Mode of Action (MOA) of Urinary Bladder Toxicity, and a “Tiered Surrogate Approach” in Risk Assessment of Data-Poor Chemicals

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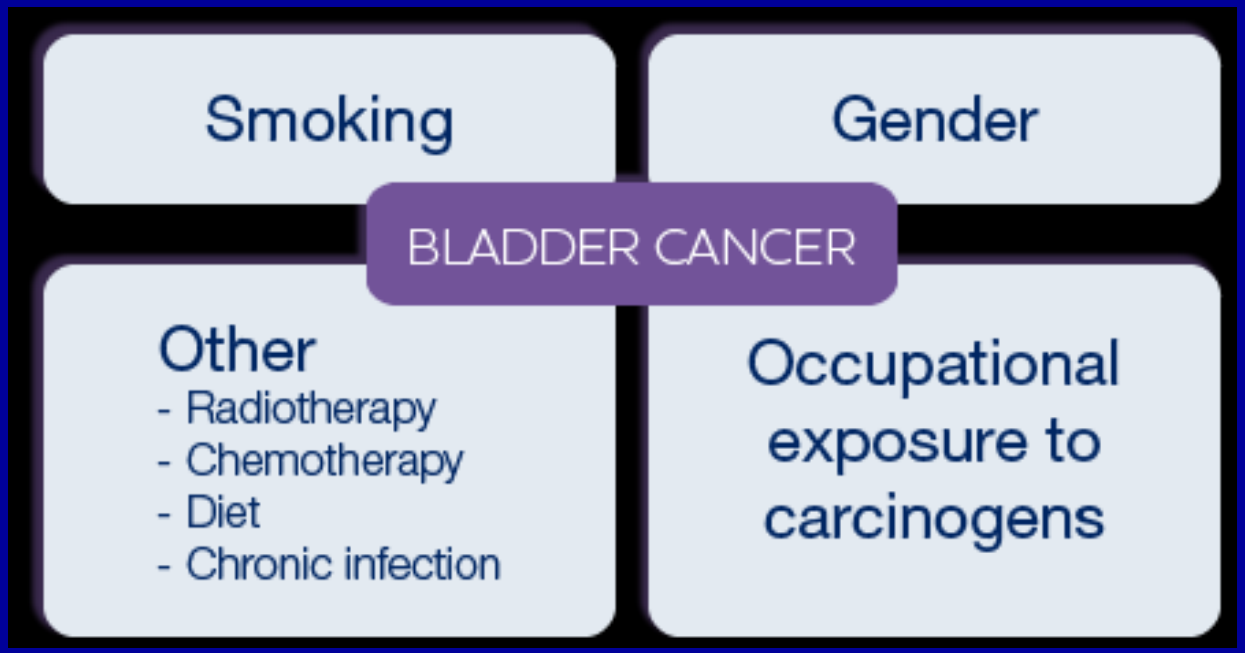
Presentation to HHAB, DPR
Date: 08/08/2016

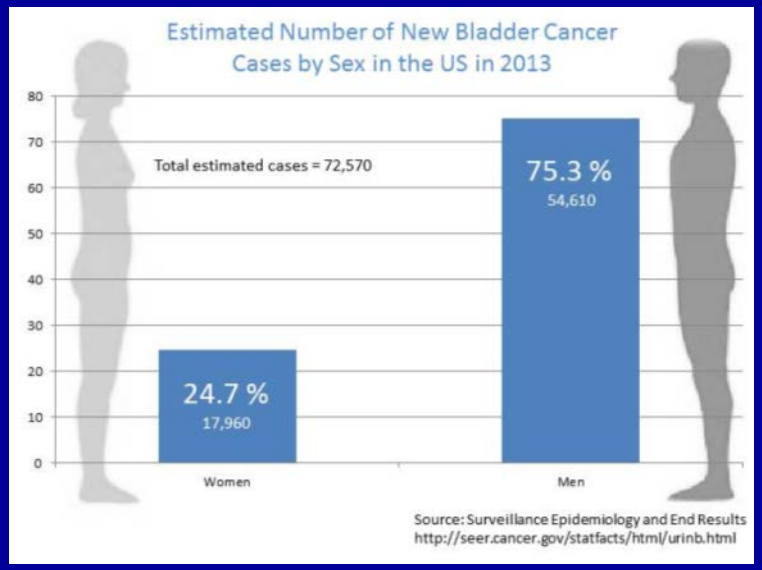
Outline

- Part 1 – MOA of urinary bladder toxicity
  - » Arsenic, a natural carcinogen
  - » Diuron, a herbicide
- Part 2 –Surrogate approach: A case study
- Part 3 – Toxicogenomics in RA

Risk factors

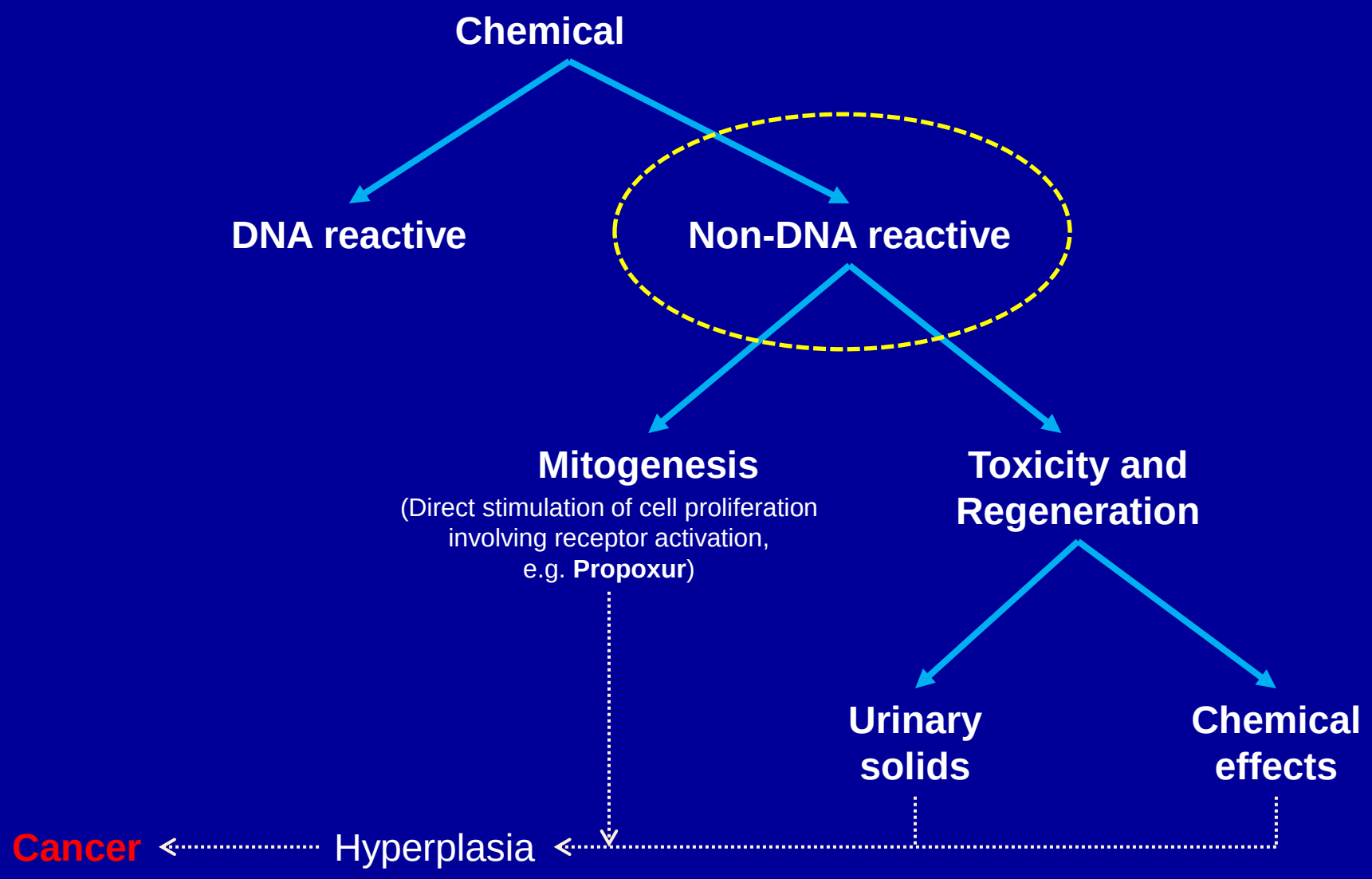


- Inorganic arsenic
- Diuron
- Pulegone (mint)
- Nicotine



Gender differences

Overview of Bladder Carcinogenesis



Definition of MOA

The term "mode of action" is defined as a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in toxicity/cancer.

- A "key event" is an empirically observable precursor step that is a necessary element of the mode of action.
  - e.g. Hyperplasia

Inorganic Arsenic

Natural environmental toxicant & a known carcinogen

Table 2. The ATSDR 2011 substance priority list.

Rank	Substance name	Points	CAS number
1	Arsenic	1665.5	007440-38-2
2	Lead	1529.1	007439-92-1
3	Mercury	1460.9	007439-97-6
4	Vinyl chloride	1361.1	000075-01-4
5	Polychlorinated biphenyls (PCBs)	1344.1	001336-36-3
6	Benzene	1332.0	000071-43-2
7	Cadmium	1318.7	007440-43-9
8	Polycyclic aromatic hydrocarbons	1282.3	130498-29-2
9	Benzo[*a*]pyrene	1305.7	000050-32-8
10	Benzo[*b*]fluoranthene	1252.4	000205-99-2

This list was generated by the ATSDR (2011) using an algorithm that translates potential public health hazards into a points-scaled system based on the frequency of occurrence at NPL Superfund sites and on toxicity and potential for human exposure.

