How to use available partial information to improve risk management decisions?
  - “Meta-hormesis” and decision analysis
    - Geometry of (possibly) J-shaped curves
  - Bounding optimal (risk-minimizing) exposure levels

What is *known* about i-As dose-response?

Not much!

- Ambiguous epidemiology
  - No big risks proved at low doses
  - Effects: Maybe none, or positive, or negative
- Animal data are suggestive
  - uncertain applicability to humans
- *In vitro* results suggest many possibilities
  - relevance to dose-response *in vivo* hard to judge

Philip Morris International project: Can we do better? (i-AS in smoking cancers?)

What affects i-As dose-response?

- Personal metabolism (methylation)
  - i-As ⇒ DMA ⇒ MMA (mono- and dimethyl acids)
  - methylated trivalent species can attack DNA
    - Uncertain relevance to lung cancer *in vivo* in humans
    - p16 inactivation (by methylation) is suggestive

- Exposures to other carcinogens
  - Co-carcinogenesis via DNA repair inhibition?
  - Crystalline silica in Chinese tin mines? (Chen 06)
  - Cigarette smoke, nutrition, UV light, etc.

Epidemiology is ambiguous / conflicting for arsenic and lung cancer

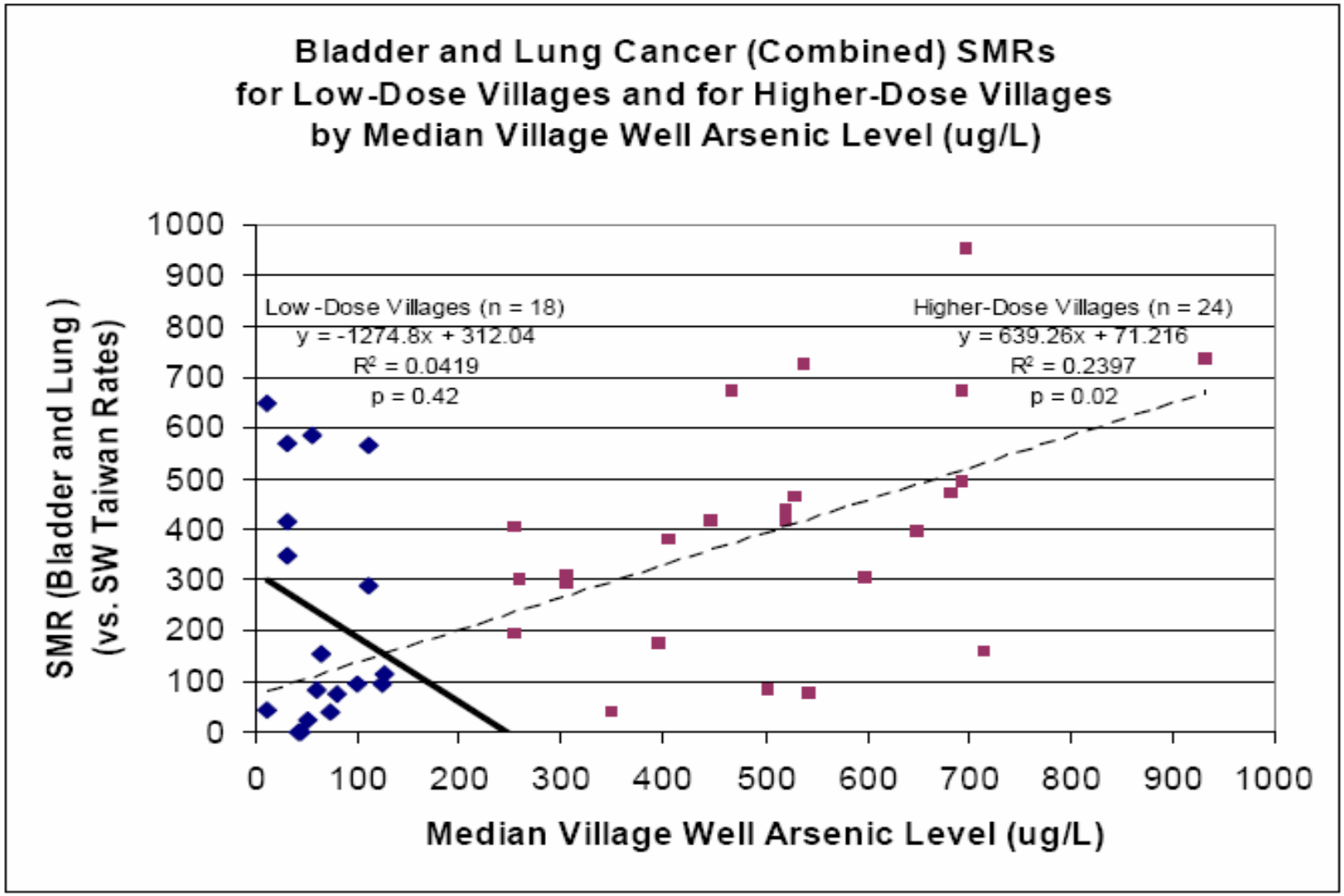
Conflicting epidemiology results

Study	Finding
Linear: "…Respiratory cancer mortality increased **linearly** with increasing cumulative exposure group, ranging from two to nine times expected"	Lee-Feldstein, 1986, for Montana smelter workers exposed to arsenic trioxide
"A **positive** dose-response relationship was found between cumulative arsenic exposure [estimated intensity but not duration] and lung cancer mortality with an overall SMR of 372... " (1989)	Jarup '89 Swedish smelter workers. **OR = 1.07** [1.02-1.1] among smokers (Lundstrom 2006)
Supralinear: "[S]tudies with quantitative data are consistent with a **supralinear** dose-response relationship." (or with smoking, mismodeled exposure errors)	Hertz-Picciotto and Smith, 1993, for Chinese miners.

Conflicting epidemiology results

Study	Finding
Supralinear: "The additional follow up confirms the earlier finding that at low doses the increments in death rates for respiratory cancer for a given increment in dose are greater than at high doses"	Enterline et al., 1995 for Tacoma, Washington copper smelters. (OCMAP effect?)
Sublinear or Hormetic (U-shaped): "Recent epidemiological studies have shown that the relative risk for cancer among populations exposed to ≤ 60 ppb As in their drinking water is often **lower** than the risk for the unexposed control population."	Snow et al, 2005 Schoen et al., 2004 (No clear effect in U.S. for drinking water.) Lamm et al., 2006 (Taiwan villages)

Lamm et al., 2006



Chemical Name: Arsenic, Inorganic
CAS Registry Number: 7440-38-2
Cancer Inhalation Risk Values (ITER Database)

Organization Name	ATSDR	Health Canada	IARC	ITER	NSF Intl	RIVM	U.S.EPA
Risk Value Name	NA	TC05	NA			TCA	RSC
Risk Value (mg/cu.m)	NA	**7.8E-3**	NA			**1E-3**	**2.3E-6**
Year	2005	1992	2002			2000	1997
Classification	NA	I	1			NA	A
Target Organ	NA	lung	NA			lung cancer	lung
Species	NA	human	NA			human	human
Study	NA	several	NA			Blom et al., 1985; Lagerkvist et al., 1984	several

Animal and *in vitro* models suggest many possibilities…

Animals models for As carcinogenicity

- "A **remarkable species diversity** in arsenic methyltransferase activity may account for the wide variability in sensitivity of humans and animals to arsenic toxicity." (Goering et al, 1999)
  - Methyltranferase activity metabolizes arsenic to mono- and dimethylated species, using S-adenosyl-methionine (SAM) as methyl-donating cofactor
- DMA **promotes lung tumors in mice** and induces SSBs in lung DNA (Kenyon and Hughes, 2001)
- "Chronic oral exposure to inorganic arsenate interferes with methylation status of **p16INK4a** and **RASSF1A** and **induces lung cancer in A/J Mice**."
  - iAs(V) exposure **increased lung tumor incidence and multiplicity** in A/J mice. Epigenetic changes of tumor suppressor genes such as p16(INK4a) and RASSF1A are involved… (Cui et al., 2006).

