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Adverse Outcome Pathway as an Integrating Framework for Systematic Review





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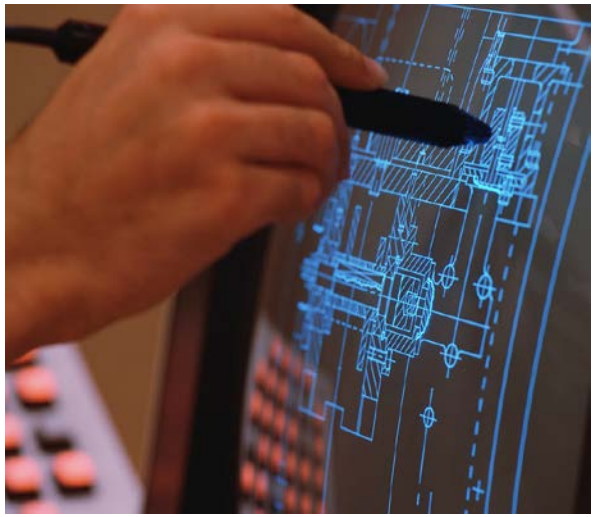

ADVERSE OUTCOME PATHWAY AS AN INTEGRATING FRAMEWORK FOR SYSTEMATIC REVIEW

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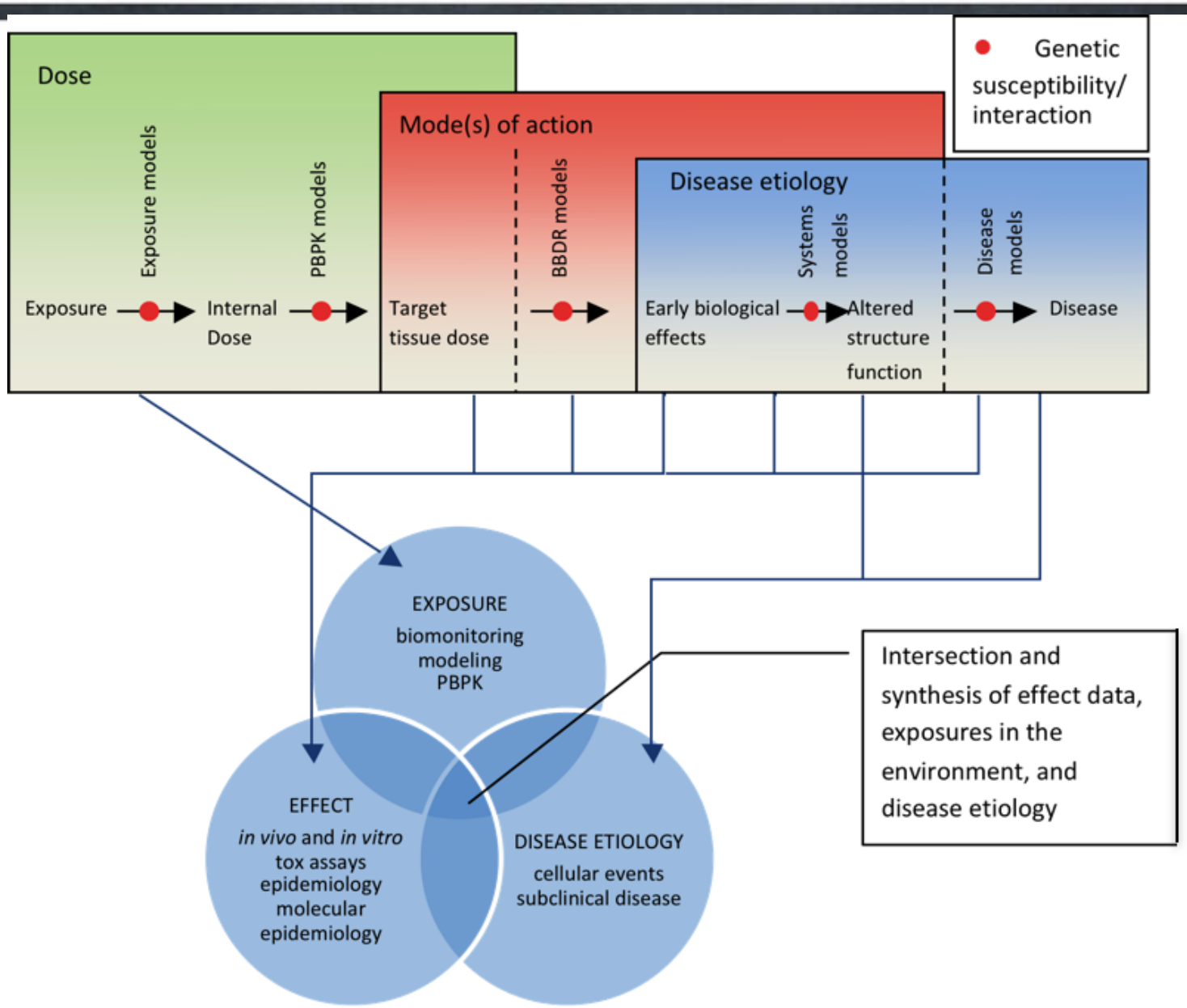
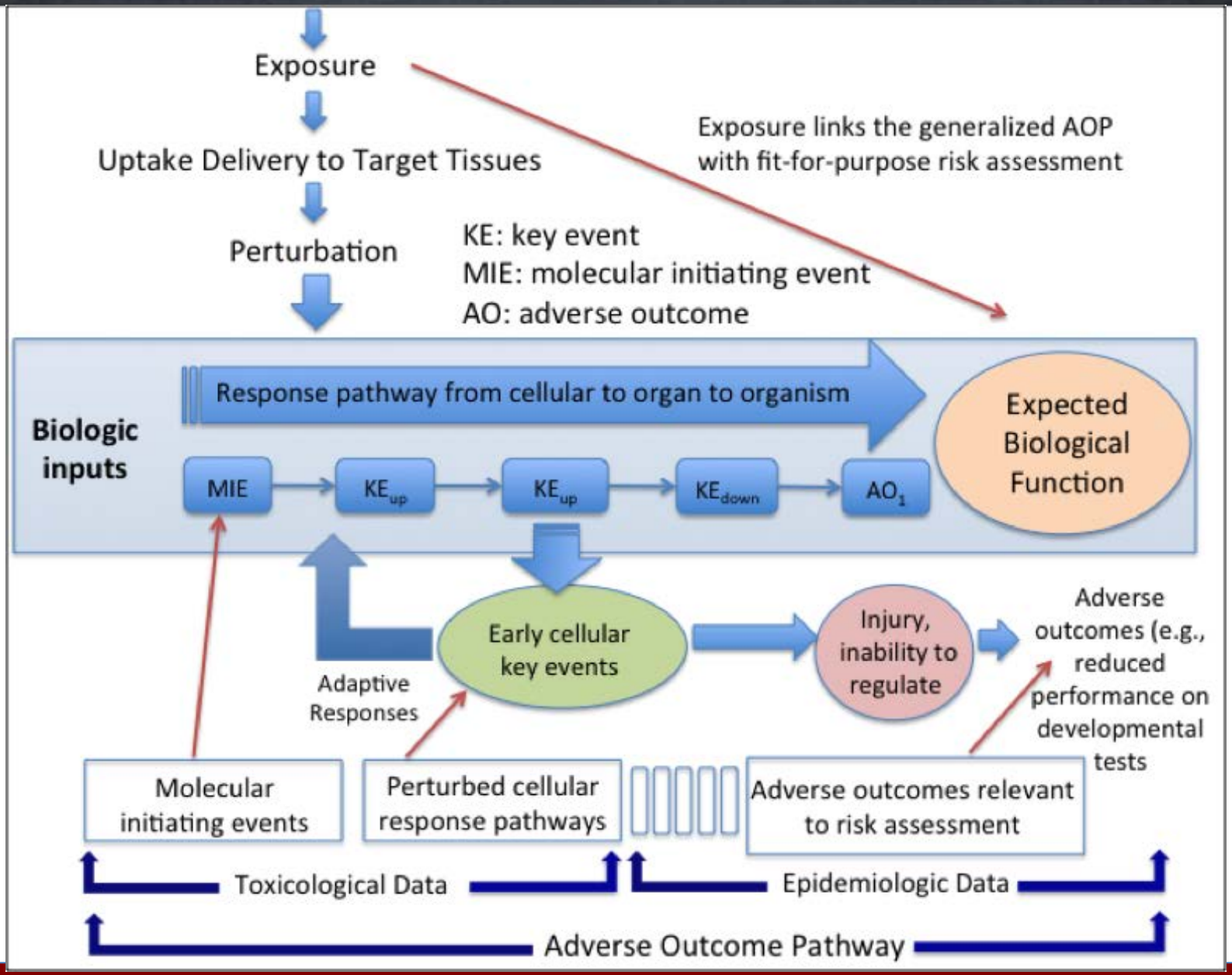
Biogeochemistry of Global Contaminants