Agency for Toxic Substances and Disease Registry (ATSDR), Atlanta, GA

Chronic Exposure

Cancer

- Lung
- U Bladder
- Skin

Non-cancerous

- Arsenicosis
- Bronchiectasis
- CVD
- Developmental

Mortality due to chronic arsenic exposure

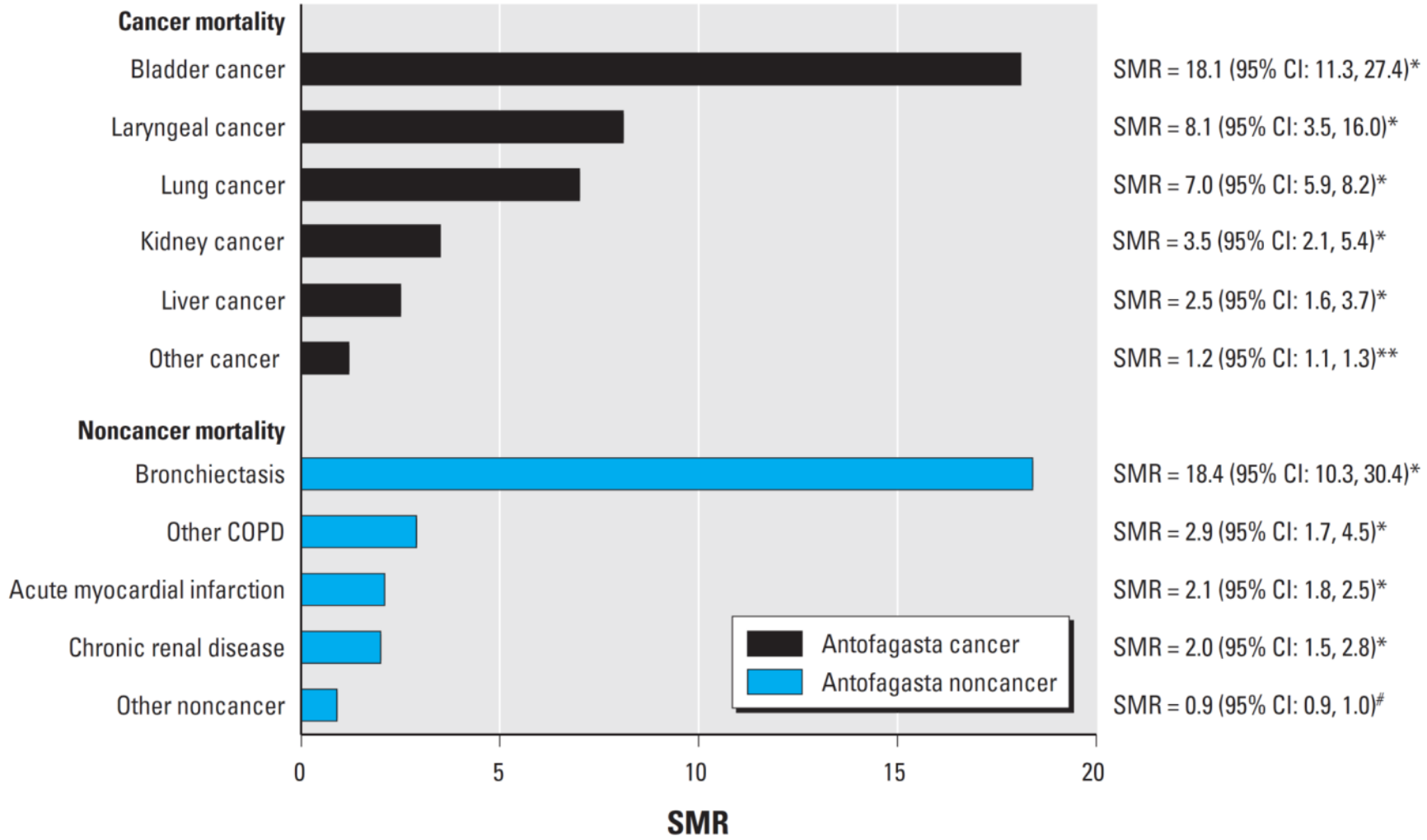
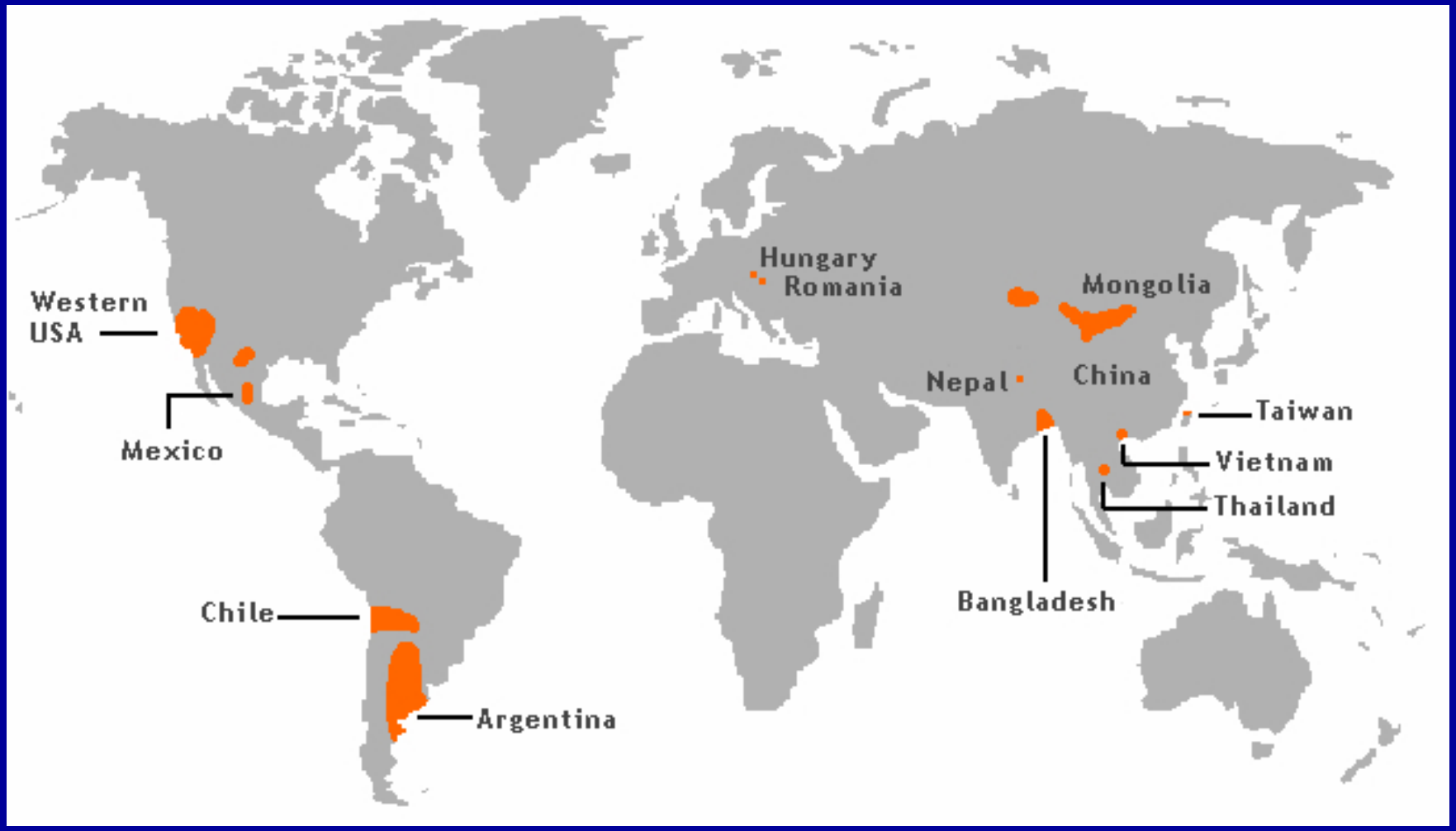
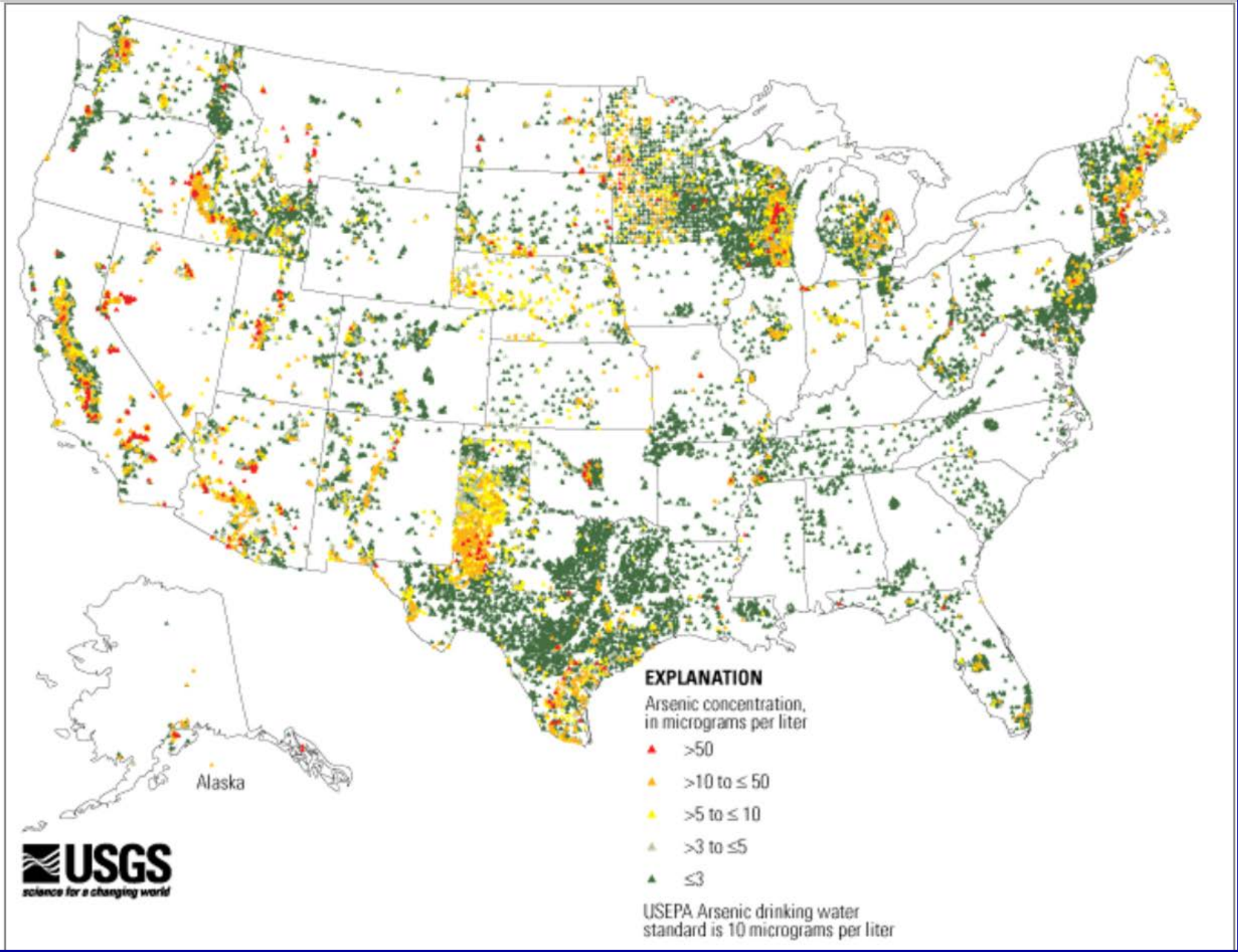


Figure 1. Summary of SMRs for 30–49-year-old males and females (pooled) who were born in Antofagasta, Chile, combining those born before and during the high-exposure period (Smith et al. 2006; Yuan et al. 2007, 2010).