Many proposed mechanisms for As toxicity and carcinogenicity…

- As → DNA methyl-transferases → **DNA hypermethylation** → inactivate tumor suppressor genes (TSGs) → cancer risk
- As → SAM⇓ → **DNA hypomethylation** → oncogenes⇑
- "These results suggest that tumor promotion due to DMA(V) administration is mediated by DMA(III) through the induction of **oxidative stress**." (Mizoi et al., 2005, for mice)
- **proteinase / anti-proteinase activity** (Josyula, 2006)
- "… low-dose As(III) could stimulate growth of tumors through a HIF [hypoxia inducible factor-1alpha]-dependent stimulation of **angiogenesis**." (Kamat et al., 2005, in mice)
- "As may act [via] interference of regulation of **DNA repair** or integrity. " (Gebel 01)
- As toxicity → **cell proliferation**⇑ (Byrd, 1996), **apoptosis**⇑, **chromosomal aberrations**⇑ (Gradecka, 2001)

… and anti-carcinogenicity

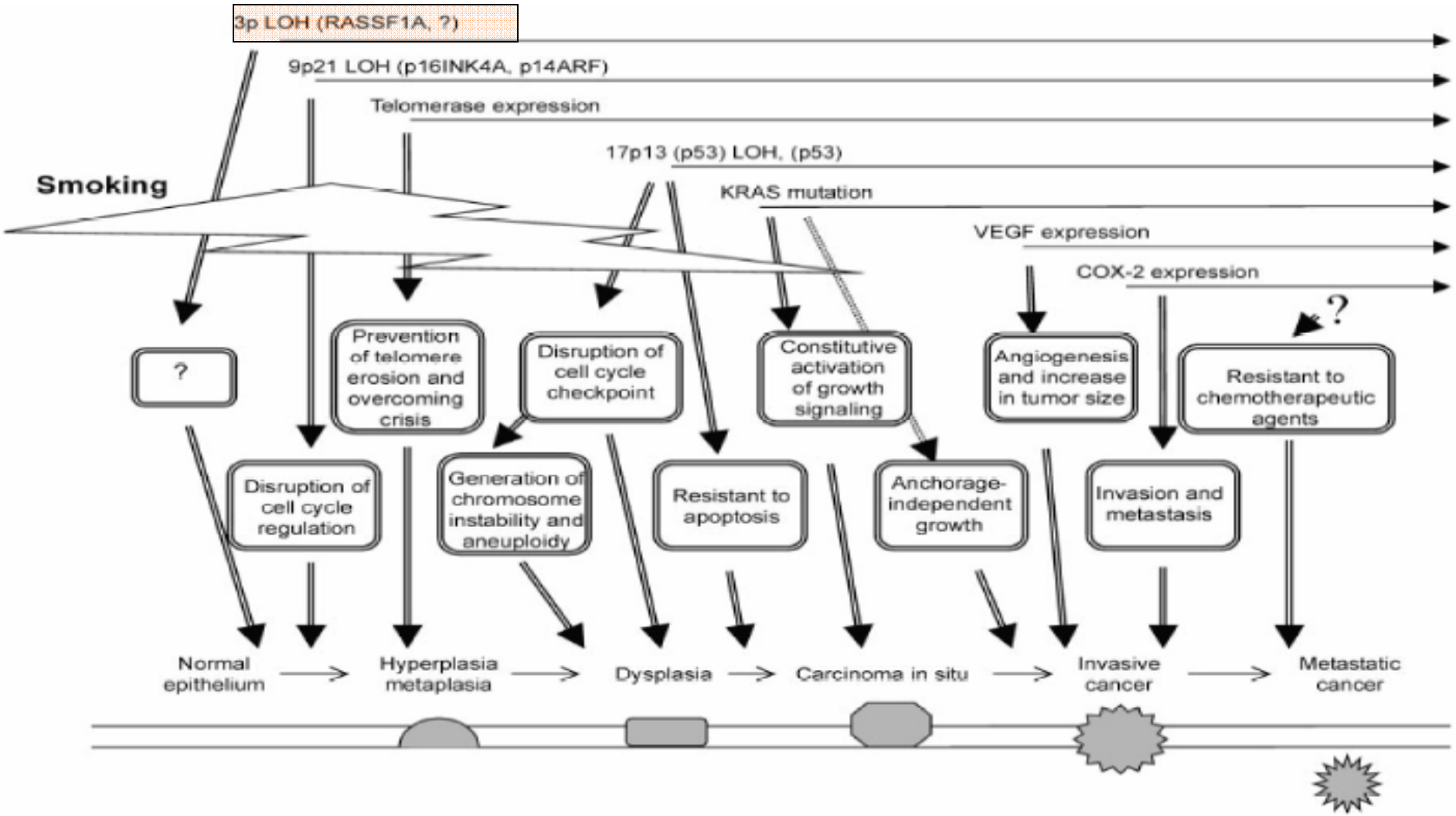
- "…Treatment of human keratinocyte and fibroblast cells with 0.1 to 1 muM arsenite (As(III)) also produces a **low dose protective effect** against oxidative stress and DNA damage" (Snow et al., 2005)
- "…Chronic low-level exposure of cells to arsenic alone or in a mixture containing arsenic, cadmium, chromium, and lead **inhibited malignant conversion**" in human keratinocyte cell line (Bae et al., 2002)
- "Synergistic effect of all-trans-retinoic acid and arsenic trioxide on **growth inhibition and apoptosis in** human hepatoma, breast cancer, and **lung cancer cells in vitro**.", Lin et al., 2005
- Pre-exposure to As for 3 weeks "reduced the size and number of **pulmonary adenomas** observed per mouse" following urethan injection (Blakley, 1987)
- Development of **tolerance/protection** via induction of GSH, HSPs, DNA repair, etc.

Implications for dose-response?

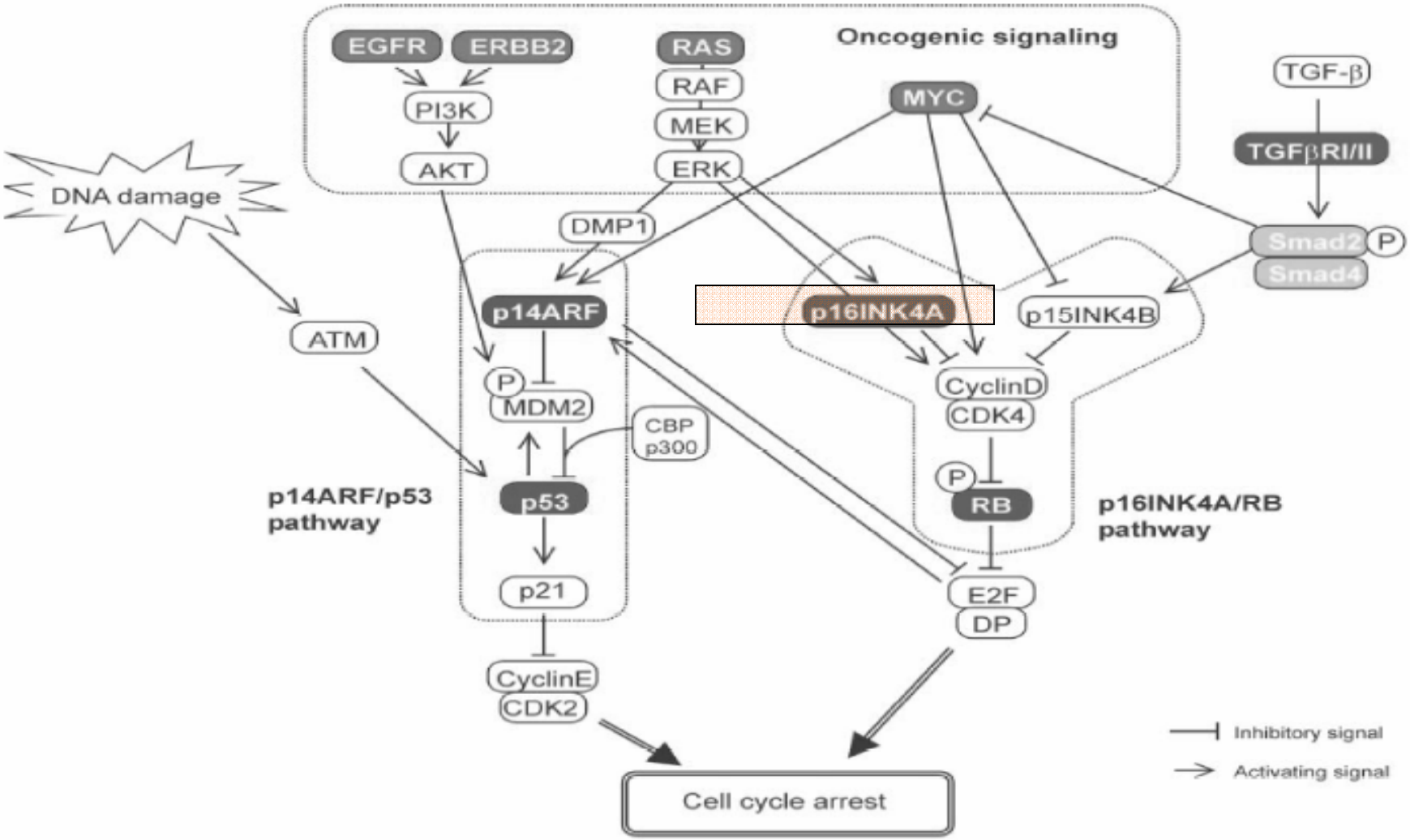
Not very clear!

How can knowledge of lung cancer biology help interpret / use the mechanistic data?

RASSF1A in lung carcinogenesis
(Osada and Takahashi, 2002)



p16INK4A in RB path affects lung tumor suppression (Osada and Takahashi, 2002)



Simplified model of lung cancer: Milestones and transition rates

Normal $\Rightarrow$ Patch $\Rightarrow$ Field $\Rightarrow$ CIS $\Rightarrow$ Tumor

CIS = “Carcinoma in situ”

Simplified model of lung cancer: Milestones and transition rates

Normal $\Rightarrow$ Patch $\Rightarrow$ Field $\Rightarrow$ CIS $\Rightarrow$ Tumor

$\uparrow$

niche kinetics $\leftarrow$ **As**

- cytotoxicity, linear protective effects (?)
  - Receptor-mediated $\rightarrow$ threshold-like for cell, linear for spatial population of niches (?)
- cell kinetics
  - "Niche" = local self-regulating compartment

Simplified model of lung cancer: Milestones and transition rates

Normal $\Rightarrow$ Patch $\Rightarrow$ Field $\Rightarrow$ CIS $\Rightarrow$ Tumor

$\uparrow$ low doses $\qquad\qquad$ $\uparrow$ high doses suppress

patch formation kinetics $\leftarrow$ **As**

- methylation rates (linear?)
- DNA repair inhibition/stimulus
- oxidative damage (protection?)
- apoptosis (protection?)