Harvard School of Engineering and Applied Sciences

Synthesizing Evidence from Exposure to Health Outcome



Adverse Outcome Pathway



Case Studies as Examples

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Review Article

A Systematic Review of Carcinogenic Outcomes and Potential Mechanisms from Exposure to 2,4-D and MCPA in the Environment

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Chlorophenoxy compounds, particularly 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-
(MCPA), are amongst the most widely used herbicides in the United States for both agric
Epidemiologic studies suggest that exposure to 2,4-D and MCPA may be associated with incr
(NHL), Hodgkin's disease (HD), leukemia, and soft-tissue sarcoma (STS). Toxicological stu
carcinogenicity, and regulatory agencies worldwide consider chlorophenoxies as not likely to l
carcinogenicity. This systematic review assembles the available data to evaluate epidemiol
exposure, and biomonitoring studies with respect to key cellular events noted in diseas
hypothesized modes of action for these constituents to determine the plausibility of a
environmentally relevant concentrations of 2,4-D and MCPA and lymphohematopoietic canc
support a genotoxic mode of action. Although plausible hypotheses for other carcinogenic m
biomonitoring data to oral equivalent doses calculated from bioassay data shows that environ
support a causal relationship. Genetic polymorphisms exist that are known to increase the ri
interaction between these polymorphisms and exposures to chlorophenoxy compounds, pa
largely unknown.

Risk Analysis DOI: 10.1111/risa.12425

Perspective

Exposure to Mixtures of Metals and Neurodevelopmental Outcomes: A Multidisciplinary Review Using an Adverse Outcome Pathway Framework

Katherine von Stackelberg,[1,2,*] Elizabeth Guzy,[2] Tian Chu,[2] and Birgit Claus Henn[2,3]

Current risk assessment guidance calls for an individual chemical-by-chemical approach that fails to capture potential interactive effects of exposure to environmental mixtures and genetic variability. We conducted a review of the literature on relationships between prenatal and early life exposure to mixtures of lead (Pb), arsenic (As), cadmium (Cd), and manganese (Mn) with neurodevelopmental outcomes. We then used an adverse outcome pathway (AOP) framework to integrate lines of evidence from multiple disciplines based on evolving guidance developed by the Organization for Economic Cooperation and Development (OECD). Toxicological evidence suggests a greater than additive effect of combined exposures to As–Pb–Cd and to Mn with any other metal, and several epidemiologic studies also suggest synergistic effects from binary combinations of Pb–As, Pb–Cd, and Pb–Mn. The exposure levels reported in these epidemiologic studies largely fall at the high-end (e.g., 95th percentile) of biomonitoring data from the National Health and Nutrition Examination Survey (NHANES), suggesting a small but significant potential for high-end exposures. This review integrates multiple data sources using an AOP framework and provides an initial application of the OECD guidance in the context of potential neurodevelopmental toxicity of several metals, recognizing the evolving nature of regulatory interpretation and acceptance.

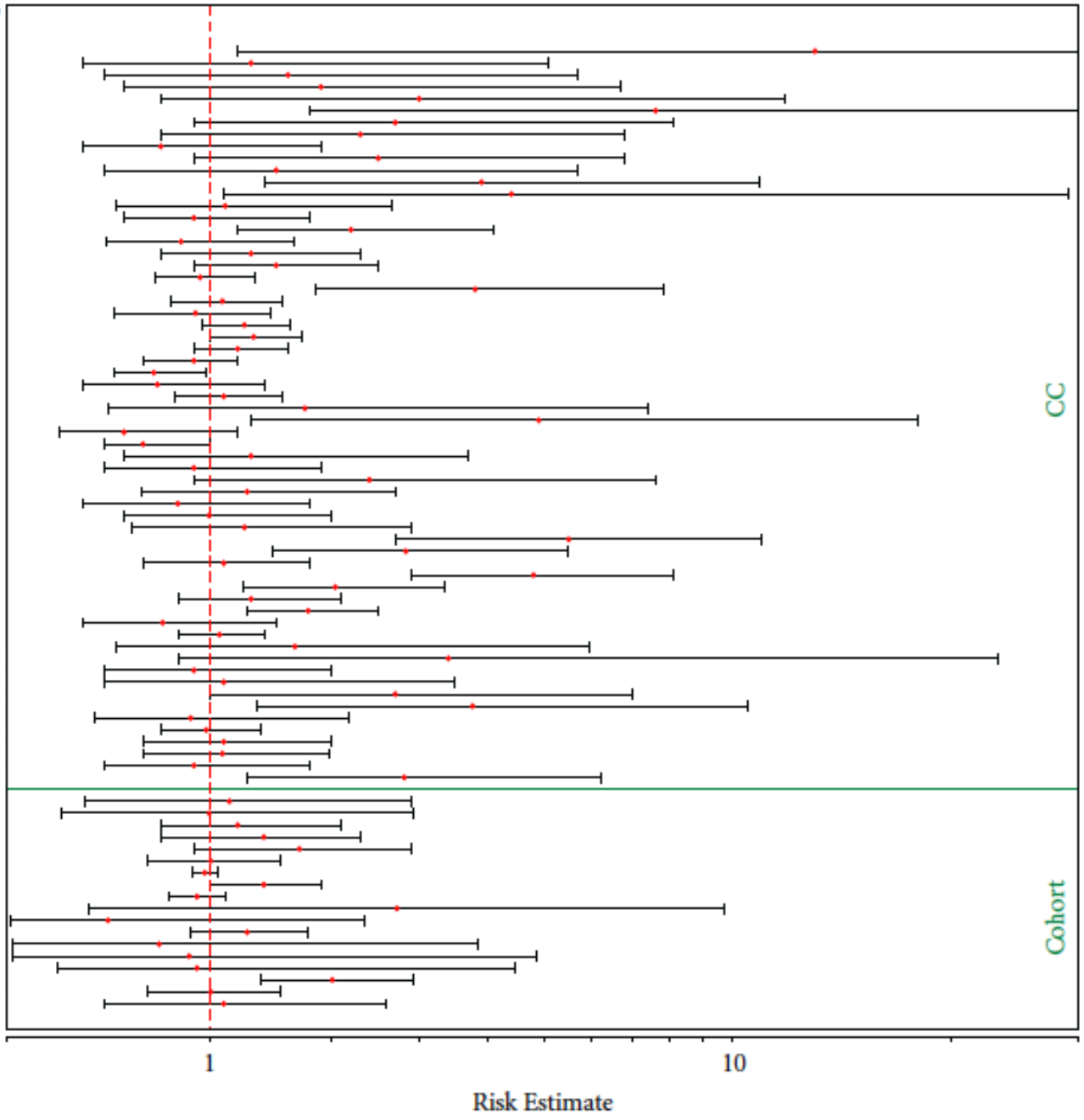
KEY WORDS: Adverse outcome pathway; arsenic; cadmium; developmental; lead; manganese; metal mixtures; systematic review

Regulatory Reviews for 2,4-D and Cancer

Table 1: Summary of Reviews of 2,4-D and/or MCPA.