Arsenic problem is global



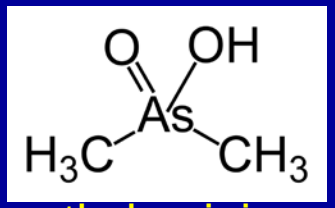
Arsenic in ground water



(Working hypothesis for inorganic arsenic)

Mode of action of DMA^V

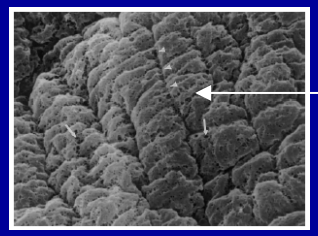
(Conclusions from – Office of Pesticide Programs)



(Dimethylarsinic acid)

DMA^{III} Metabolite

Urothelial Toxicity



Urinary bladder from a female F344 rat treated with 100 ppm DMA^V

Sustained

Regenerative Proliferation

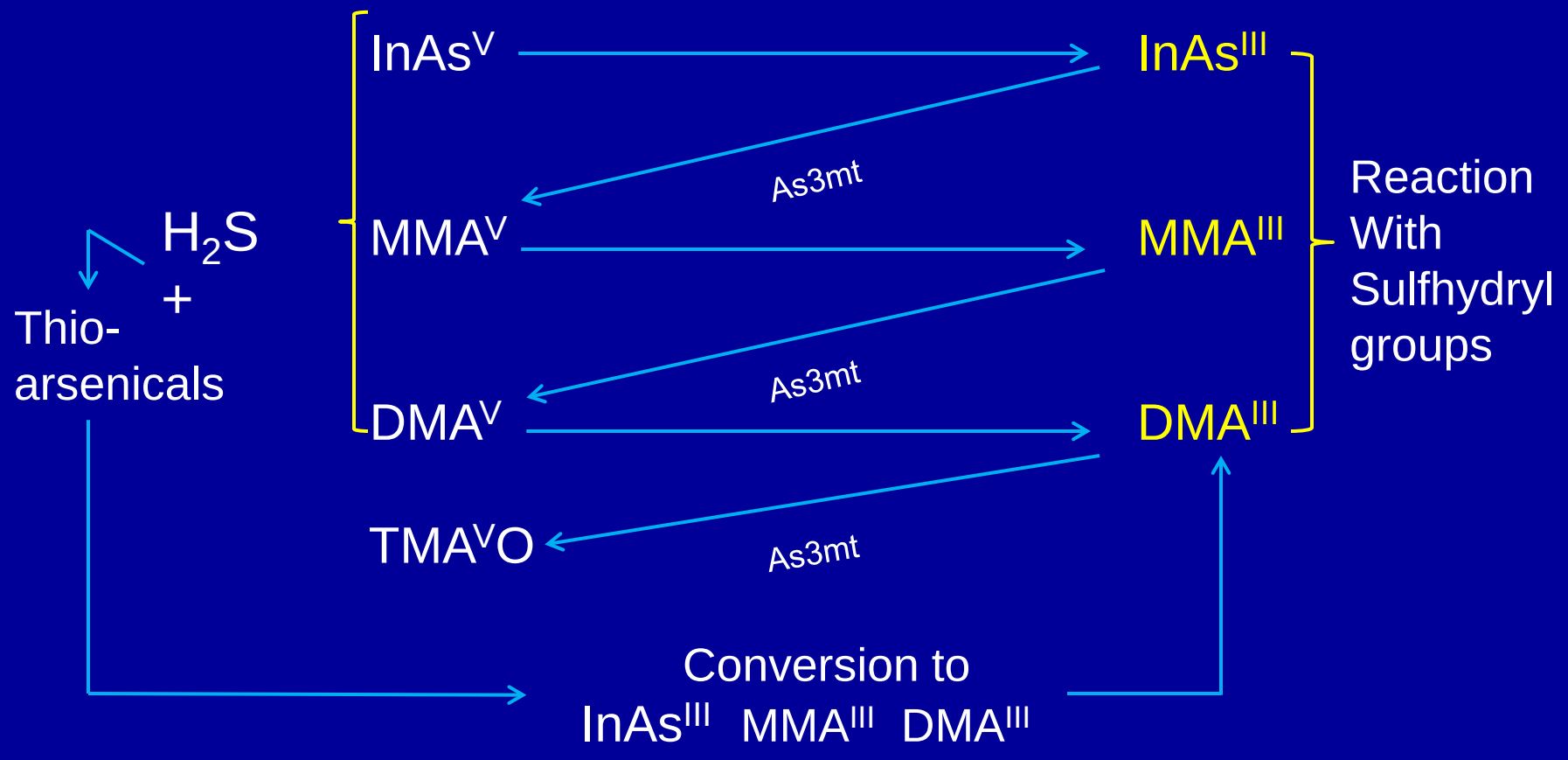
Hyperplasia

Urinary Bladder Tumors

EPA (Dellarco)

Arnold et al, 2006

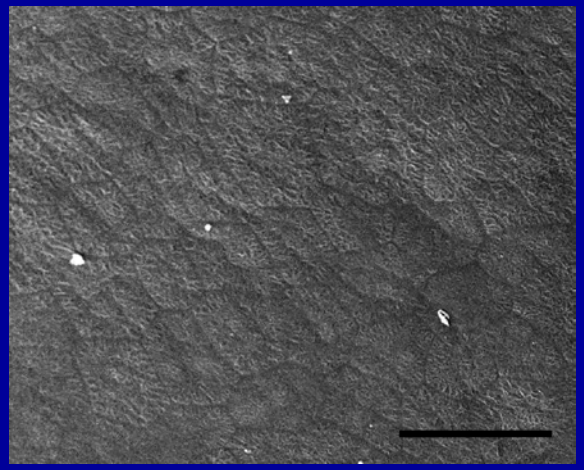
Generation of toxic trivalent arsenicals



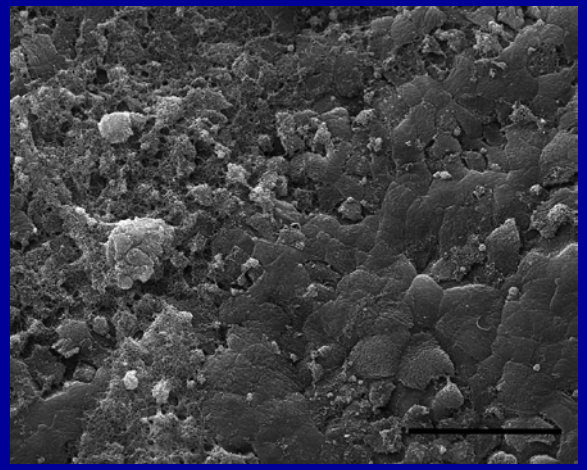
Major species in urine in mammals – DMA (DMA^{III} + DMA^{V})

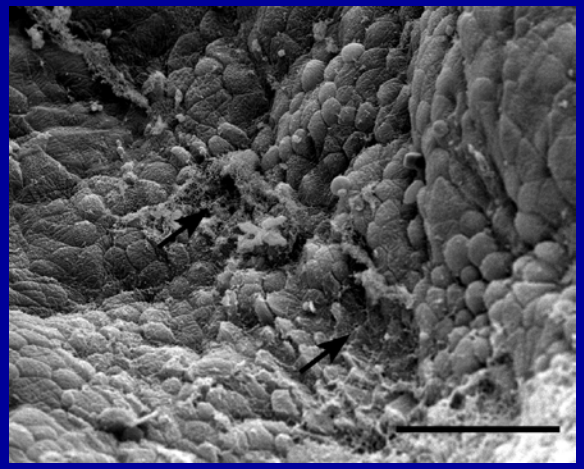
Urothelial cytotoxicity and proliferation induced by inorganic arsenic

Control

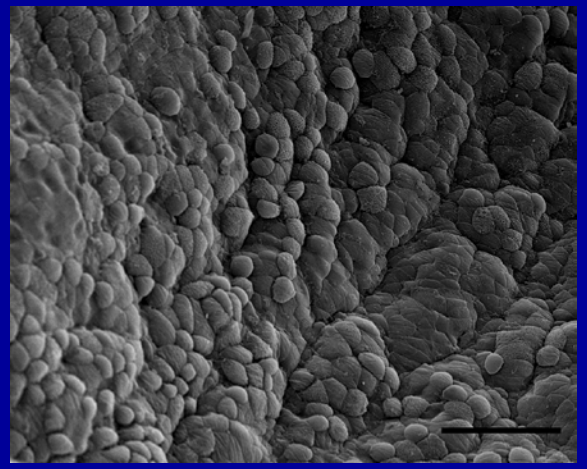


Necrosis



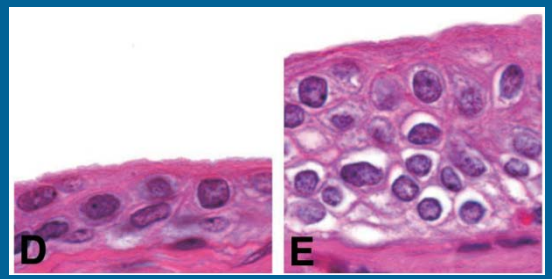


Sustained effect
(Necrosis + Regeneration)