Model: Cell mutations stochastically diffusing within niches (Nowak et al.)

- *If* stem cell compartment sizes, transit divisions, division rates etc. have evolved to maintain homeostasis while minimizing cancer risk…
- *Then* changing these variables (in any direction) increases cancer risk (until adaptation occurs).
  - Convex ("J-shaped") dose-response
  - Not necessarily hormetic (negative at low exposures)
- Biology $\rightarrow$ two rate-limiting hits (TSG, chromosomal instability, CIS) $\rightarrow$ approximately linear-quadratic risks at low doses.

  Possible Implication: As dose increases, ~quadratic harm overtakes ~linear protection.

Local regulation of niche homeostasis favors chromosomal instability (CIN) (Michor *et al*, 2003)

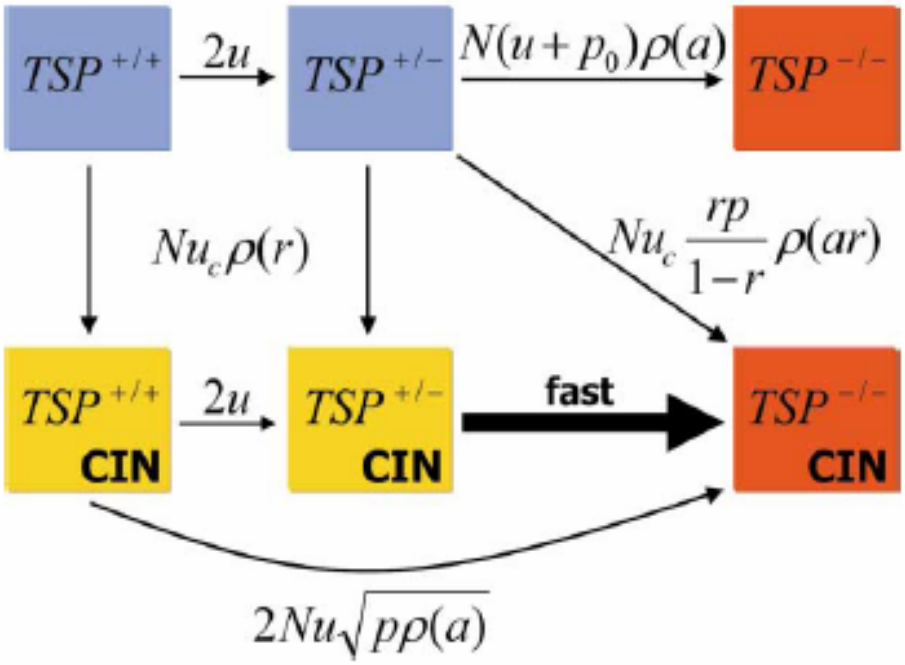


Figure 1. Processes and Rates

(A) Cancer initiation via a tumor suppressor (TSP) gene with or without chromosomal instability (CIN). The mutation rate per gene is given by u. The rate of loss of heterozygosity (LOH) in normal and CIN cells is given by p_0 and p, respectively. We have $p >> p_0$. CIN mutations are acquired at a rate of u_c.

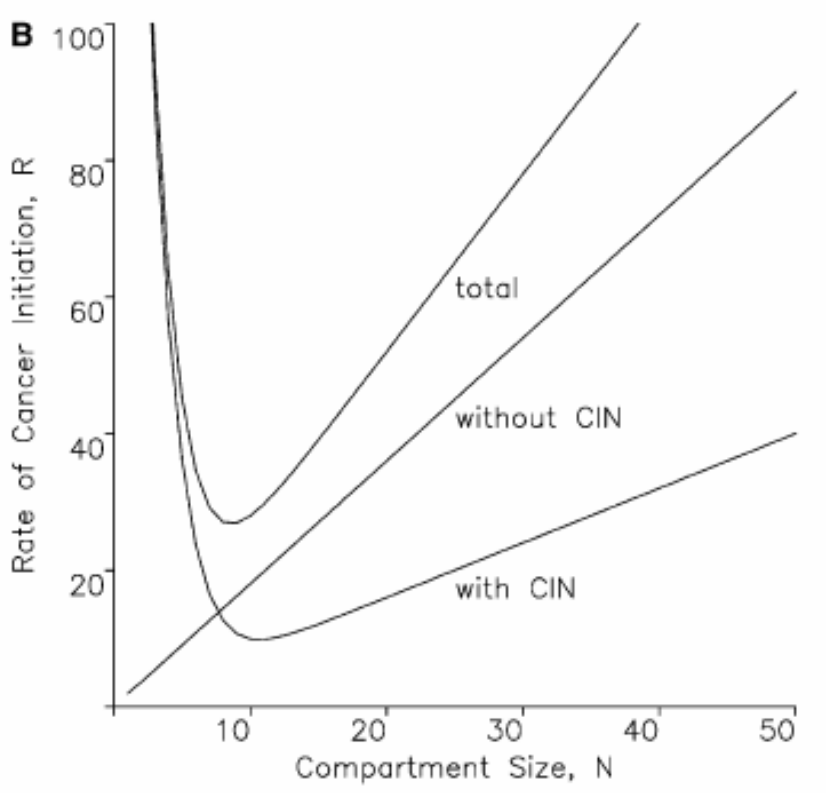


Figure 2. The Effect of Compartment Size on the Frequency of CIN

≥ 2 rate-limiting hits → U-shaped optimal compartment size (Michor et al., 2003)