Reference	Evaluation	Conclusions
Regulatory reviews or reviews in support of regulatory activities		
US EPA, SAB [27]	Science Advisory Board consultation on carcinogenicity of 2,4-D	"Data are not sufficient to conclude that there is a cause and effect relationship between exposure to 2,4-D and non-Hodgkin's lymphoma"
US EPA [28]	4th carcinogenicity of 2,4-D peer review	Not classifiable as to carcinogenicity
WHO/IARC [29]	Evaluations of carcinogenic risk	Inadequate and/or limited for 2,4-D specifically and chlorophenoxy compounds generally
US EPA [5]	Health Effects Division Carcinogenicity Peer Review Committee (2,4-D)	"Evidence is inadequate and cannot be interpreted as showing either the presence or absence of a carcinogenic effect."
European Commission [30]	Review report for 2,4-D	Proposed uses have no harmful effects on animal or human health; no evidence of carcinogenicity
US EPA [3, 4]	Risk assessments and reregistration decision for MCPA	Limited evidence for carcinogenicity
US EPA [8]	Risk assessments and reregistration decision for 2,4-D	Group D, not classifiable as to carcinogenicity
Health Canada PMRA [31]	Reregistration decision for 2,4-D	No evidence of carcinogenicity
Health Canada PMRA [32]	Reregistration decision for MCPA	No evidence of carcinogenicity
Health Canada [9]	MCPA in drinking water	Not considered a carcinogen
77FR23135 [33]	Response to NRDC petition to revoke 2,4-D registration	No new evidence that would suggest registration should be revoked

Reference	NHL OR	Cases	Controls	Contam
Hardell et al. 1994	13 (1.2–360)	3	1	2,4-D
Hour et al. 1986	1.3 (0.3–5.1)	3	16	2,4-D
Hour et al. 1986	1.6 (0.4–5.7)	4	16	2,4-D
Hour et al. 1986	1.9 (0.5–6.7)	4	16	2,4-D
Hour et al. 1986	3 (0.7–11.8)	4	9	2,4-D
Hour et al. 1986	7.6 (1.8–32.3)	5	6	2,4-D
Hour et al. 1986	2.7 (0.9–8.1)	6	17	2,4-D
Hour et al. 1986	2.3 (0.7–6.8)	6	17	2,4-D
Miligi et al. 2003	0.7 (0.3–1.9)	6	NR	2,4-D
Hour et al. 1986	2.5 (0.9–6.8)	7	22	2,4-D
Miligi et al. 2003	1.5 (0.4–5.7)	7	NR	2,4-D
Hour et al. 1986	3.9 (1.4–10.9)	8	15	2,4-D
Miligi et al. 2005	4.4 (1.1–29.1)	9	3	2,4-D
Kogevinas et al. 1995	1.11 (0.46–2.65)	12	56	2,4-D
Miligi et al. 2005	0.9 (0.5–1.8)	17	18	2,4-D
Hour et al. 1986	2.2 (1.2–4.1)	24	78	2,4-D
Hartge et al. 2005	0.82 (0.41–1.66)	24	18	2,4-D
Hardell and Eriksson 1999	1.3 (0.7–2.3)	43	62	2,4-D
Zahm et al. 1990	1.5 (0.9–2.5)	43	98	2,4-D
Hohenadel et al. 2011	0.94 (0.67–1.33)	49	187	2,4-D
Mills et al. 2005	3.8 (1.85–7.81)	60	NR	2,4-D
McDuffie et al. 2005	1.09 (0.76–1.56)	64	186	2,4-D
Hartge et al. 2005	0.91 (0.45–1.45)	86	59	2,4-D
McDuffie et al. 2005	1.25 (0.96–1.62)	110	293	2,4-D
McDuffie et al. 2001	1.32 (1.01–1.73)	111	293	2,4-D
Cantor et al. 1992	1.2 (0.9–1.6)	118	231	2,4-D
De Roos et al. 2003	0.9 (0.6–1.2)	123	314	2,4-D
Hartge et al. 2005	0.66 (0.45–0.98)	165	162	2,4-D
Wooch et al. 1989	0.68 (0.3–1.4)	181	196	2,4-D
Hartge et al. 2005	1.1 (0.78–1.55)	257	161	2,4-D
Fritschi et al. 2005	1.75 (0.42–7.38)	3	5	all
Persson et al. 1989	4.9 (1.3–18)	6	6	all
Lenz and Savitz 1995	0.5 (0.2–1.2)	6	79	all
Lenz and Savitz 1995	0.6 (0.4–1)	10	98	all
Miligi et al. 2003	1.3 (0.5–3.7)	11	NR	all
Orsi et al. 2009	0.9 (0.4–1.9)	11	25	all
Miligi et al. 2003	2.4 (0.9–7.6)	13	6	all
Eriksson et al. 2008	1.27 (0.59–2.7)	15	13	all
Lenz and Savitz 1995	0.8 (0.3–1.8)	15	118	all
Miligi et al. 2003	1 (0.5–2)	18	NR	all
Kogevinas et al. 1995	1.25 (0.54–2.9)	19	85	all
Hardell et al. 1994	5.5 (2.7–11)	25	24	all
Eriksson et al. 2008	2.83 (1.47–5.47)	32	13	all
Miligi et al. 2003	1.1 (0.6–1.8)	32	28	all
Hardell et al. 1981	4.8 (2.9–8.1)	41	24	all
Eriksson et al. 2008	2.04 (1.24–3.36)	47	26	all
Pearce et al. 1986	1.3 (0.8–2.1)	51	92	all
Hohenadel et al. 2011	1.78 (1.27–2.5)	63	133	all
Wooch et al. 1989	0.71 (0.3–1.5)	181	196	all
Wooch et al. 1987	1.07 (0.8–1.4)	576	694	all
Eriksson et al. 2008	1.66 (0.46–5.96)	6	4	MCPA
Miligi et al. 2005	3.4 (0.8–23.2)	7	3	MCPA
De Roos et al. 2003	0.9 (0.4–2)	8	16	MCPA
Miligi et al. 2003	1.1 (0.4–3.5)	8	NR	MCPA
Hardell and Eriksson 1999	2.7 (1–7)	12	11	MCPA
Eriksson et al. 2008	3.76 (1.35–10.5)	15	5	MCPA
Kogevinas et al. 1995	0.88 (0.36–2.18)	15	76	MCPA
McDuffie et al. 2005	0.98 (0.7–1.37)	15	33	MCPA
McDuffie et al. 2001	1.1 (0.6–2)	17	46	MCPA
McDuffie et al. 2005	1.09 (0.6–1.98)	17	46	MCPA
Miligi et al. 2005	0.9 (0.4–1.8)	18	19	MCPA
Eriksson et al. 2008	2.81 (1.27–6.22)	21	9	MCPA
Zahm 1997	1.14 (0.31–2.91)	cohort	study	2,4-D
Burns et al. 2001	1 (0.21–2.92)	cohort	study	2,4-D
Burns et al. 2011	1.2 (0.7–2.1)	cohort	study	2,4-D
Burns et al. 2011	1.4 (0.7–2.3)	cohort	study	2,4-D
Burns et al. 2011	1.7 (0.9–2.9)	cohort	study	2,4-D
Wiklund et al. 1987a	1.01 (0.63–1.54)	cohort	study	all
Wiklund et al. 1988b	0.97 (0.89–1.06)	cohort	study	all
Correa et al. 1989	1.4 (1.0–1.9)	cohort	study	all
Wigle et al. 1990	0.92 (0.75–1.11)	cohort	study	all
Coggon et al. 1991	2.72 (0.33–9.73)	cohort	study	all
Asp et al. 1994	0.42 (0.01–2.35)	cohort	study	all
Kogevinas et al. 1997	1.27 (0.88–1.78)	cohort	study	all
'T Mannetje et al. 2005	0.69 (0.02–3.84)	cohort	study	all
'T Mannetje et al. 2005	0.87 (0.02–4.87)	cohort	study	all
Boers 2010 Factory A	0.92 (0.19–4.47)	cohort	study	all
Jones et al. 2009	2.01 (1.38–2.95)	cohort	study	all
Wiklund et al. 1989	1.01 (0.63–1.54)	cohort	study	MCPA
Lynge 1998	1.1 (0.4–2.6)	cohort	study	MCPA