Hyperplasia

Control Treated



Hyperplasia
(H&E staining)

Mode of Action of Inorganic Arsenic

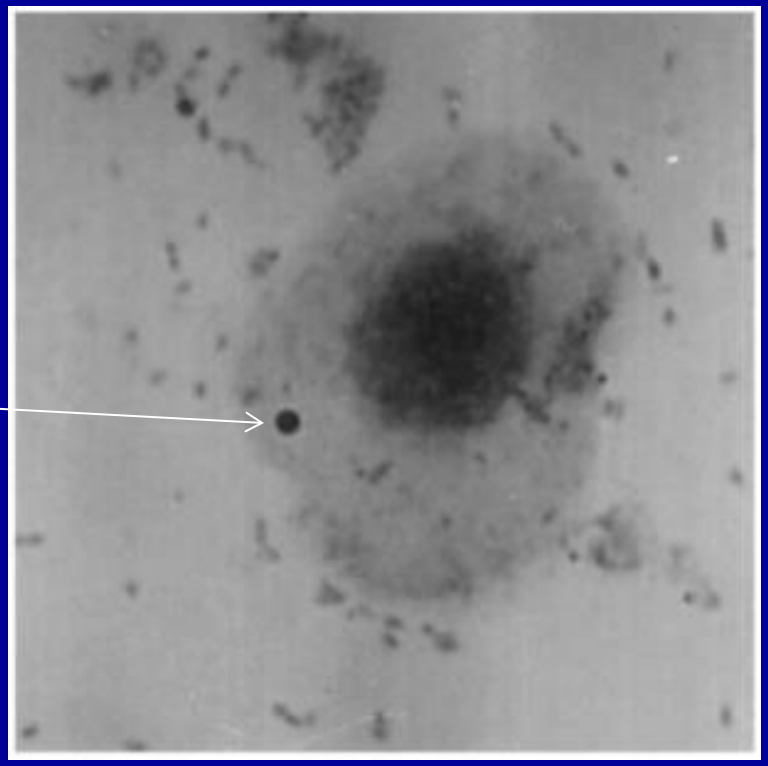
Key Event	Rat / Mice	Humans
Exposure to high dose	+	+
Generation of trivalent arsenicals	+	+
Concentration and excretion in urine/target tissue	+	+
Reacts with critical thiol-containing proteins	+	+
Cytotoxicity & cell death	+	+
Regenerative proliferation	+	+
Tumors	+	+

NOAEL 1 ppm

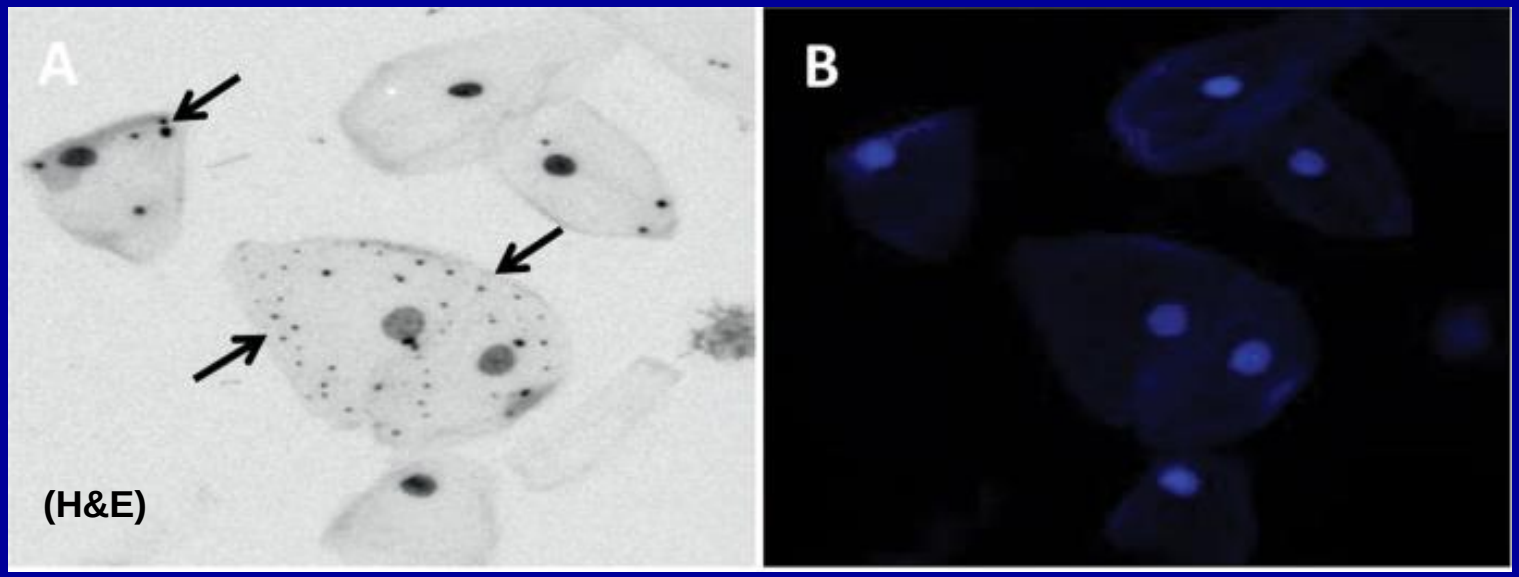
Genotoxicity Issue of Inorganic Arsenic

- No direct damage to DNA
- Inhibition of DNA repair
  - At high concentrations
  - Could be due to trivalents binding to proteins
- Indirect damage to DNA
  - *In vitro* at higher concentration that are cytotoxic and not attainable in humans
  - Suggestion of increased micronuclei formation
    - Are these Arsenic-rich inclusions or micronuclei?

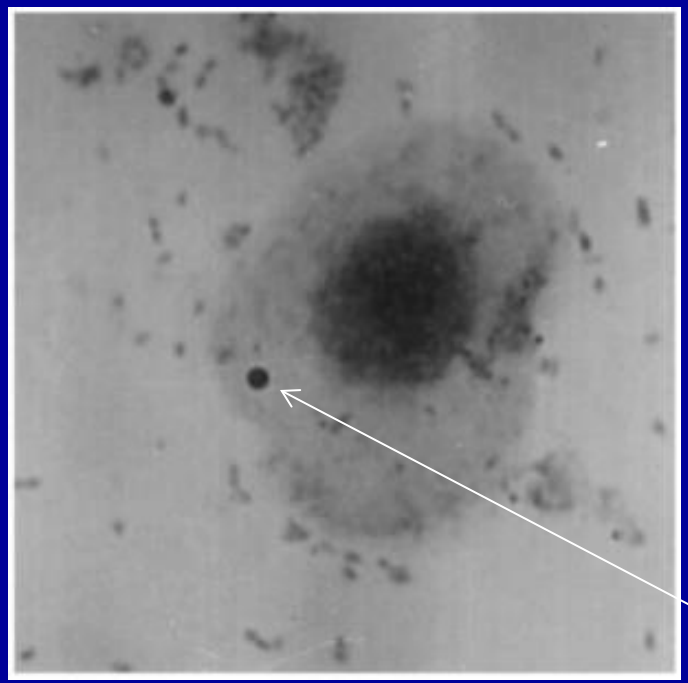
Basu et al, 2002

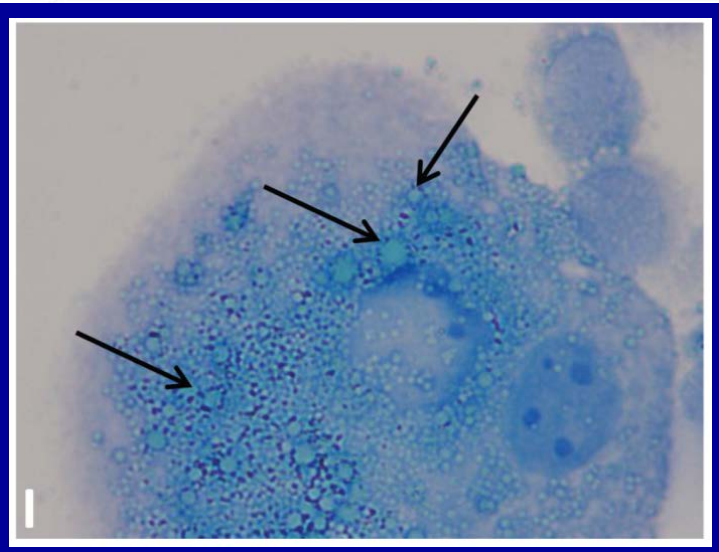


Inclusions in **human** PML patients treated with ATO (*Wedel et al, 2013).* Similar findings in mice *(Dodmane et al, 2014)*