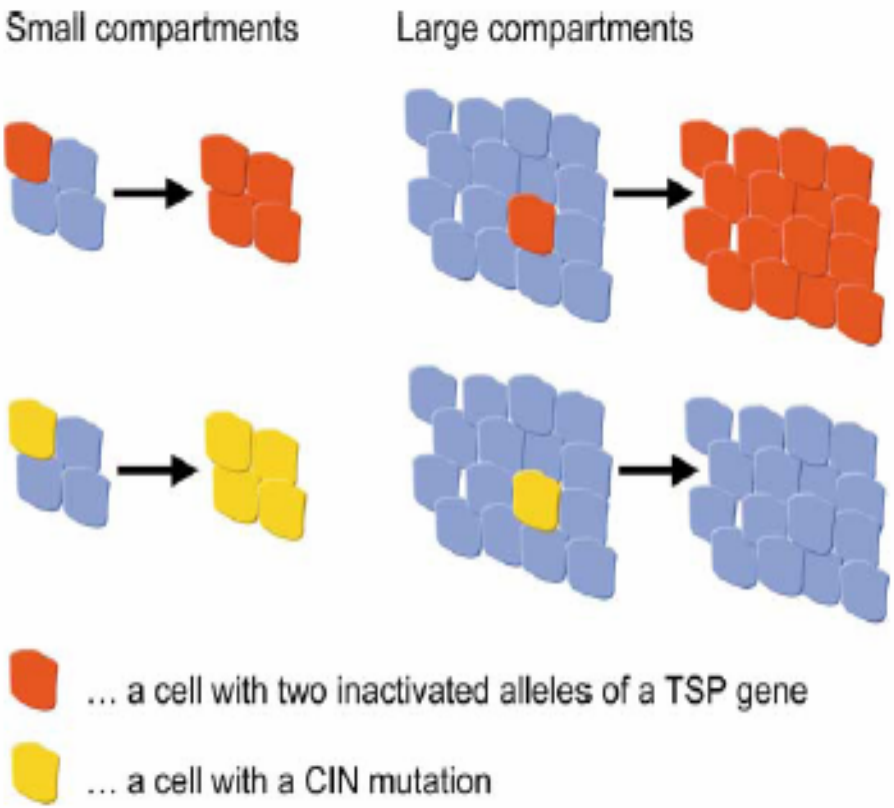


Figure 3. Fixation Probability with and without CIN

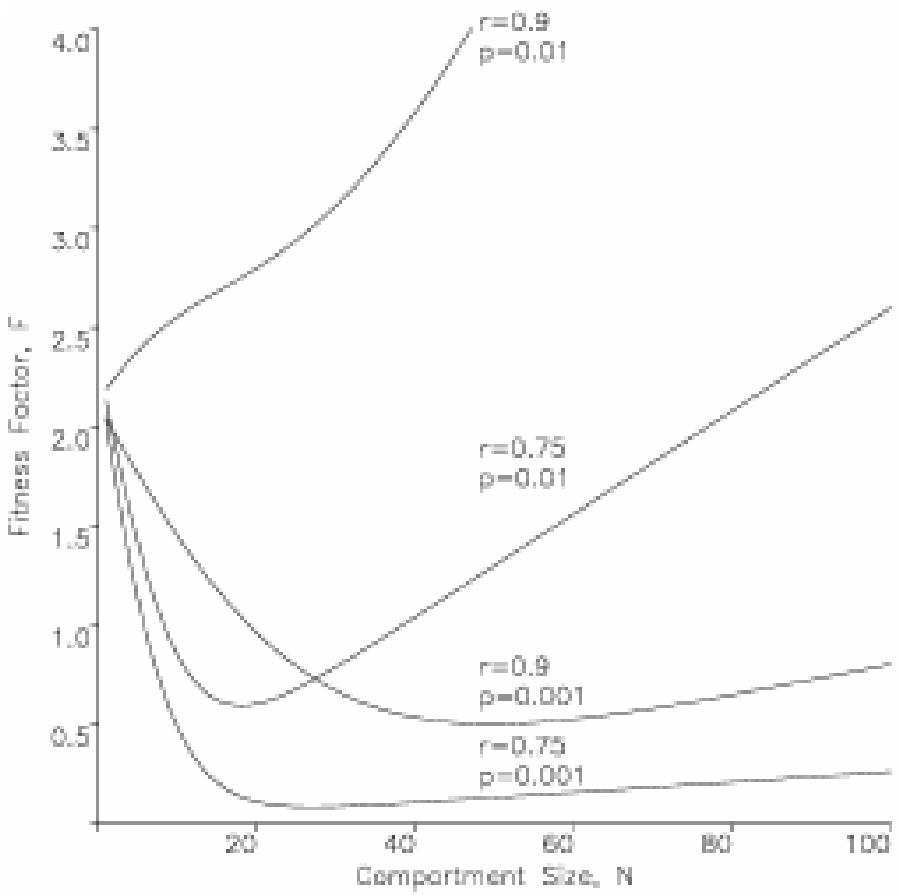


Figure 4. The Risk of Cancer Initiation as a Function of the Compartment Size

So what?

Implications for dose-response?

- Cytotoxicity-mediated effects…
  - Typically have thresholds
  - Usually convex (for good evolutionary reasons)
- Possible protective effects
  - Low-dose protective effects *without* high thresholds?
    - Methylation effects, receptor-mediated in spatial populations
  - High-dose (cytotoxic, e.g., apoptosis of altered cells) protective effects probably have thresholds
- "Anti-hormesis" effects on angiogenesis and lung tumor growth and metastasis in mice at high (therapeutic) doses (Soucy et al., 2005)
- **Sum of effects: J-shaped at low doses?** (maybe)

Should hypothesized mechanisms change risk management?

- “Consideration of arsenic's plausible mechanisms and evidence from epidemiological studies **support the use of nonlinear methods, either via biologically based modeling** or use of a margin-of-exposure analysis, to characterize arsenic risks.” (Schoen et al., 2004)
- Is this true?
- *Even if it is true… How to do it?*

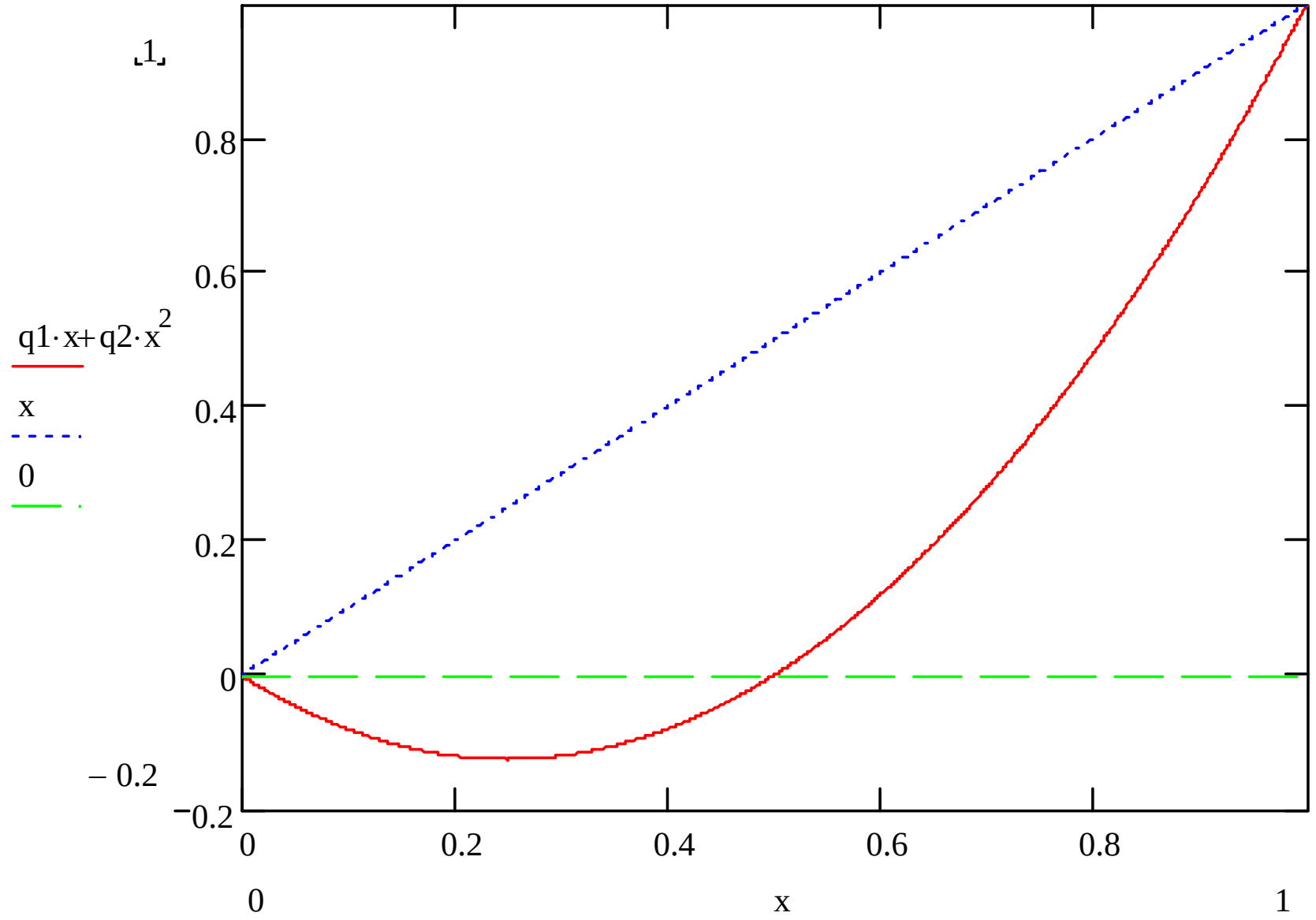
How can math help?

Useful quantitative implications of qualitative properties
(such as “may be J-shaped”)

Framework

- Observed data:
  - Exposure = x, Risk = M(x)
  - Scale to (x, M(x)) = (1, 1) without loss of generality
  - x = exposure level
  - M(x) = expected tumors/lifetime at exposure x
  - Without loss of generality, excess risk curve also goes through the origin: [x, M(x)] = (0, 0)
- Assume that *shape* of curve is uncertain
  - Might be linear or convex (or concave)
  - “Unknown probabilities for unknown curves”
- What conclusions can we draw?