- 5% LCL
- 95% UCL
- Estimate

Putative AOP – Generalized Schematic of NHL

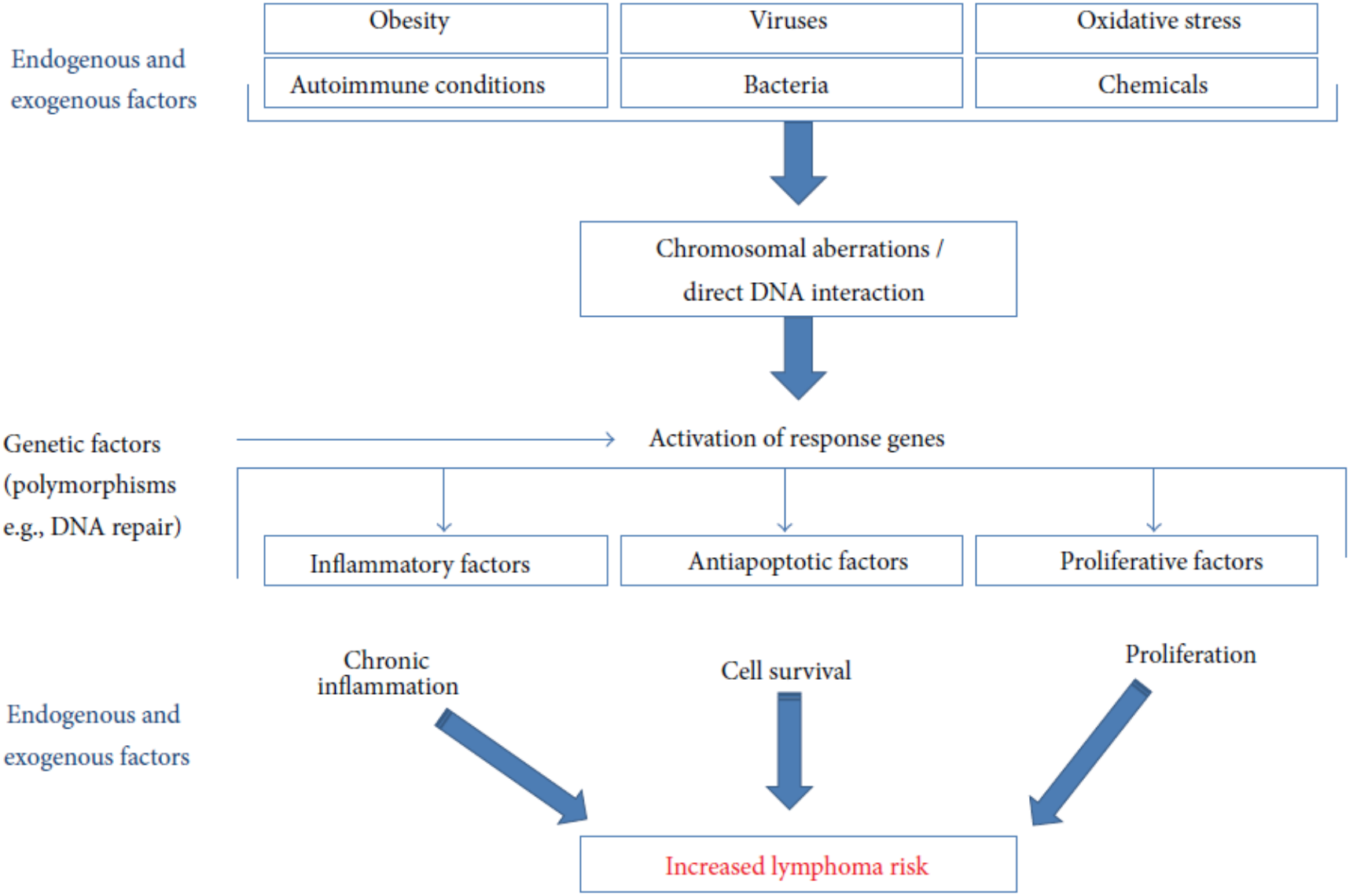


FIGURE 5: Schematic of general pathways leading to increased risk of developing lymphoma.

Genetic Abnormalities in NHL

Foreign antigen independent → Foreign antigen dependent → Terminal differentiation

Bone marrow → Peripheral lymphoid tissue

B cells	Somatic mutations	Corresponding lymphoma	Genetic abnormalities	Percent of cases
Stem cell	None			
Pro B cell	None			
Pre B cell	None	B-cell lymphoblastic lymphoma (B-LBL)		
Immature B cell	None			
Mature naïve B cell	None	Mantle cell lymphoma	t(11; 14)(q13; q32)	>95%
		Chronic lymphocytic leukemia		
Germinal center	Introduction of somatic mutations	Burkitt lymphoma (BL)	t(8; 14)(q24; q32)	80%
			t(8; 22)(q24; q11)	5%
			t(2; 8)(p11; q24)	15%
		Follicular lymphoma (FL)	t(14; 18)(q32; q21)	90%
			t(2; 18)(p11; q21)	5%
			t(18; 22)(q21; q11)	5%
		Lymphocyte-predominant Hodgkin lymphoma		
		Diffuse large cell B-cell lymphoma (B-LBL)	der(3)(q27)	35%
			t(14; 18)(q32; q21)	30%
			t(3; 14)(p14.1; q32)	Unknown
			t(3; 14)(q27; q32)	35%
			t(8; 14)(q24; q32)	10%
			t(8; 22)(q24; q11)	5%
		Hodgkins disease		
Memory B cell	Somatic mutations	Marginal zone B-cell lymphoma; chronic lymphocytic leukemia	t(9; 14)(p13; q32)	50%
Plasma cell	Somatic mutations	Plasmacytoma/myeloma		
		Anaplastic large T-cell lymphoma	t(2; 5)(p23; q35)	60% adults; 85% children

Molecular Epidemiology for 2,4-D

- Urinary 2,4-D levels not correlated with chromosome aberration frequency (although significant genomic instability)
- Exposure-related effects observed were reversible and temporary (*Source: Garry et al. EHP 2001)*
- t(14;18)-positive NHL cases have larger relative risks from agricultural exposures than t(14;18)-negative cases, but show no association with 2,4-D or chlorophenoxys (*Source: Chiu and Blair J Agromed 2009; Schroeder et al. Epi 2001; Agopian et al. J Exp Med 2009)*
- Significant evidence of genetic polymorphisms that contribute to NHL risk (*Source: Hill et al. Blood 2006; Kelly et al. Cancer Epidemiol Biomarkers Prev 2010)*
- t(14;18) occurs frequently in general public *(Source: Bende et al. Leukemia 2007; Janz et al. Genes Chrom Canc 2003)*

Summary of Toxicological Studies

- 2,4-D and MCPA are capable of interacting with cellular functions
  - Cell proliferation
  - CD38 – MCPA – marker in NHL
  - Mitotic arrest
- Show an impact on immunological parameters in humans exposed *in vivo* but effects are transient and reversible
- Less evidence for direct DNA interaction
  - Equivocal results *in vivo*
  - Some positive *in vitro* but did not come up in ToxCast
- Commercial formulations rather than pure product tend to show positive results
- No evidence for difference in toxicity across salts, acid, ester

Reverse Dosimetry to Obtain Urinary Concentrations Associated with *in vivo/in vitro* Assays to Compare to Biomonitoring Data

$$\text{Oral Equivalent Dose} = AC_{50} \bullet \frac{1\,\text{mg/kg} - \text{d}}{C_{ss}\,(\mu\text{M})}$$