DAPI Staining



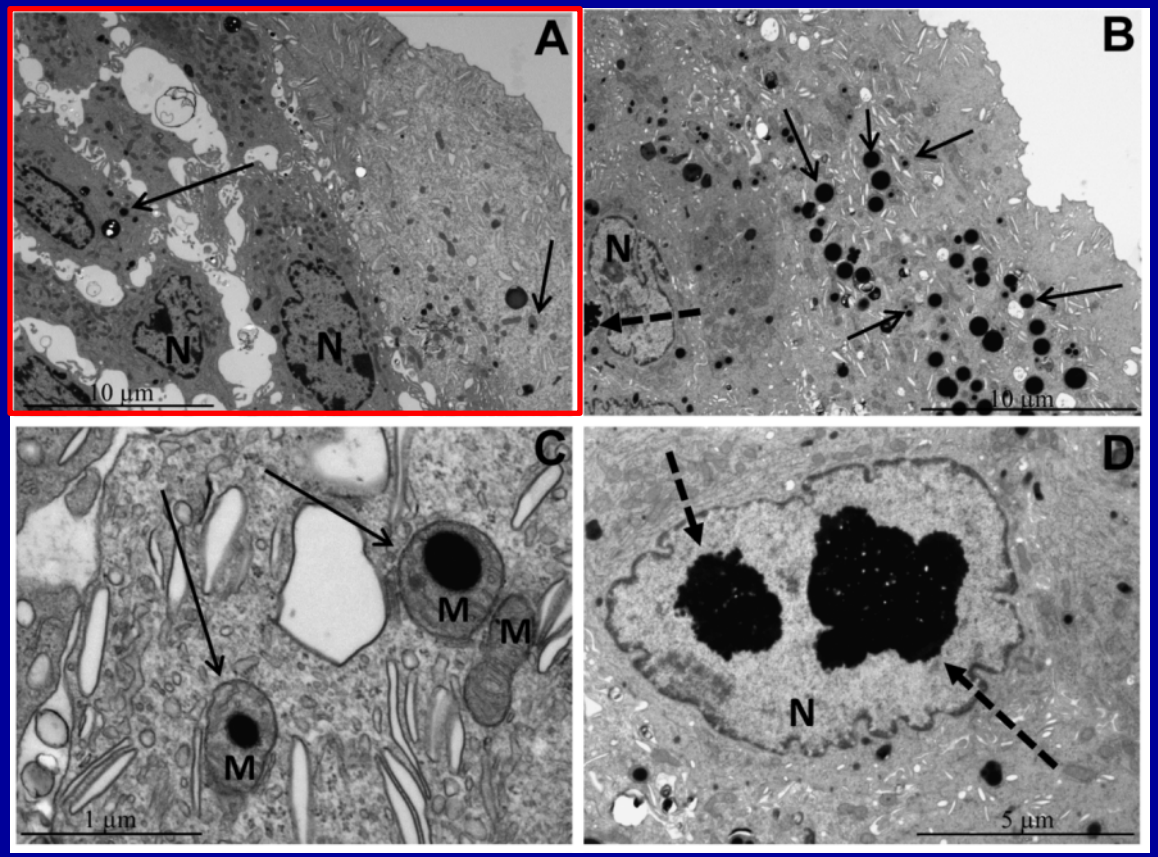


Light microscopy: Giemsa staining of scraped urothelial cells from treated mice

"Micronuclei" *Basu et al, 2002*

As3mt KO Mice

Control



25 ppm As^{III}

Summary on arsenic

- Inorganic arsenic (iAs) induces urothelial cytotoxicity, necrosis and consequent regeneration leading to hyperplasia, ultimately tumors
- There is concordance of multiple key events from rodents to humans
- iAs is sequestered as inclusions in urothelial cells, which appear similar to “micronuclei”

Work on Diuron
(Herbicide)

Mode of action of diuron-induced urinary bladder toxicity and cancer

Diuron is closely related
- Linuron
- Propanil

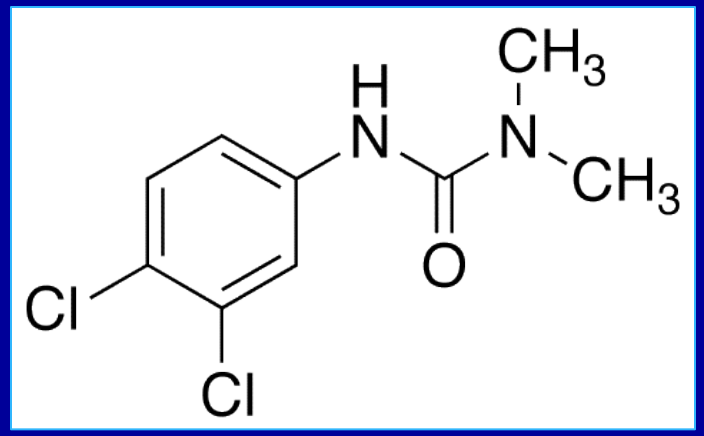


Table 14: Toxicity Endpoints for Diuron Risk Assessment

Route / Duration	NOAEL (mg/ kg/day)	Effect	Study	Uncertainty Factors and Safety Factors
Short- and intermediate-term Dermal	No systemic toxicity following repeated dermal dosing at 1200 mg/kg/day was seen in the dermal toxicity study. Also, there is no developmental concern. No hazard was identified and no quantitative assessment is required.			
Long-term Dermal[a] (greater than six months)	1.0 (LOAEL)	Evidence of hemolytic anemia and compensatory hematopoiesis.	Chronic toxicity/carcinogenicity study in rats	Interspecies: 10x Intraspecies: 10x FQPA: 1x Use of LOAEL instead of a NOAEL: 3x
Short-term Inhalation[b]	10	Decreased body weight and food consumption	Developmental toxicity study in rabbits	Interspecies: 10x Intraspecies: 10x FQPA: 1x
Intermediate-term Inhalation[b]	1.0	Altered hematological parameters observed at six months	Chronic toxicity/carcinogenicity study in rats	Interspecies: 10x Intraspecies: 10x FQPA: 1x
Long-term Inhalation[b]	1.0 (LOAEL)	Evidence of hemolytic anemia and compensatory hematopoiesis	Chronic toxicity/carcinogenicity study in rats	Interspecies: 10x Intraspecies: 10x FQPA: 1x Use of a LOAEL instead of a NOAEL: 3x
Cancer	Known/likely human carcinogen $Q_1^* = 1.91 \times 10^{-2}$	Urinary bladder carcinoma in both sexes of the Wistar rat, kidney carcinomas in the male rat (a rare tumor), and mammary gland carcinomas in the female NMRI mouse	Carcinogenicity study in rats and mice	

a An oral endpoint was used for dermal exposure: dermal absorption factor of 4% of oral exposure shall be used.
b An oral endpoint was used for inhalation exposure: inhalation exposure assumed equivalent to oral exposure.

Key events of toxicity by Diuron

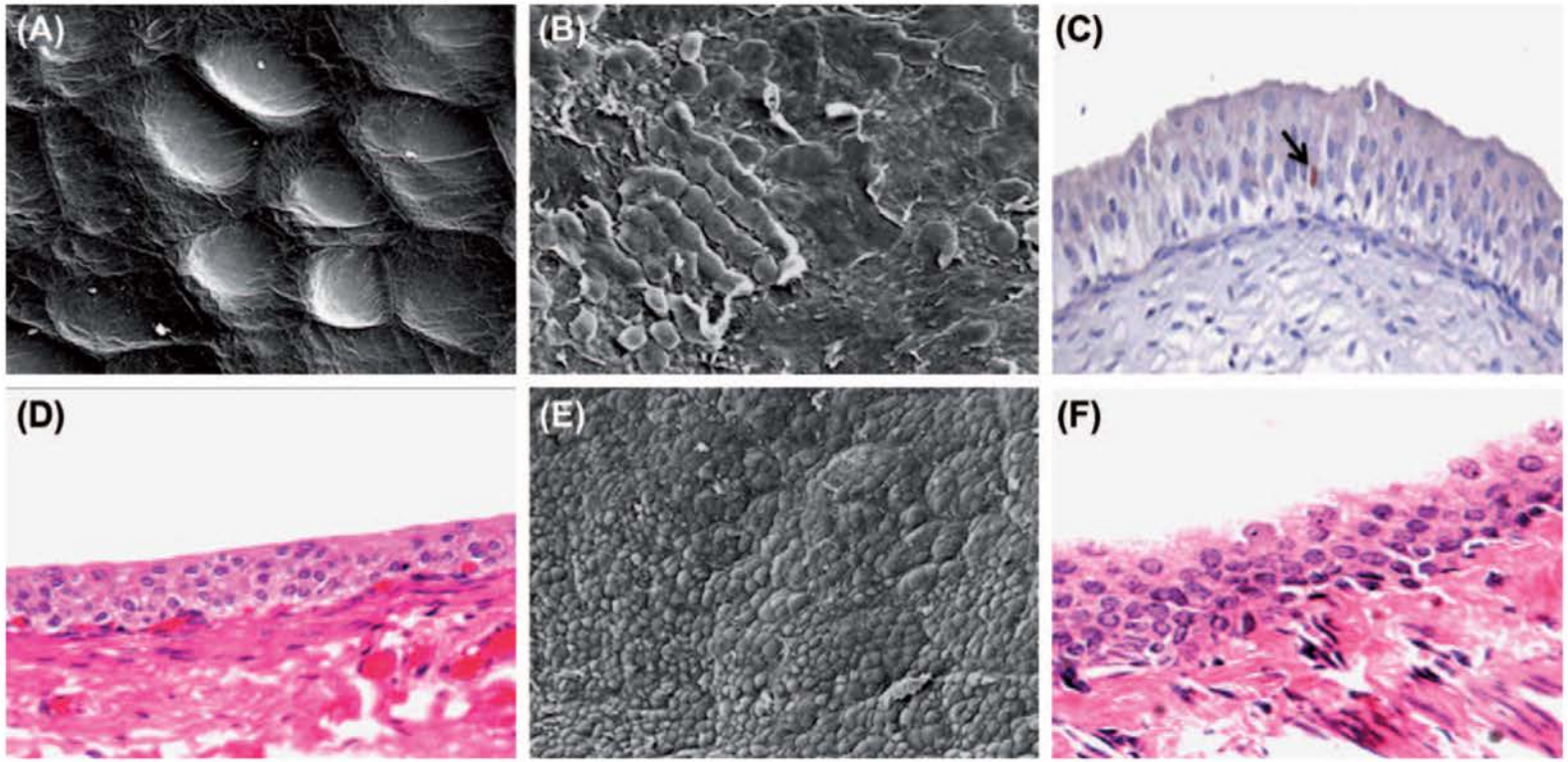
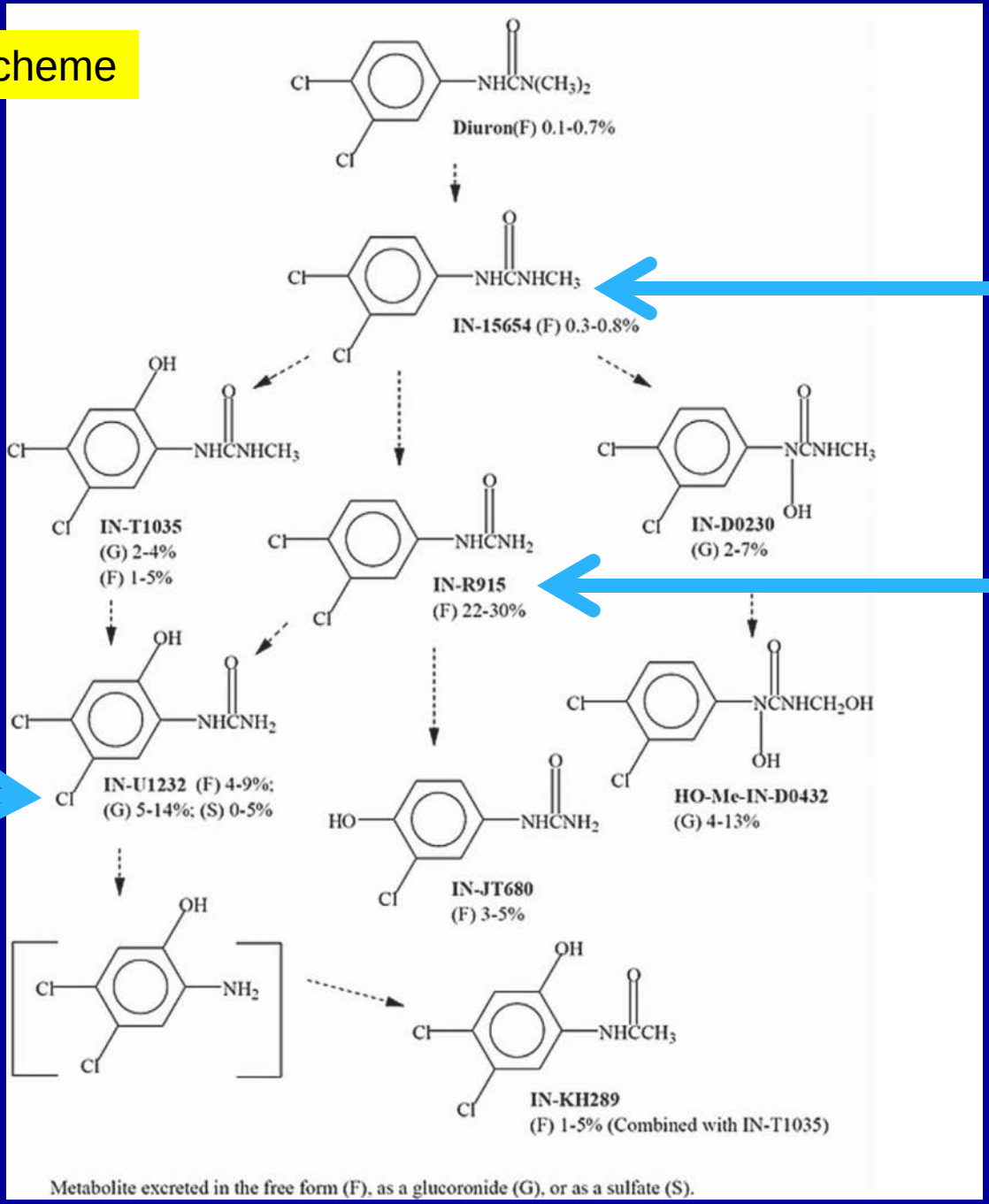