Two possible dose-response curves

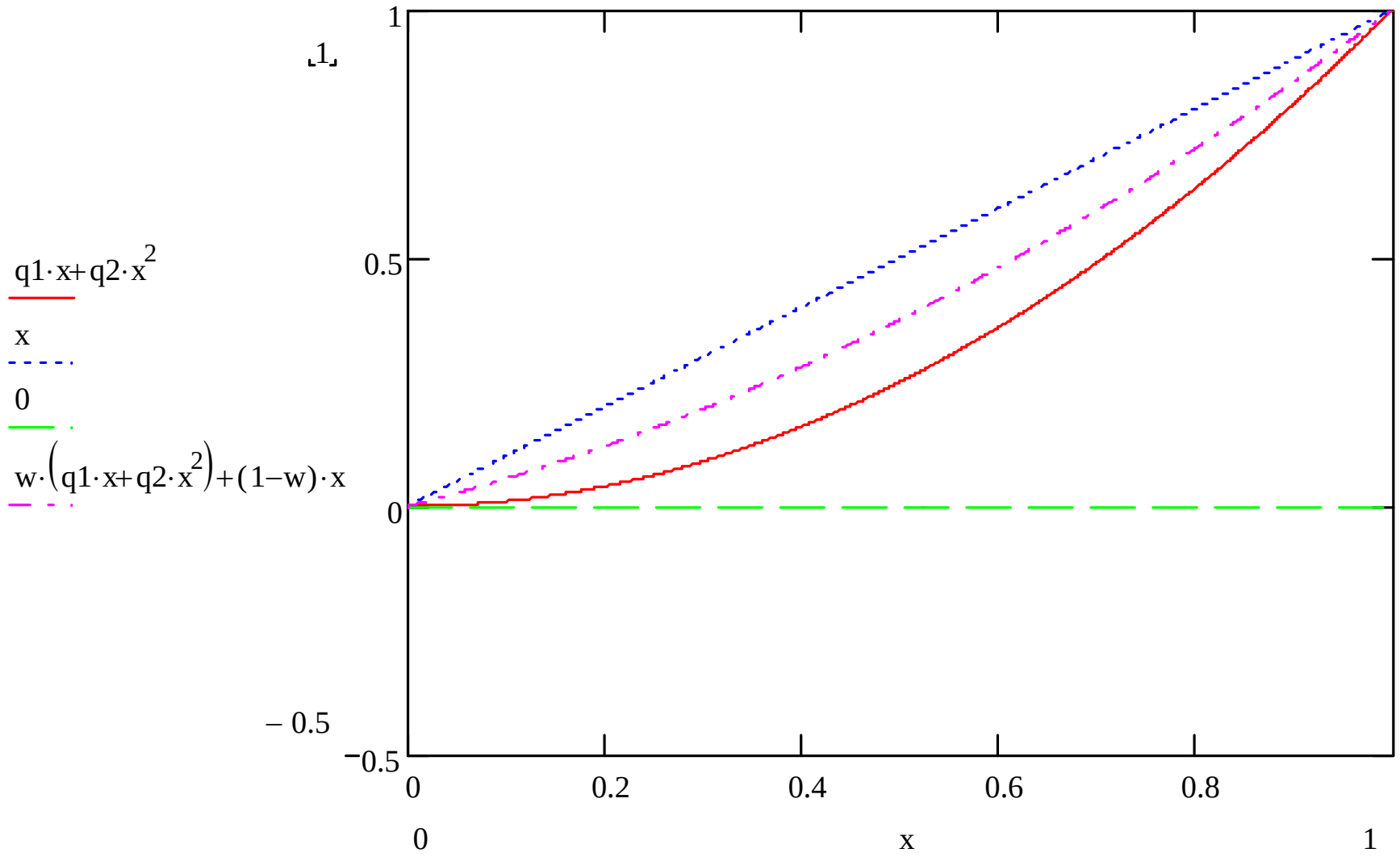


Result 1 (“Meta-hormesis”)

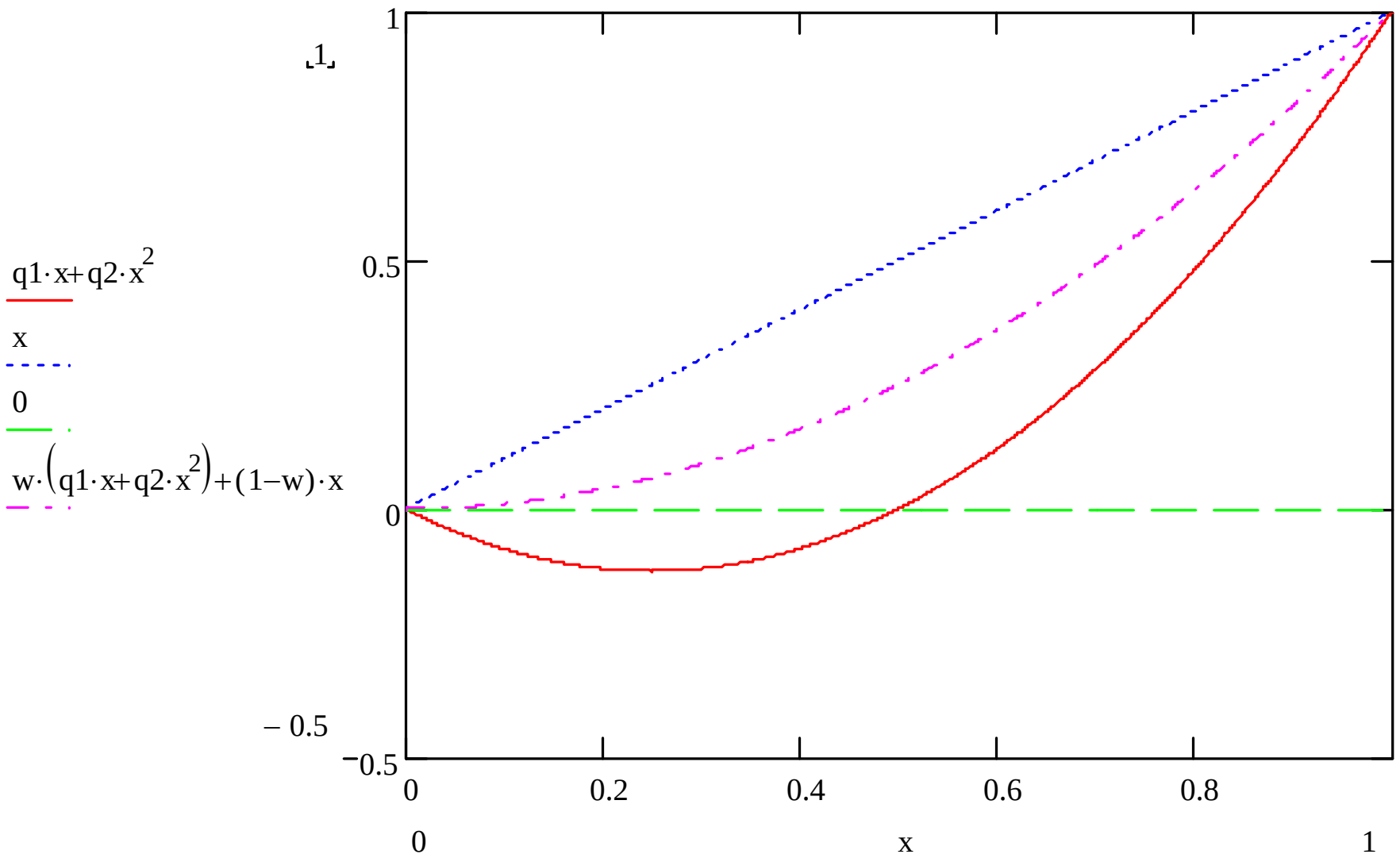
If the dose-response curve is *sufficiently uncertain* (high enough variance of slope at the origin) and its possible shapes are linear or upward-curved (J-shaped, convex), then to minimize expected risk, a rational decision-maker should set exposure levels *as if* this uncertain dose-response relation were *known to be hormetic.*

– Uncertainty pushes probability mass to extremes of allowed range; expected slope must be negative.