Source: Wetmore et al. Tox Sci 2011

Predicted concentration in urine at oral equivalent dose in µg/L:

Source: Aylward et al. Reg Tox Pharm 2008

$$C_{urine} = \frac{OED \bullet BW}{V_{24hr}}$$

Compare to biomonitoring data

Use other reference values from bioassays

Evaluate underlying assays with respect to disease etiology

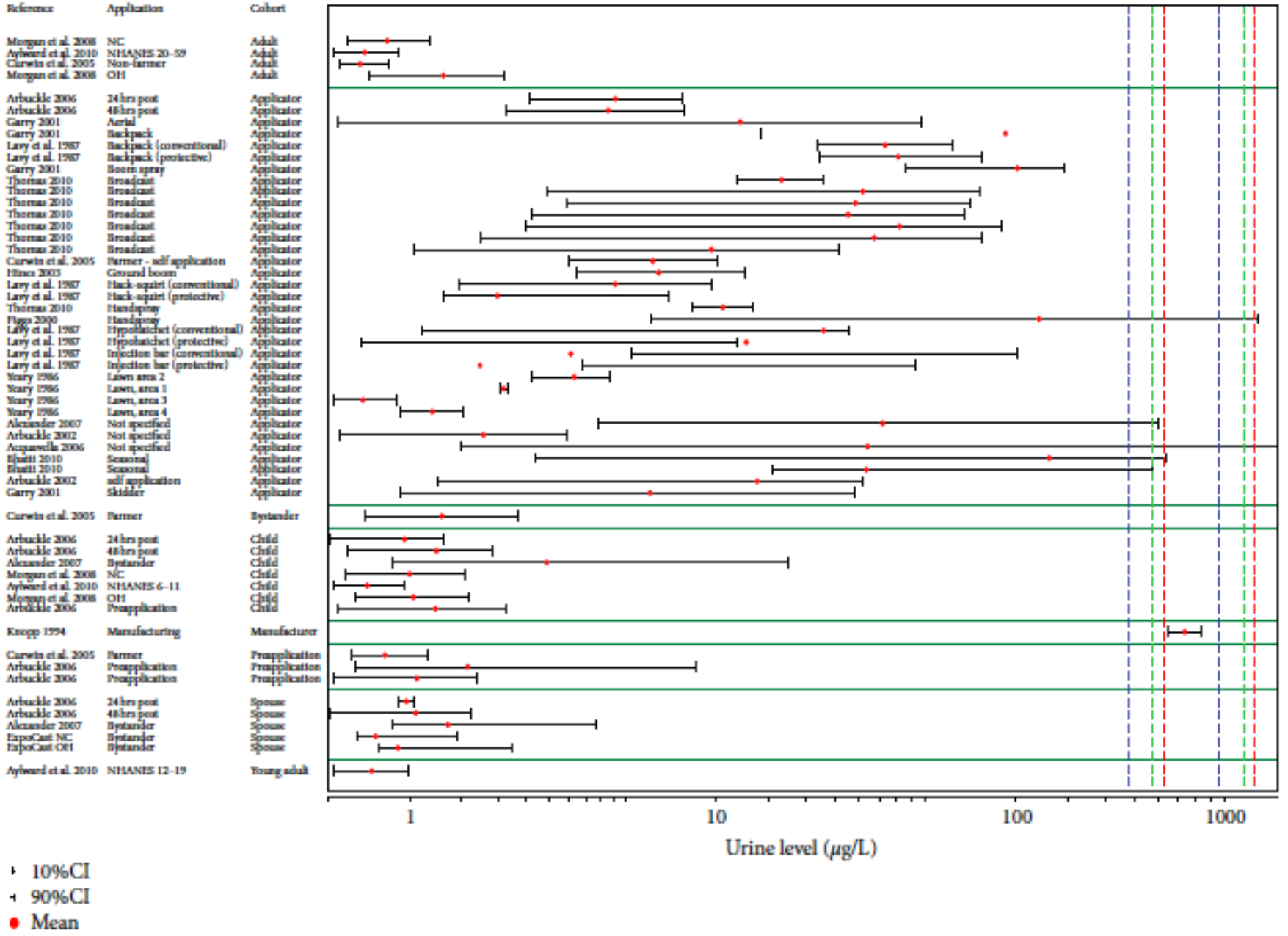


FIGURE 7: Biomonitoring data for 2,4-D in the context of backcalculated urine levels. Reference lines: green = men 575 μg/L (RfD), men 1170 μg/L (ToxCast reverse dosimetry). Blue = women 480 μg/L (RfD), women 960 μg/L (ToxCast reverse dosimetry). Red = child 630 μg/L (RfD), child 1250 μg/L (ToxCast reverse dosimetry) (see for review [26, 124, 154, 201, 214, 215, 218–227]).

Exposure to Mixtures of Metals

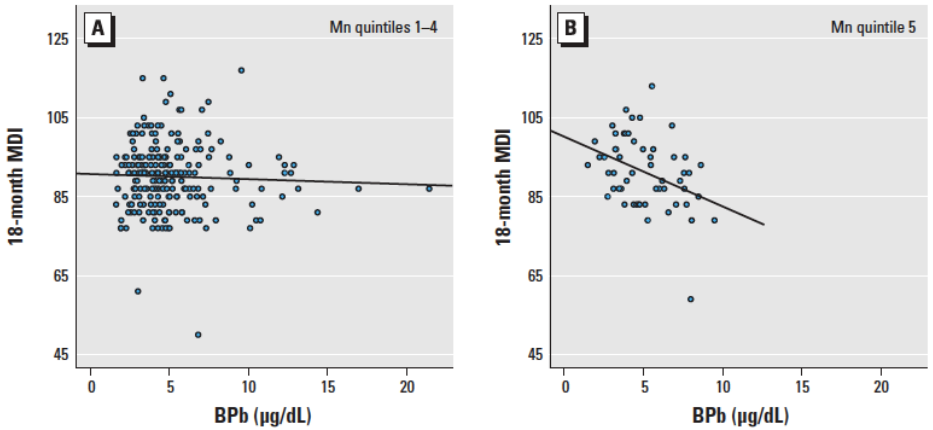


Figure 1. Scatterplots and regression lines of 12-month blood lead (BPb) and 18-month MDI among children with 12-month blood manganese (Mn) in quintiles 1–4 (*A*) and quintile 5 (*B*).