Figure 2. Key events of carcinogenic MOA for diuron: (A) swollen cells observed using SEM, ×200; (B) necrosis and exfoliation foci observed using SEM, ×600; (C) cell proliferation, labeled with BrdU (*arrow*), ×40; (D) regenerative hyperplasia observed using light microscopy, H&E×40; (E) regenerative hyperplasia observed using SEM; piling up of rounded cells, ×400; (F) simple hyperplasia in kidney pelvis, observed using light microscopy, H&E×40.

Metabolism Scheme



DCPMU

DCPU

2-OH-DCPU

DCA

Relative cytotoxicity of metabolites and their concentration in urine

(Units in µM)	Diuron	DCPU	2-OH-DCPU	DCPMU	DCA
Conc. in rat urine	54 ± 35	>488	125 ± 60	13 ± 5	35 ± 16
Cytotoxicity in -					
Rat - MYP3 cells IC_{50}	-	185	230	104	213
Human - 1T1 Cells IC_{50}	-	157	329	224	72

- DCPU - the major metabolite in rat urine
- DCPMU - most potent in rats
- DCA - most potent in humans

Concordance Analysis

Table 3. Concordance analysis of diuron mode of action key events between rats and humans.

Key event	Rat	Human
Exposure levels	High and continuous levels	Low incidental levels
Metabolites generation	Primarily DCPU and 2-OH-DCPU, less DCPMU, very low levels of DCA	Primarily DCPU, very low levels of DCA
Metabolites excretion and concentration in urine	Primarily DCPU and 2-OH-DCPU	Primarily DCPMU, less DCPU
Cytotoxicity	Primarily DCPU and DCPMU, others considerably less based on IC_{50}	Exposures not capable of generating sustained concentrations of cytotoxic metabolites in the urine
Regenerative proliferation and hyperplasia	Present by 7 days after 2500 ppm diuron exposure by diet	No (prediction based on absence of sustained urothelial cytotoxicity)
Tumors	2 years, 2500 ppm diuron exposure by diet	No (prediction based on absence of sustained urothelial cytotoxicity and regenerative hyperplasia)

Conclusions on MOA

- Chemical agent-induced urothelial cytotoxicity, resulting in necrosis, regenerative proliferation and hyperplasia, ultimately leading to bladder tumors is a common MOA for arsenic and Diuron and many other chemicals

PART -2

My work at -
National Center for Environmental Assessment
(U.S. EPA, NCEA, Cincinnati, OH)

Human Health Risk Assessment of Data-Poor Chemicals by a Tiered Surrogate Approach: A Case Study

Disclaimer: The views expressed in this presentation are those of the presenter and do not necessarily reflect the views and policies of the U.S. EPA.

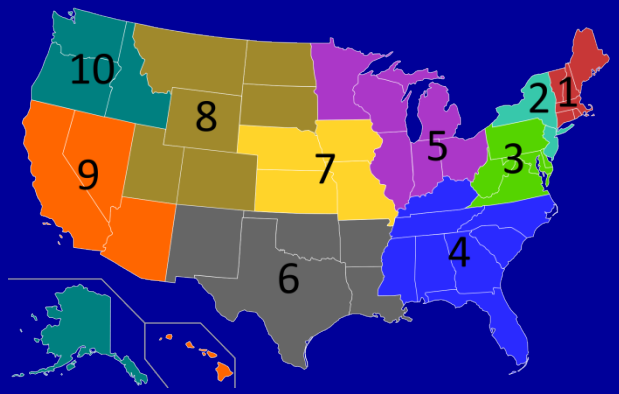
Superfund Health Risk Technical Support Center (STSC)

- Established in 1987 by three offices of EPA
  - Office of Solid Waste and Emergency Management (OSWER – renamed as Office of Land and Emergency Management [OLEM]),
  - Office of Research and Development ORD, and
  - Regional Superfund Office
- Managed by NCEA at Cincinnati, Ohio

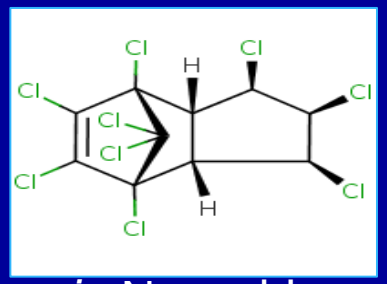
<u>Key lines of Support:</u>

- Preparation of Provisional Peer-Reviewed Toxicity Value (PPRTV) assessments
- Technical support to EPA regional scientists – *This case study is an example*
- Support for interpretation of EPA publications

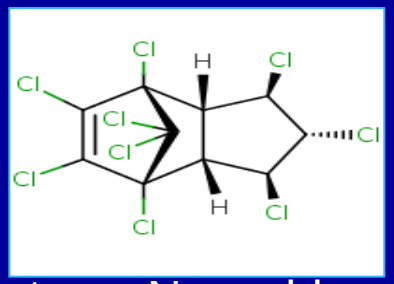
Case Study: A Request from Region 2



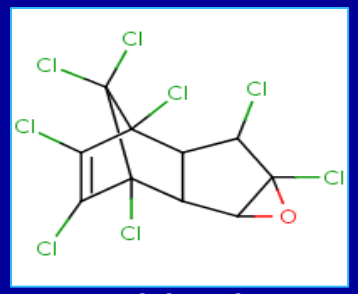
- The problem –
  - <u>*cis*-nonachlor, *trans*-nonachlor and oxychlordane</u> were detected at the Lower Passaic Superfund Site, which have limited toxicity data to derive human health risk reference values.