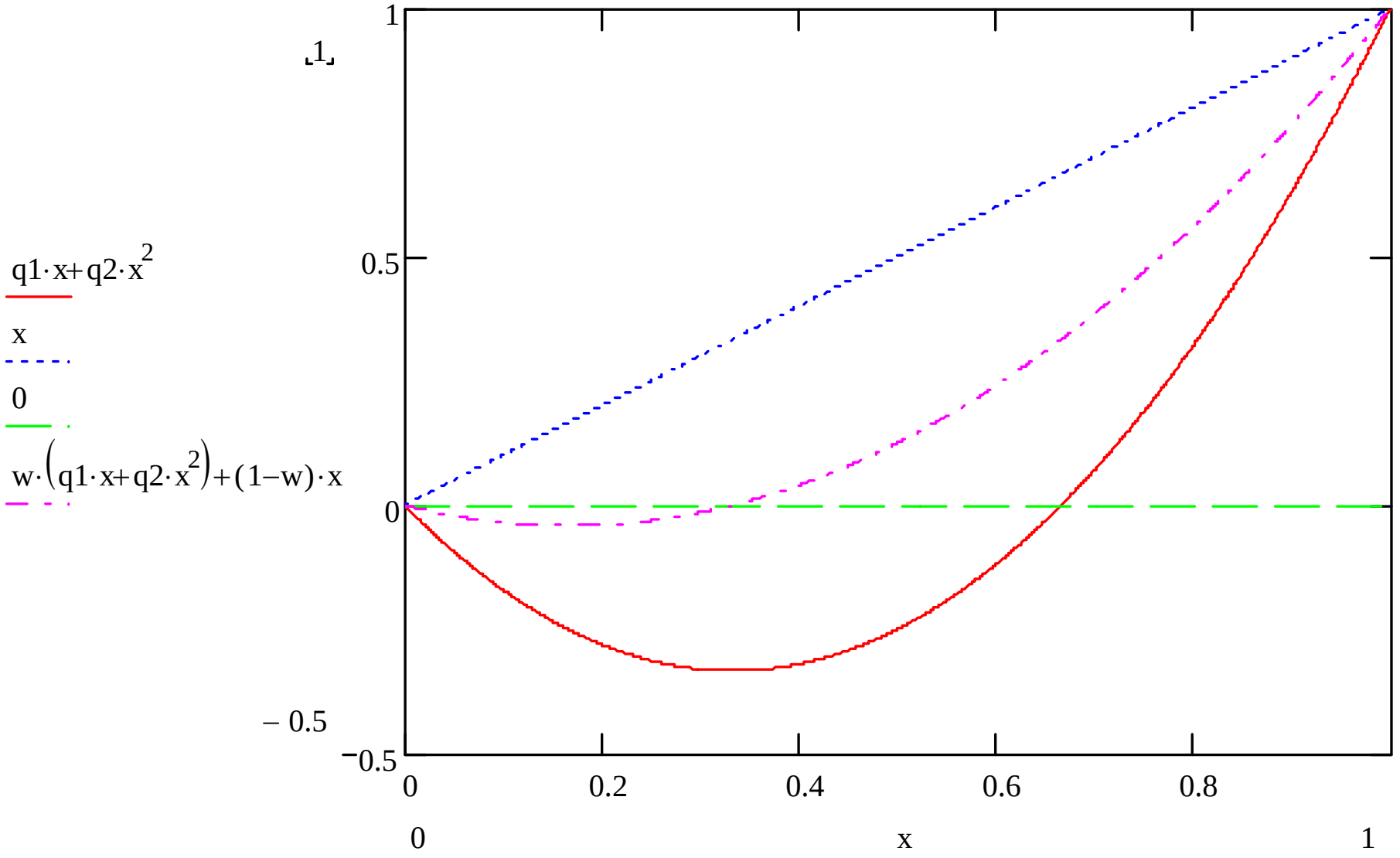
Small uncertainty



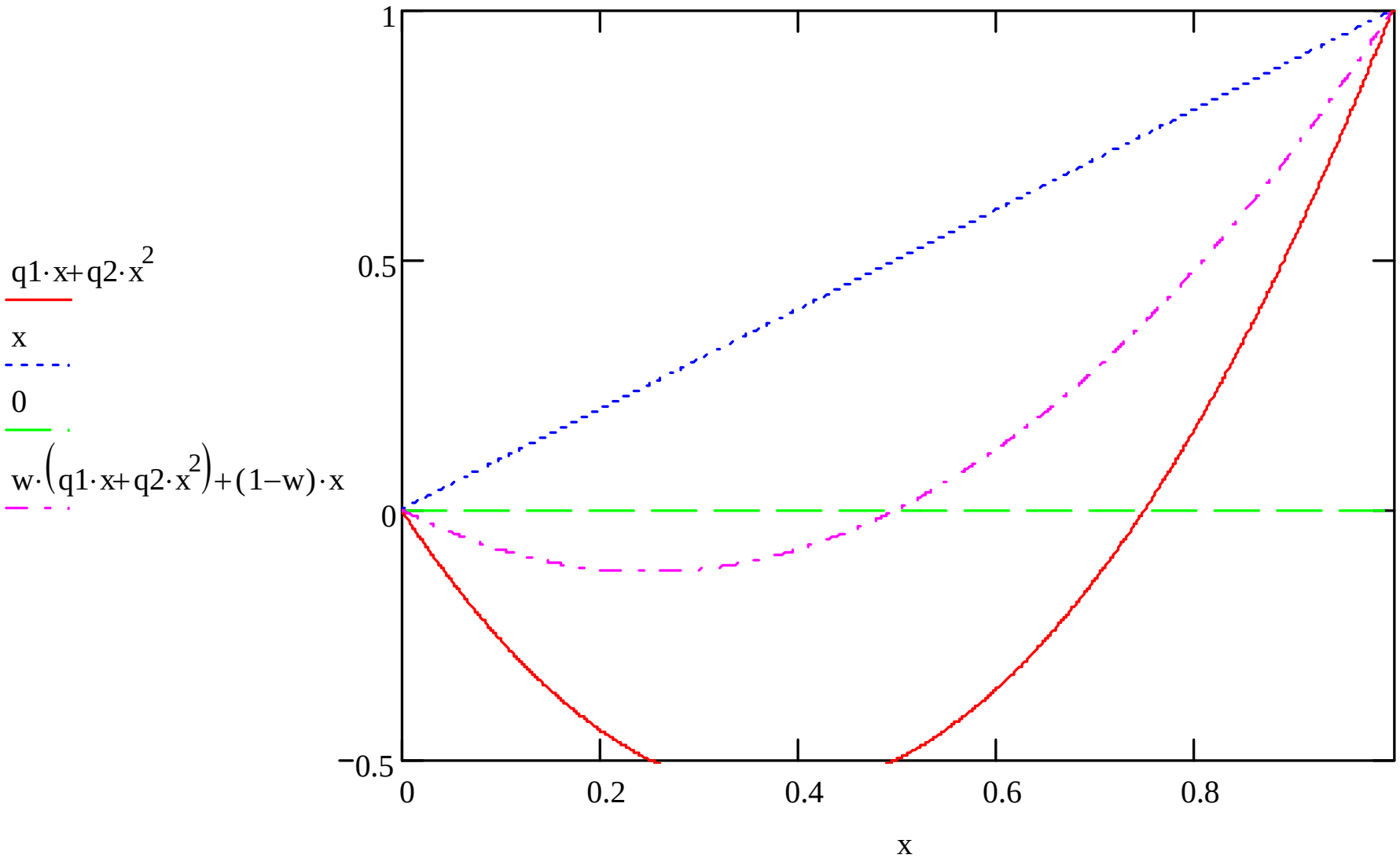
Larger uncertainty



Large enough uncertainty



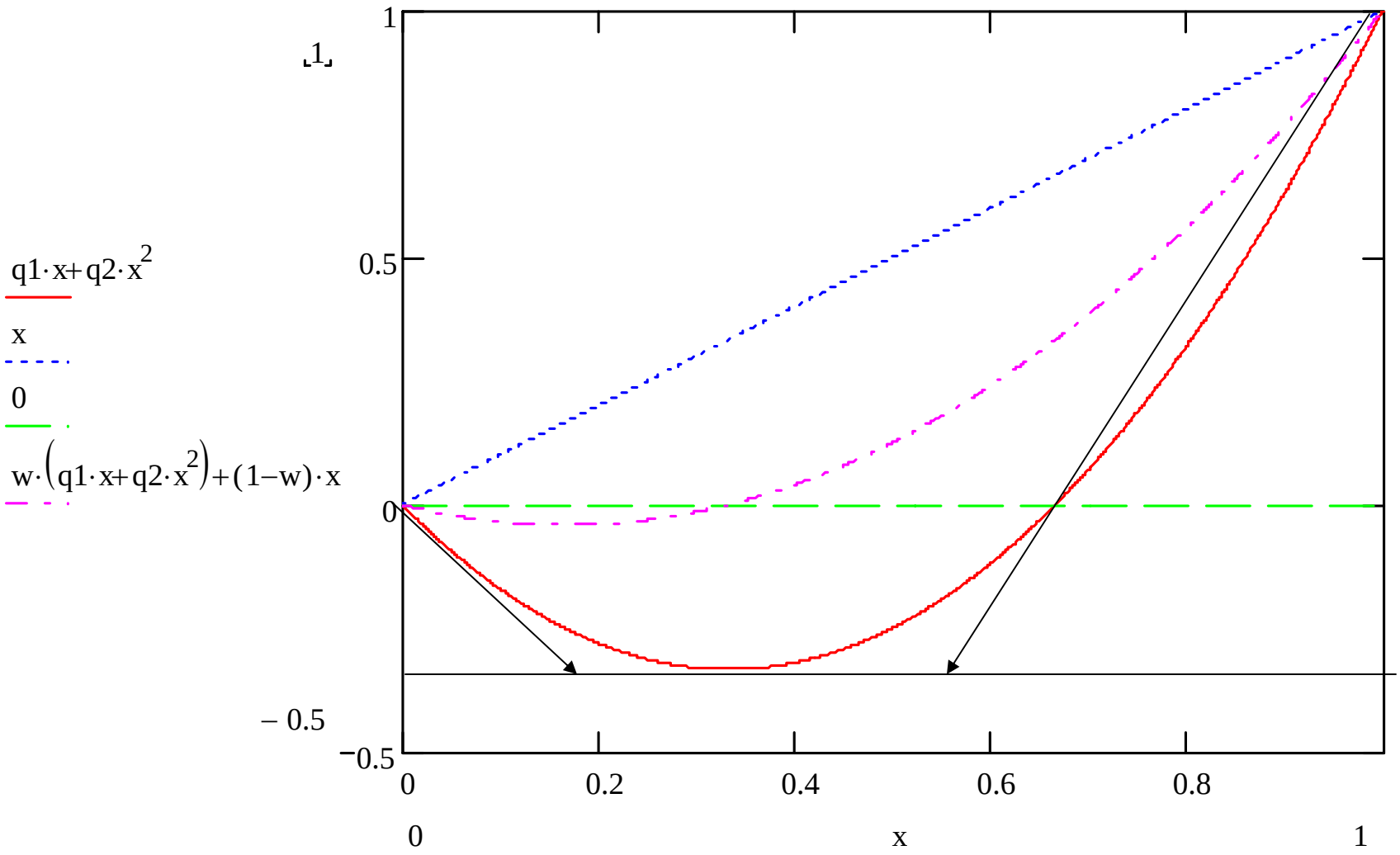
“Flat minimum” optimal dose



Result 2: Bounding optimal exposure in J-shaped models

- If the *slope at the origin* can be bounded (via model and approximate data) and the size of the *reduction in risk* due to hormesis can be bounded (e.g., “at least x% reduction”), then a *lower bound on the optimal dose* can be quantified.
- If the no-adverse effect level (NAEL) can also be bounded (“Not more than X ppm”), then an *upper bound on the optimal dose* can also be quantified.