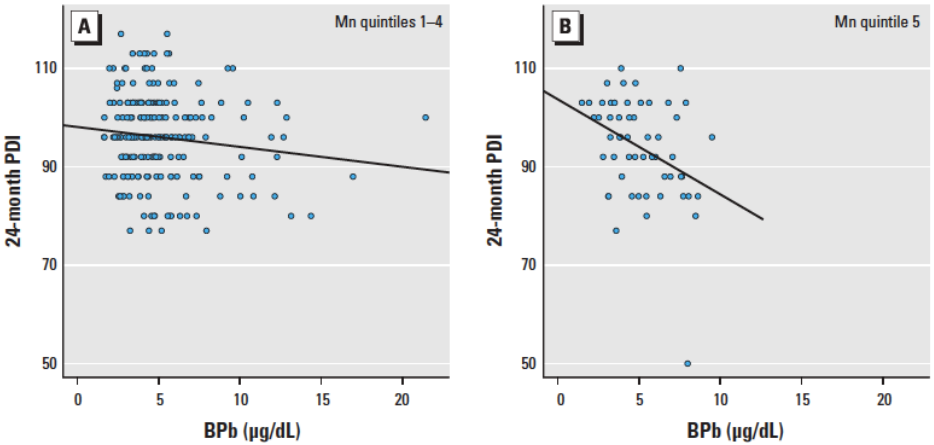
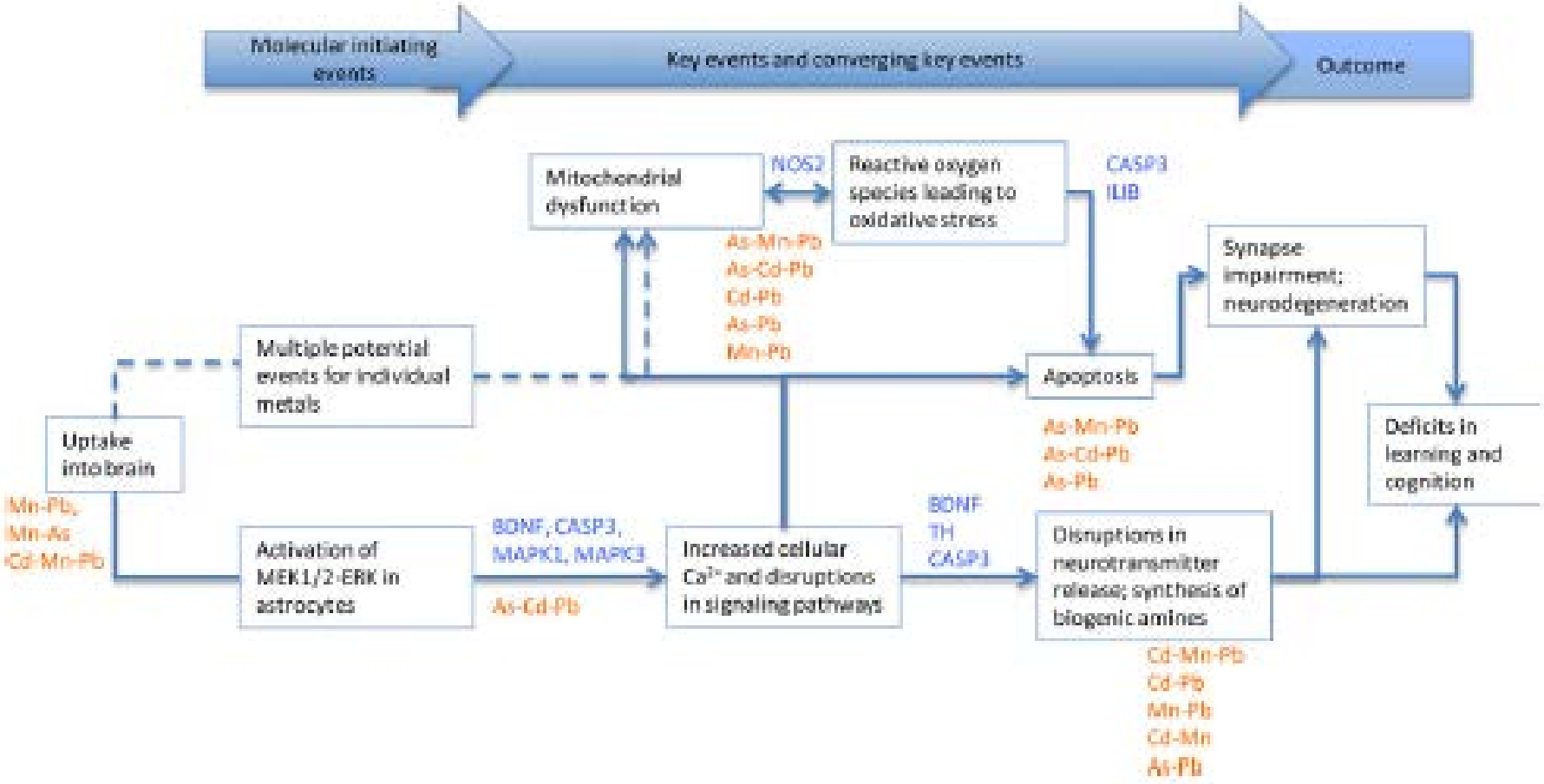


Figure 2. Scatterplots and regression lines of 12-month blood lead (BPb) on 24-month PDI among children with 12-month blood manganese (Mn) in quintiles 1–4 (*A*) and quintile 5 (*B*).

- Superfund Research Program at Harvard
- Mexico city cohort n=493
- Previous study showed U-shaped association between Mn and neurodevelopmental outcomes at 12 mos (*Claus-Henn et al. 2010, Epidemiology, 21(4):433-439*)
- Exposure to both Pb and Mn associated with greater cognitive deficits than to either constituent individually *(Claus-Henn et al. 2012, EHP, 120(1):126-131)*

- Evidence for less or greater than additive effect
  - Independent or additive
  - Antagonistic
  - Synergistic
- Multiple exposures leading to a common but non-specific health outcome
  - Reduced performance on a battery of tests including WISC, Bayley's, etc.
- Frame for the AOP is normal neuronal development and opportunities for perturbations
- May be similar or different mechanisms
- AOPwiki and associated OECD guidance

Strawman for Greater than Additive Effects



Biological Plausibility and Essentiality

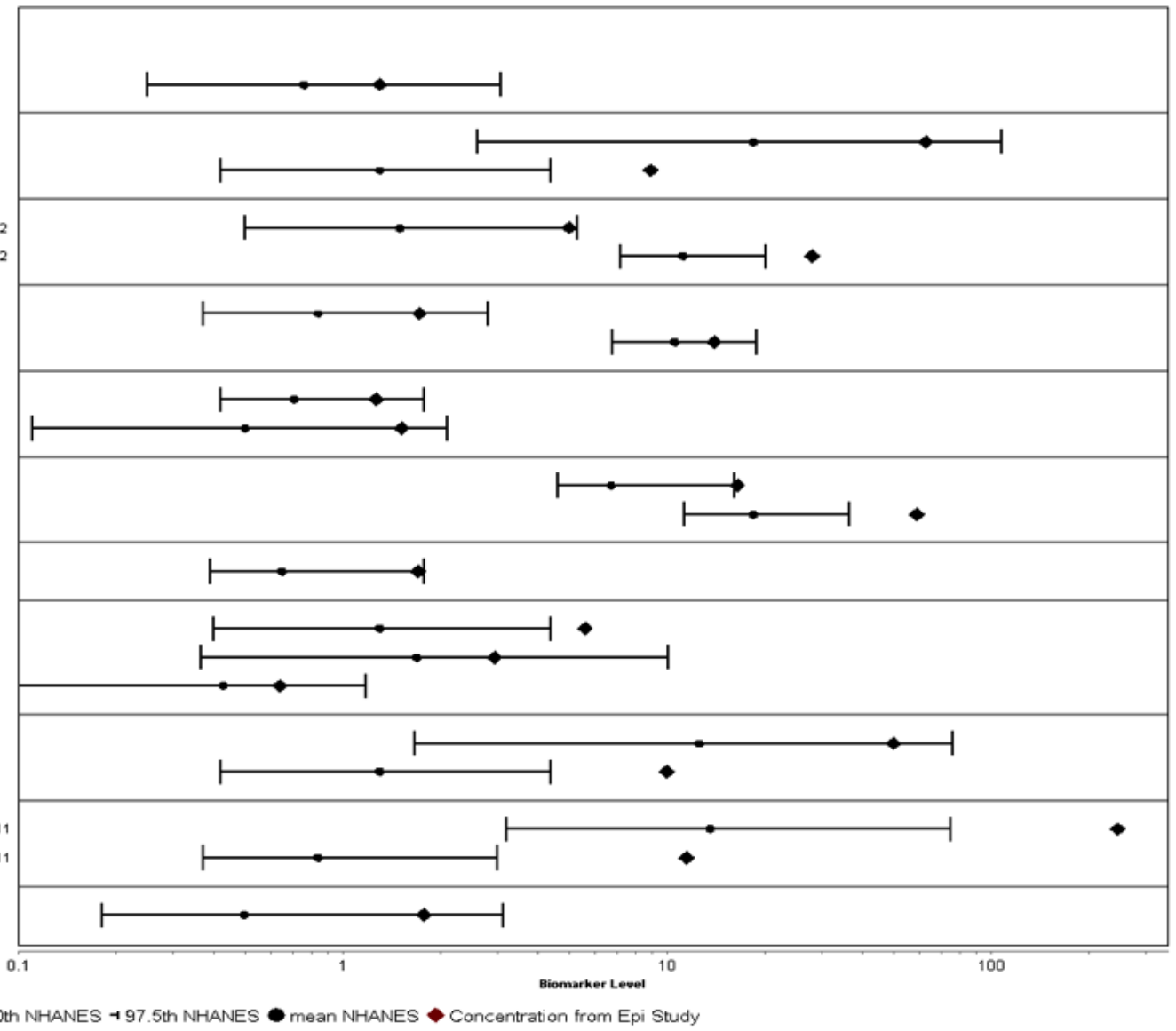
1. Support for Biological Plausibility	Strong	Moderate
MIE: Activation of MEK1/2, ERK in astrocytes	As–Cd–Pb: 63 As: 121 Cd: 122, 123, 124 Mn: 125–127 Pb: 128	
KE: Increased cellular Ca^{2+}	As–Cd–Pb: 60, 63 As: 135, 136, 137 Cd: 92, 123, 124, 138 Mn: 125 Pb: 96, 97	
KE: Reactive organic species and oxidative stress in brain tissue		As–Mn–Pb: 68 As–Cd–Pb: 63, 64 Cd–Pb: 42 As–Pb: 55 As: 121, 123, 135, 136, 137, 139, 140, 141 Cd: 92, 122, 134 Mn: 98, 123, 125, 138, 142 Pb: 97, 143
KE: Apoptosis of astrocytes or neurons	As–Cd–Pb: 60–63 As–Pb: 55 As: 121, 139, 137, 141 Cd: 92, 139, 138, 124 Mn: 153, 125, 126, 152 Pb: 96, 112, 154	
KE2: Disruptions in neurotransmitter release		Cd–Mn–Pb: 33 Cd–Pb: 38, 40, 47 As–Pb: 54 Cd–Mn: 59 Mn–Pb: 50 As: 157, 135, 142 Cd: 123, 124 Mn: 98, 99, 144 Pb: 96, 97, 112, 143, 154
AO: Learning and cognition disorders		As–Pb: 81, 84 reported qualitative interaction, 76–79 reported synergistic Mn–Pb: 14, 87 As–Mn: 15