cis-Nonachlor



trans-Nonachlor

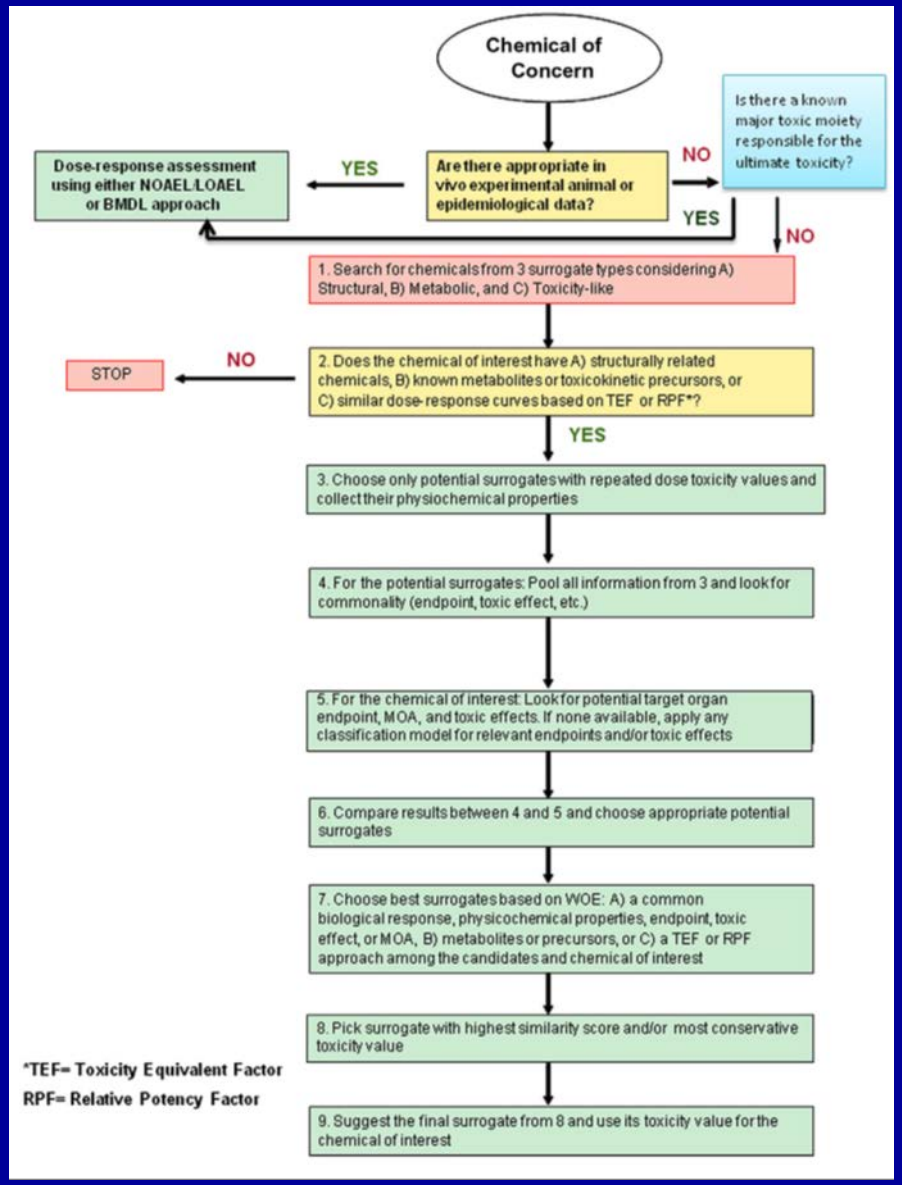


Oxychlordane

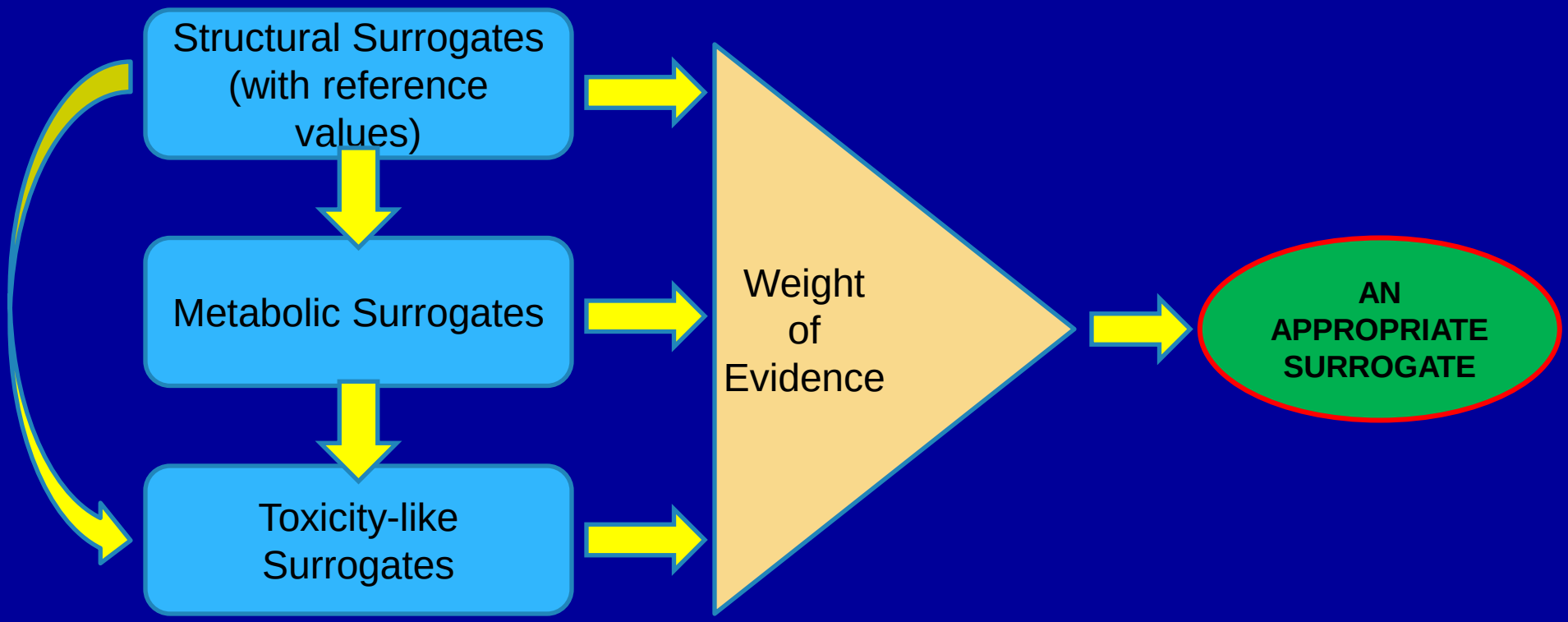
- Goal of the analysis -
  - Identify <u>an appropriate surrogate</u> for noncancer and/or cancer effects.

Tiered Surrogate Approach

Wang et al., 2012. Application of computational toxicological approaches in human health risk assessment. I. A tiered surrogate approach. *Regulatory Toxicology & Pharmacology. 63:10-19*



Three Tiers of Analysis



Nonachlor (*cis* and *trans*) and Candidate Analogs

	Test chemicals		Structural Analogs							
	cis-Nonachlor	*trans*-Nonachlor	Chlordane	#Technical Chlordane	Aldrin	Heptachlor	Dieldrin	Endrin	Heptachlor epoxide	Endosulfan
CASRN	5103-73-1	39765-80-5	57-74-9	12789-03-6	309-00-2	76-44-8	60-57-1	72-20-8	1024-57-3	115-29-7
DSSTox Similarity Score (%)	100	100	97	97	94.3	87.1	60.2	60.2	57.6	53.6
¥ChemID Plus Similarity Score (%)	100	100	98	ǂ	90.7	98	70.9	70.9	75.6	68.3
Oral chronic RfD available?	No	No	Yes		Yes	Yes	Yes	Yes	Yes	Yes
Reference	None	None	(U.S.EPA, 1997)		(U.S.EPA, 1991c)	(U.S.EPA, 1991e)	(U.S.EPA, 1991d)	(U.S.EPA, 1989)	(U.S.EPA, 1991f)	(U.S.EPA, 1994)

¥ Sourced from ChemIDPlus

ǂ Not available

#Technical Chlordane: 12 major compounds make up 67% of the composition. Remaining 135 compounds make up 33%.

Oxychlordane and Candidate Analogs

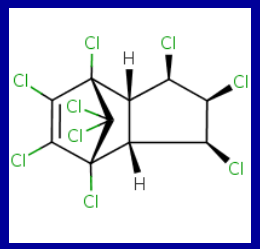
	Test chemical	Structural Analogs					
	Oxychlordane	Chlordane	Technical Chlordane	Heptachlor epoxide	Dieldrin	Endrin	Aldrin
CASRN	27304-13-8	57-74-9	12789-03-6	1024-57-3	60-57-1	72-20-8	309-00-2
DSSTox Similarity Score (%)	100	49.5	49.5	86.5	69.6	69.6	49.5
¥ChemID Plus Similarity Score (%)	100	69.8	ǂ	93.8	86.4	86.4	75
Oral chronic RfD available?	No	Yes		Yes	Yes	Yes	Yes
Reference	Not available	(U.S.EPA, 1997)		(U.S.EPA, 1991f)	(U.S.EPA, 1991d)	(U.S.EPA, 1989)	(U.S.EPA, 1991c)

¥Sourced from ChemIDPlus database

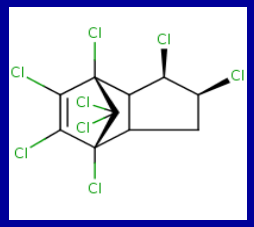
ǂ Not available

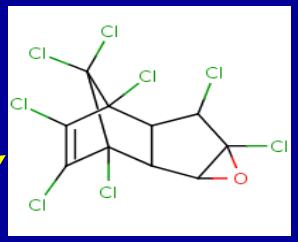
Metabolism of Nonachlor (*cis* & *trans*) to Oxychlordane

cis-nonachlor

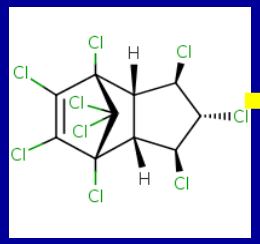


***cis*-chlordane**

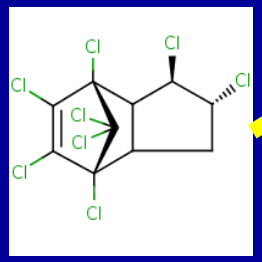




Oxychlordane



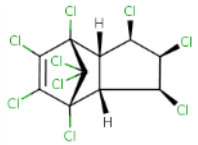
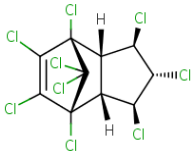
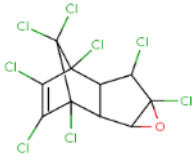
trans-nonachlor



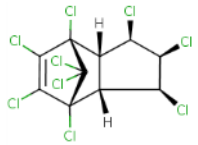
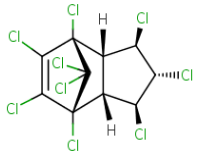
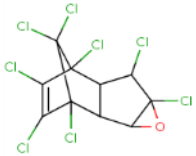
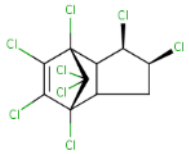
***trans*-chlordane**

[Modified from: ATSDR, 1994; Kania-Korwel and Lehmler, 2013; Tashiro and Matsumura, 1977, 1978]