Bounding the optimal dose



Properties of exposure bounds for hormesis (J-shaped) model

- More uncertainty about slope at origin $\rightarrow$ lower-bound on optimal exposure shifts left
  - Policy trade-off: Uncertainty creates meta-hormesis, but too much uncertainty weakens it.
- More conservative (smaller) bound on size of risk reduction due to hormesis $\rightarrow$ wider uncertainty bounds for optimal exposure
- Higher NAEL $\rightarrow$ higher upper-bound

Extensions for Co-carcinogenesis and variability

Dealing with variability

Arsenic many act through or interact with…

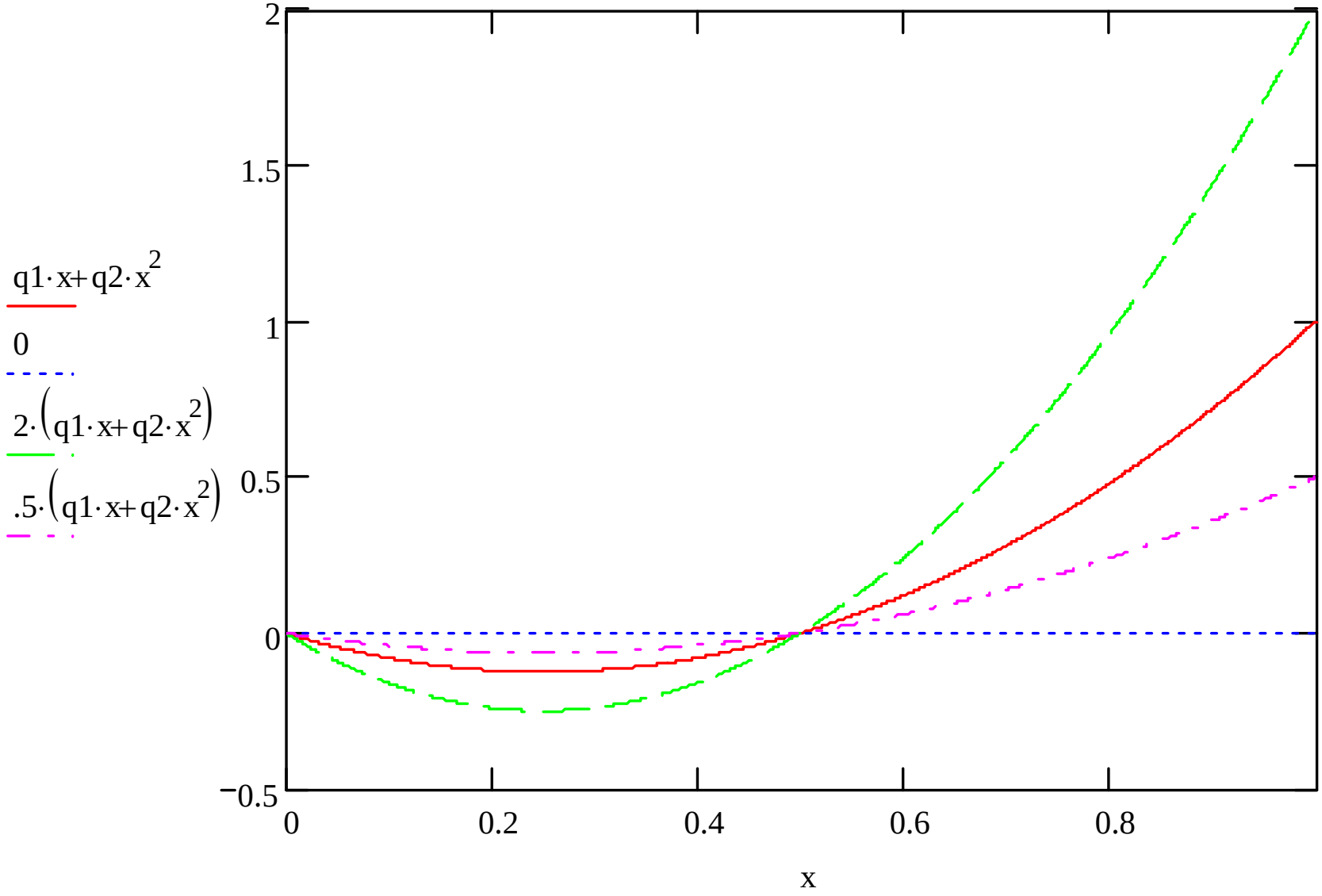
- **Co-carcinogens** (unknown patterns of individual **co-exposures**)
- Different individual **susceptibilities**
  - Genetic **polymorphisms**
- Different individual ages (DNA methylation)

How can we rationally manage risks from As if we don’t know all these other factors?

As interactions and co-carcinogenesis

Agent	Reference
Crystalline silica?	Chen, 2006
B(a)P	Evans, 2004
Smoking	Chen, 2004; Szymczak, 1997; many others
Diet/nutrition (and SES)	
MspI CYP1A1 *2A genotype	Adonis et al., 2005
Ultraviolet light	Rossman, 2002 (mice); Mudipalli et al., 2005, keratinocytes

A: Flat minimum principle!



Conclusions

- No one knows exactly how As causes lung cancer in people… or over what dose range.
  - But there are some good bets: DMA, methylation, p16 inactivation…
- Mathematical models of lung carcinogenesis suggest useful possible properties (e.g., J-shape) of dose-response relation
- These properties allow (a) Meta-hormesis result for risk management decisions; and (b) Bounds on optimal exposures (minimizing expected risk) in many situations.

Thanks!

Acknowledgments

- Philip Morris International (PMI)
- Edward Sanders, PMI, colleague and project sponsor
  - Projects on biologically-based and computational risk modeling of lung cancer; dose-response modeling for arsenic in cigarette smoke
- Bill Huber, Quantitative Decisions, collaborating on proving/extending results
- Paolo Ricci, Tom Macdonald, collaborating on risk management implications of hormesis

Tumor Stem Cell Niche Hypothesis (Baguley, 2006)

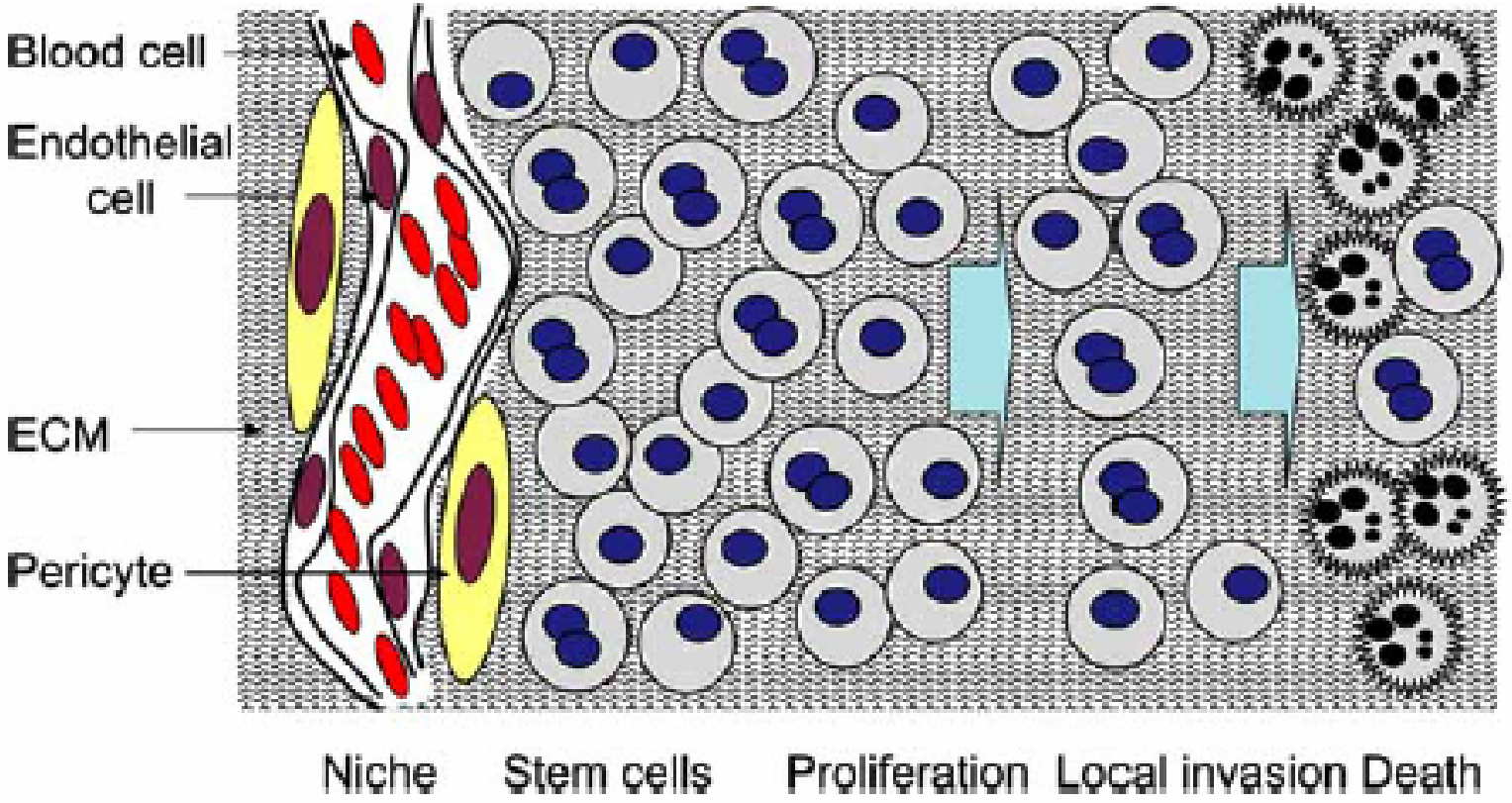


Fig. (2). Diagram of a tumor stem cell niche. As with the normal stem cell niche, it contains vascular endothelial cells, pericytes and the extracellular matrix, but the tumor stem cells are all in cycle rather than in G_0-phase. As each stem cell undergoes cell division, one daughter cell remains as a stem cell while the other leaves the niche to proliferate further and to migrate locally into tissue. The proliferating cells cannot revert to a non-cycling state, are therefore unable to undergo complete differentiation, and eventually die, probably by apoptosis.