2. Support for Essentiality of KEs	Strong	Moderate
MIE: Activation of MEK1/2, ERK in astrocytes	117, 119–121	
KE: Increased cellular Ca^{2+}	130, 131, 132, 133, 149	
KE: Reactive organic species and oxidative stress in brain tissue	118, 129, 141	
KE: Apoptosis of astrocytes or neurons	113, 152, 158	
KE2: Disruptions in neurotransmitter release	101, 111, 155, 156, 159	
3. Empirical Support for KERs	**Strong**	**Moderate**
Key event relationships	Empirical evidence/data is insufficient to develop regulatory values for synergistic effects of metal mixtures.	

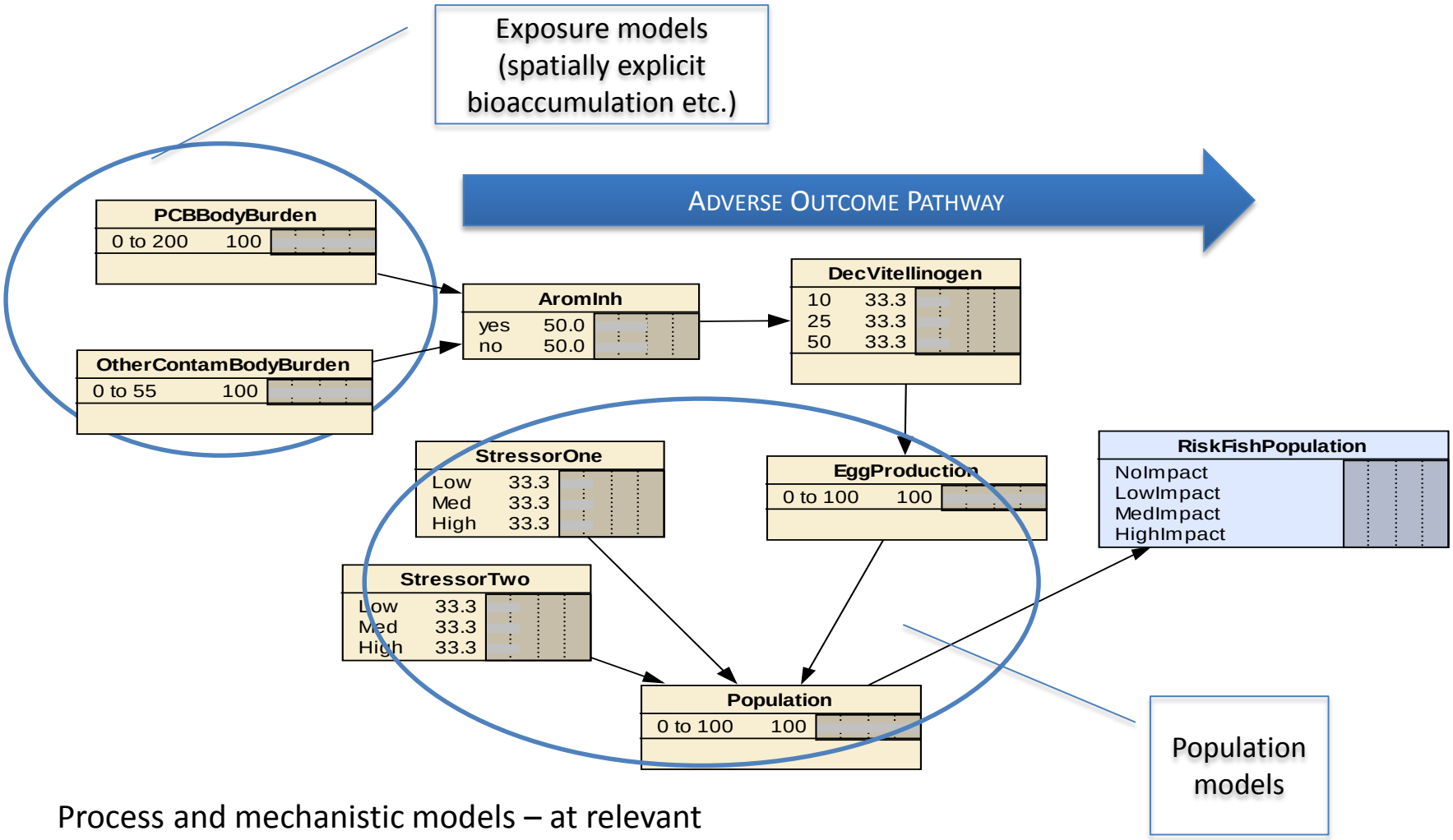
Numbers refer to reference number.

Comparison of Levels from Epi Studies to Biomonitoring Data

Cohort	Epi Exposure	Contam	Units	Reference
children 6-8	creat-adj urine	Pb	µg/g creat	Adams et al. 2009
children 6-9	creat-adj urine	As	µg/g creat	Calderon et al. 2001
children 6-9	blood	Pb	µg/dL	Calderon et al. 2001
children 1-2	blood	Pb	µg/dL	Claus Henn et al. 2012
children 1-2	blood	Mn	µg/L	Claus Henn et al. 2012
children 8-11	blood	Pb	µg/dL	Kim et al. 2009
children 8-11	blood	Mn	µg/L	Kim et al. 2009
women 18-45	blood	Pb	µg/dL	Kim et al. 2013
women 18-45	blood	Cd	µg/L	Kim et al. 2013
women 18-45	cord blood	Pb	µg/L	Lin et al. 2013
women 18-45	cord blood	Mn	µg/L	Lin et al. 2013
children 11-14	blood	Pb	µg/dL	Lucchini et al. 2012
children 6-10	blood from hair	Pb	µg/dL	Marlowe/Moon et al.
children 6-10	hair	As	mg/kg	Marlowe/Moon et al.
children 6-10	hair	Cd	mg/kg	Marlowe/Moon et al.
children 6-8	urine	As	µg/L	Rosado et al. 2007
children 6-8	blood	Pb	µg/dL	Rosado et al. 2007
children 8-11	creat-adj urine	As	µg/g creat	Wasserman et al. 2011
children 8-11	blood	Pb	µg/dL	Wasserman et al. 2011
children 11-14	hair	As	mg/kg	Wright et al. 2006