Styblo et al., 2002

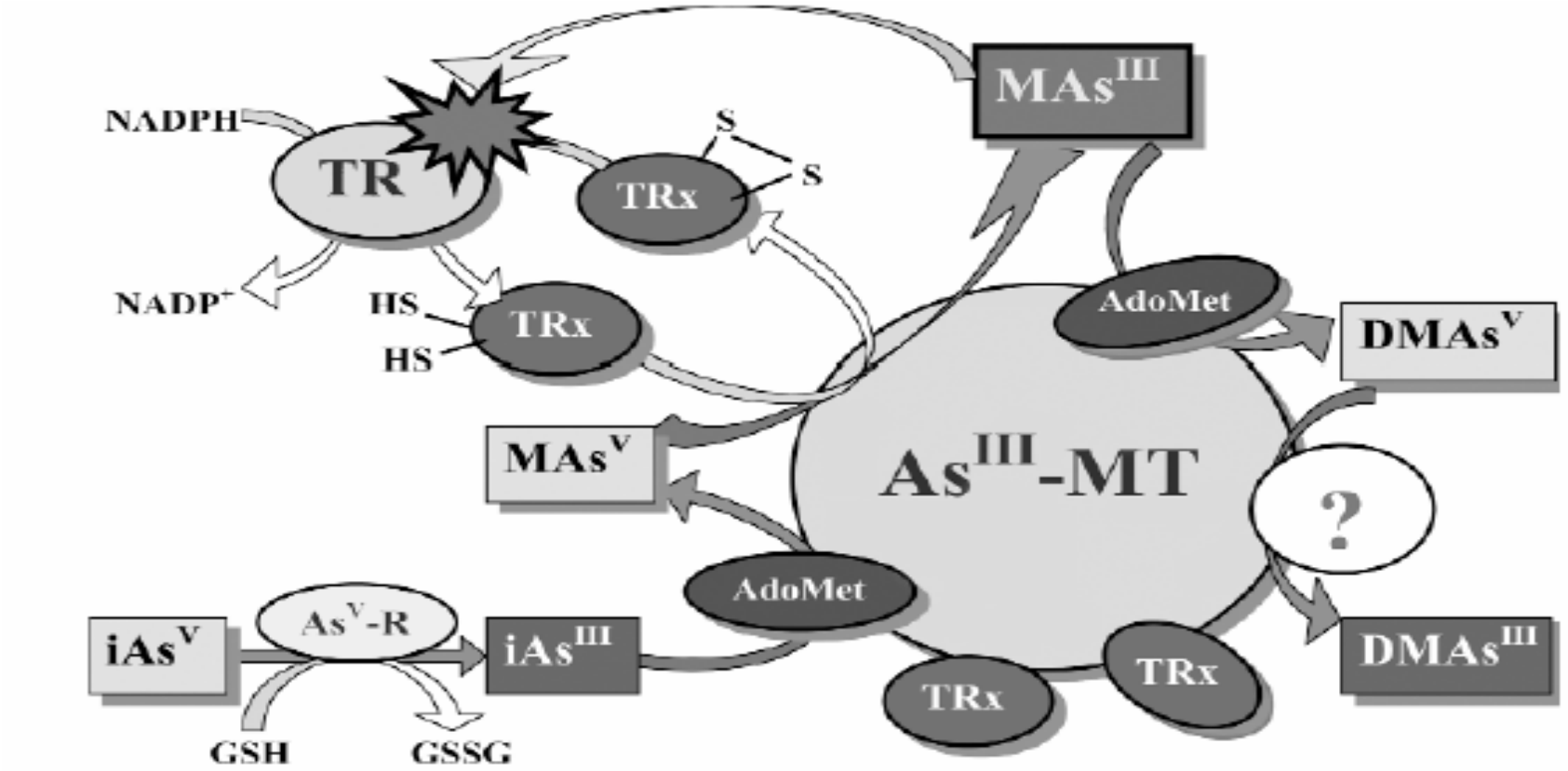


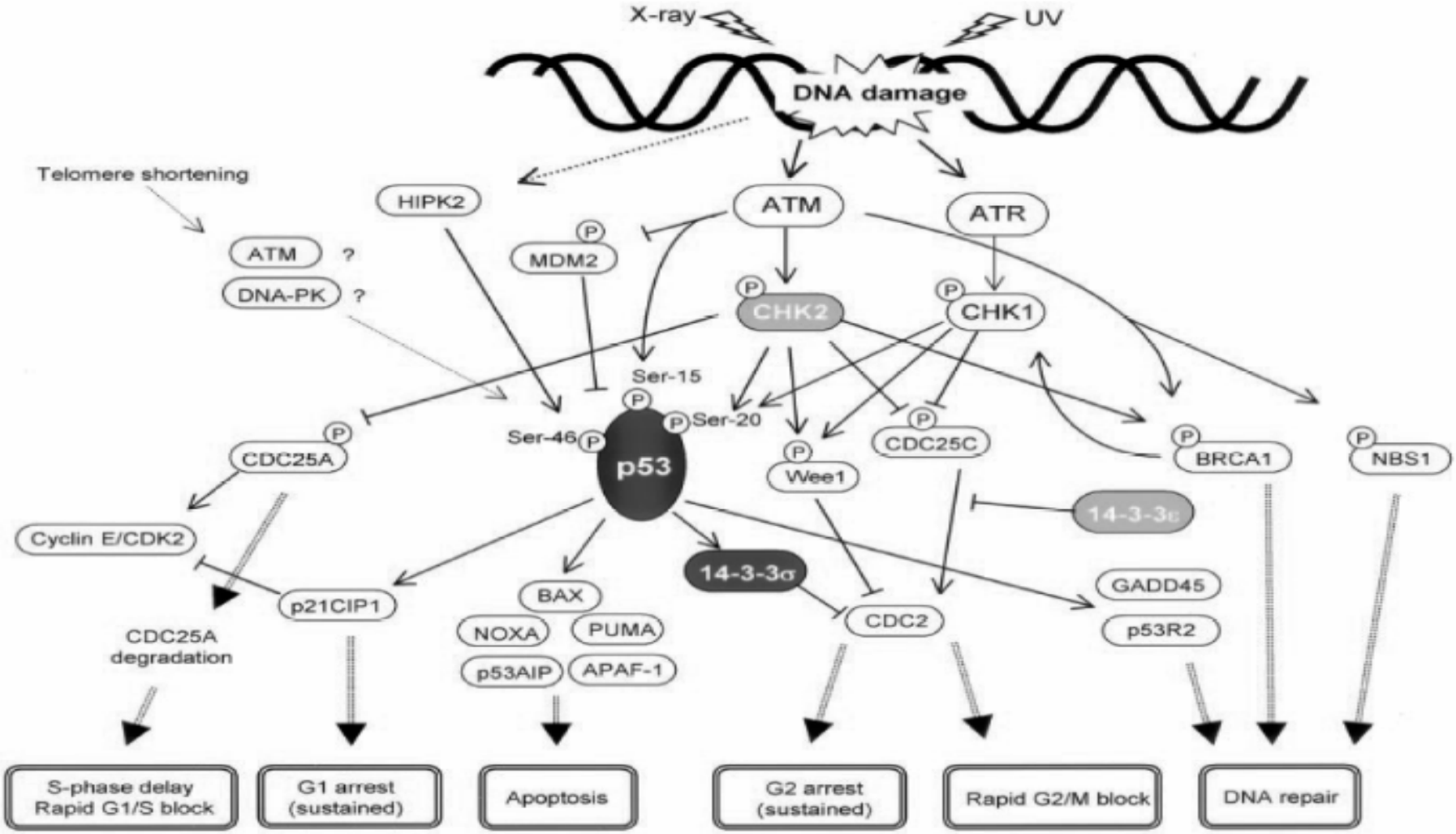
Figure 6. Hypothetical mechanism of the methylation of iAs by As^{III} methyltransferase: the role of Trx and TR. As^{V}-R, As^{V} reductase; As^{III}-MT, As^{III} methyltransferase.

Some proposed mechanisms of arsenic carcinogenesis (Schoen et al., 2004)

Plausible carcinogenic mechanisms of inorganic arsenic

Mechanism	Source of information
Oxidative injury	In vitro studies Animal studies Epidemiological studies
Disruption of DNA methylation	In vitro studies Animal studies
Inhibition of DNA repair enzymes	In vitro studies Epidemiological studies
Chromosomal damage	In vitro studies Animal studies Epidemiological studies
Modulation of signal transduction pathways and alteration of gene transcription	In vitro studies Animal studies

p53 acts on arrest, apoptosis, repair



Without suppression, abnormal growth can proceed (via EGFR, K-RAS, etc.)