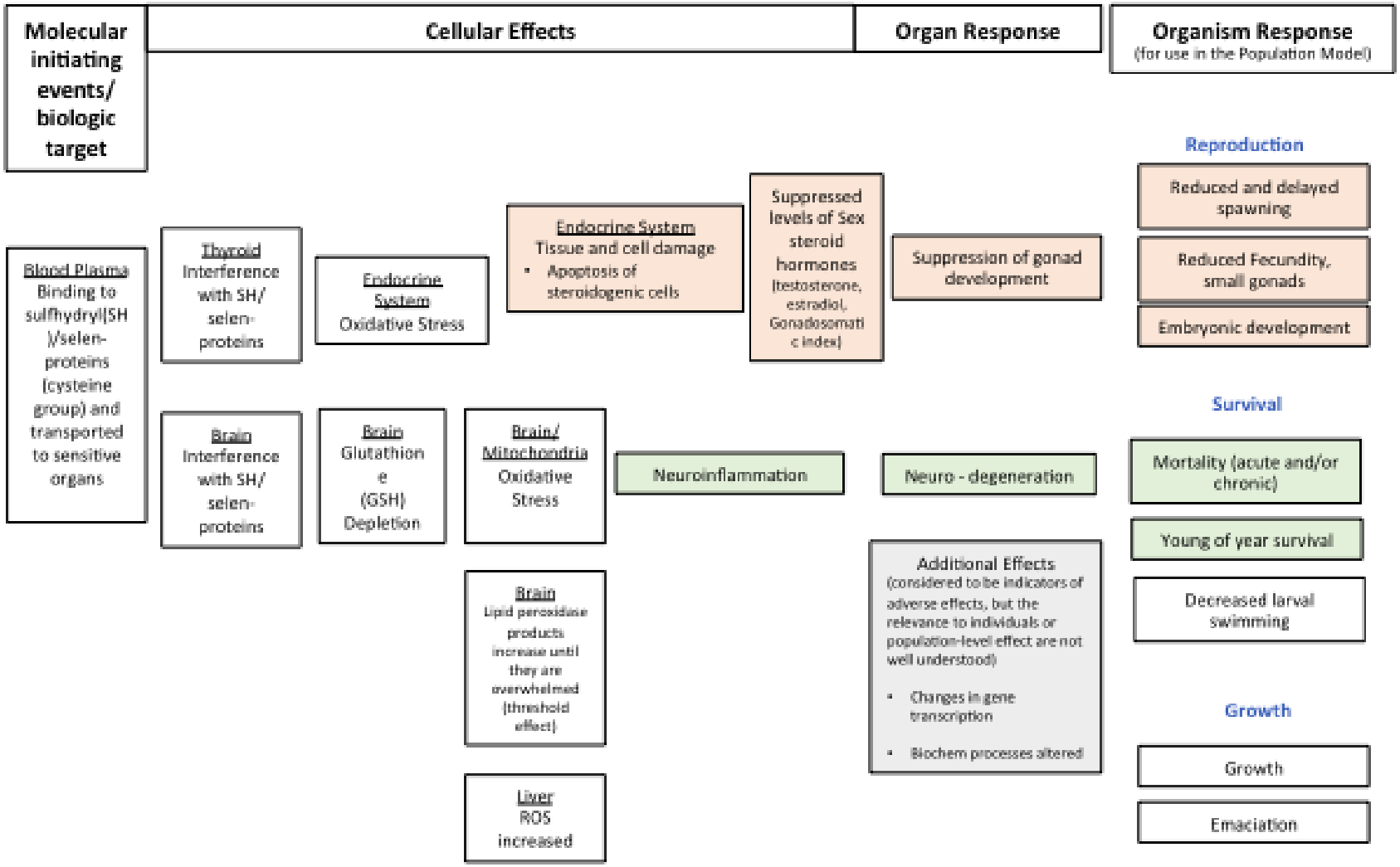


Moving Towards Quantitative Modeling

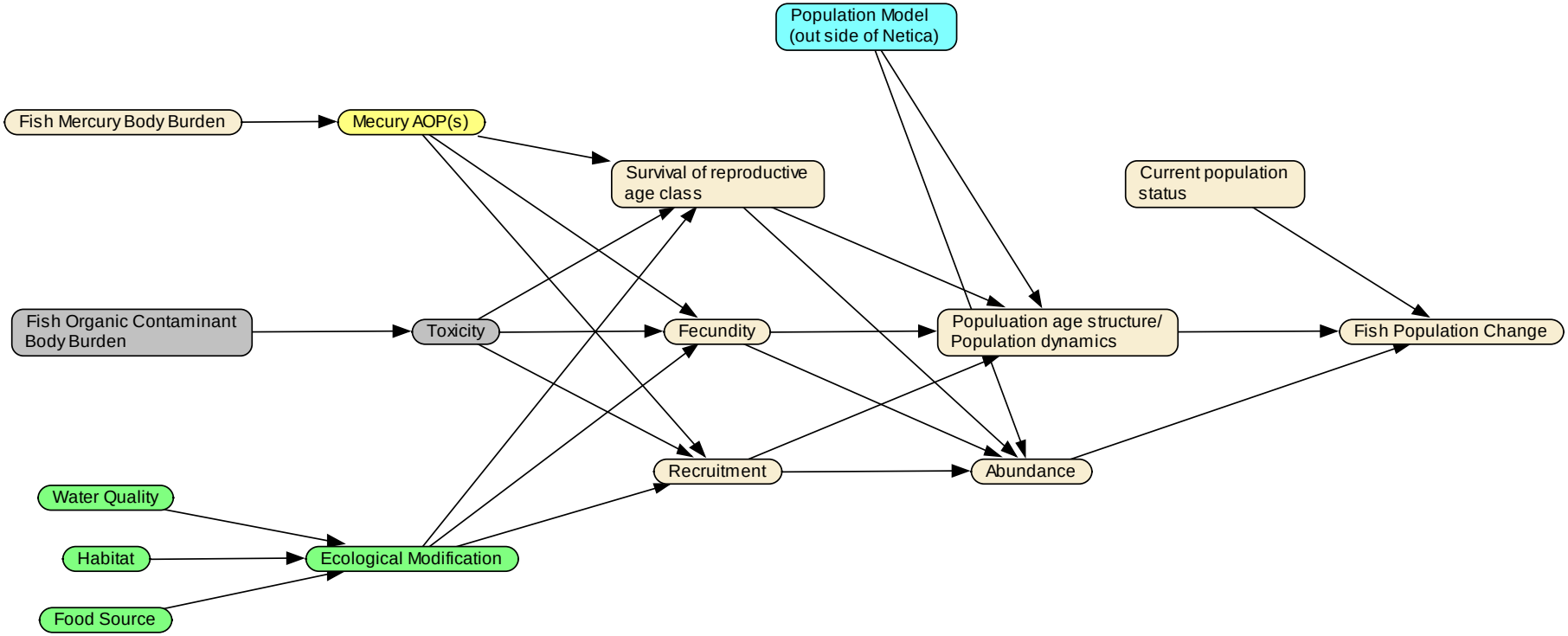


Process and mechanistic models – at relevant spatial and temporal scales – can be incorporated at any stage

Putative AOP for Mercury Based on Human Health



Initial BayesNet – Relative Risk Model



Conclusions

- AOPs provide a context for data from systematic reviews
- Important that the initial literature review-data extraction follows a structured, transparent approach
  - Toxicological
  - Epidemiologic
  - Exposure and biomonitoring
  - Health outcome etiology
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