Available Repeated Dose Toxicity Data

Chemical	Test Chemicals		
	***cis*-Nonachlor**	***trans*-Nonachlor**	**Oxychlordane**
Structure			
Major toxic residues/ metabolites in tissues	*cis*-nonachlor, **oxychlordane**	*trans*-nonachlor, **oxychlordane**	**Oxychlordane**
Toxic Effects	Based on 28-day study in rats: **hepatocellular hypertrophy**, increased liver-to-body weight (BW) ratio, increased kidney-to-BW ratio in males	Based on 28-day and 90-day studies in rats: **hepatocellular hypertrophy**, Kidney and thyroid changes–epithelia changes	Based on 28-day study in rats: **hepatocellular hypertrophy**, thymus-necrosis, epithelial changes
Reference	Bondy et al., 2000	Bondy et al., 2004; Bondy et al., 2000	Bondy et al., 2003

Available Repeated Dose Toxicity Data

Chemical	Test Chemicals			(Potential)Appropriate Surrogate	
	***cis*-Nonachlor**	***trans*-Nonachlor**	**Oxychlordane**	**Technical Chlordane**	**Chlordane**
Structure					
Major toxic residues/ metabolites in tissues	*cis*-nonachlor, **oxychlordane**	*trans*-nonachlor, **oxychlordane**	**Oxychlordane**	**Oxychlordane** (major), heptachlor, *trans*-nonachlor, *cis*-nonachlor	Chlordane, **Oxychlordane**
Toxic Effects	Based on 28-day study in rats: **hepatocellular hypertrophy**, increased liver-to-body weight (BW) ratio, increased kidney-to-BW ratio in males	Based on 28-day and 90-day studies in rats: **hepatocellular hypertrophy**, Kidney and thyroid changes–epithelia changes	Based on 28-day study in rats: **hepatocellular hypertrophy**, thymus-necrosis, epithelial changes	**Critical Effects:** Hepatic necrosis, which included fatty degeneration and **hypertrophy**. (104-week mouse oral study) Other effects: Microsomal enzyme induction	
Reference	Bondy et al., 2000	Bondy et al., 2004; Bondy et al., 2000	Bondy et al., 2003	U.S.EPA, 1997; Bondy et al., 2005 (rats)	

Weight of Evidence:

NONCANCER effects

Chlordane is an appropriate surrogate for *cis*-nonachlor, *trans*-nonachlor and oxychlordane

- ✓ High structural similarity
- ✓ Common metabolic pathway
- ✓ Similar toxicity

Weight of Evidence:

CANCER effects

Chlordane is an appropriate surrogate for *cis*-nonachlor, *trans*-nonachlor and oxychlordane

- ✓ Chlordane is considered a “probable” carcinogen (U.S. EPA, 1997)
- ✓ Chlordane-induced liver toxic effects are suggested to be preneoplastic effects (U.S. EPA, 1997)
- ✓ Oxychlordane is the major metabolite of chlordane, and the test chemicals *cis*- and *trans*-nonachlor (U.S. EPA, 1997)
- ✓ It is imperative to suggest that the oxychlordane-generating compounds *cis*- and *trans*-nonachlor might induce liver toxicity and possibly tumors similar to chlordane (U.S. EPA, 1997)

Issue of Potency

Incidence of Hepatocyte Hypertrophy in Rats Gavaged for up to 28 Days					
Chemical	Dose (mg/kg-day)				
	0	0.25	1.0	2.5	25
Female rats					
Technical Chlordane[a]	0/7	0/7	NT	**0/7**	7/7*
***cis*-Nonachlor[a]**	0/7	0/7	NT	0/7	6/6*
***trans*-Nonachlor[a]**	0/7	2/7	NT	7/7*	4/4*
Oxychlordane[b]	0/10	0/10	0/10	9/10*	NT
Male rats					
Technical Chlordane[a]	0/7	0/7	NT	**0/7**	7/7*
***cis*-Nonachlor[a]**	0/7	1/7	NT	3/7	7/7*
***trans*-Nonachlor[a]**	0/7	2/7	NT	4/7	7/7*

[a]Bondy et al. (2000); [b]Bondy et al. (2003); NT = not tested; *Significantly different from incidence in control group ($p \leq 0.05$) based on Fisher's Exact test

Accounting for potency differences by the Relative Potency Factor (RPF) method*

$$RPF = \frac{\text{EDx of The Index Chemical}}{\text{EDx of test chemical}}$$

Where:
EDx = Effective Dose (e.g. ED_{50}, BMD_{50})

(*As defined in - Supplementary Guidance for Conducting health Risk Assessment of Chemical Mixtures, U.S. EPA, 2000)

Derivation of Relative Potency Factors (RPFs)		
Chemical	Average BMD_{50} (mg/kg-day)	RPF = (Average BMD_{50} of Index Chemical[a]) / (Average BMD_{50} of data-poor chemical)
Female rats		
Technical Chlordane	12.22	1.0
***cis*-Nonachlor**	12.27	1.0
***trans*-Nonachlor**	0.38	32.2
Oxychlordane	2.19	5.6
Male rats		
Technical Chlordane	12.22	1.0
***cis*-Nonachlor**	2.52	4.8
***trans*-Nonachlor**	1.64	7.5
[a]Technical chlordane		

Relative potency:

trans-Nonachlor **>>** Oxychlordane **>** *cis*-Nonachlor **>** Technical Chlordane

Waste and Cleanup Risk Assessment

Provisional Peer Reviewed Toxicity Values for Superfund (PPRTV)

You will need Adobe Reader to view some of the files on this page. See EPA's PDF page to learn more.

Contents
- PPRTV Home
- Quickview
- PPRTV Compare
- User's Guide
- Definitions
- What's New
- PPRTV Derivation Support Documents

Derivation Support Documents

Chemical	CAS Number	Last Updated
Tungsten	7440-33-7	2015
Trimethylpentene, 2,4,4-	25167-70-8	2015
Trifluoroethane, 1,1,1-	420-46-2	2015
Sodium Tungstate Dihydrate	10213-10-2	2015
Sodium Tungstate	13472-45-2	2015
Picric Acid (2,4,6-Trinitrophenol)	88-89-1	2015
Lewisite	541-25-3	2015
Diundecyl Phthalate	3648-20-2	2015
Diethylene Glycol Monomethyl Ether	111-77-3	2015
Dichloropropene, 1,1-	563-58-6	2015
Chlorooctadecane, 1-	3386-33-2	2015
Chloronitrobenzene, p-	100-00-5	2015

Uncertainty factors

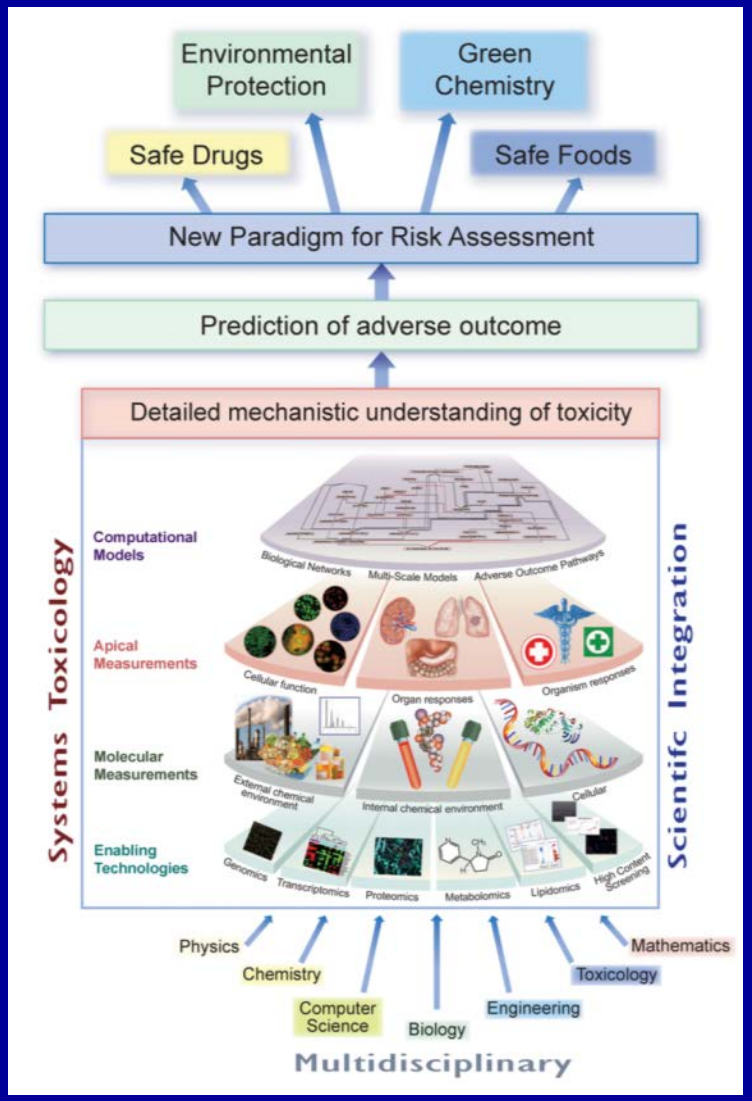
Table A-6. Comparison of Uncertainty Factors for Picric Acid and 1,3,5-Trinitrobenzene for the Chronic p-RfD			
	Picric Acid	**1,3,5-Trinitrobenzene**	**Comments**
UF_A	3	10	The UF_A for picric acid has been reduced from 10 to 3 based on the calculation of a HED through the application of a default DAF. The U.S. EPA (1997) assessment for 1,3,5-trinitrobenzene was performed prior to the EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b), therefore, a UF_A of 10 was applied to account for inter-species extrapolation.
UF_D	10	1	A UF_D of 10 for picric acid reflects unknown and unaccountable database deficiencies, including the lack of information on potential developmental and reproductive effects. The UF_D for 1,3,5-trinitrobenzene was reduced from 10 to 1 due to the available information from systemic, developmental and reproductive studies that support hematological toxicity as the most sensitive effect.
UF_H	10	10	NA
UF_L	1	1	NA
UF_S	1	1	NA
UF_C	300	100	NA

Applications of surrogate approach

- Inform regulatory decisions for data-poor chemicals
  - Derive a reasonable screening reference value for data-poor chemicals, which otherwise would not have any
  - Predict potential toxic effects of data-poor chemicals

- To reduce resources needed to conduct toxicity studies and generate data to derive risk values
  - Time, cost, and number of animals

Part 3
Use of toxicogenomics information in RA

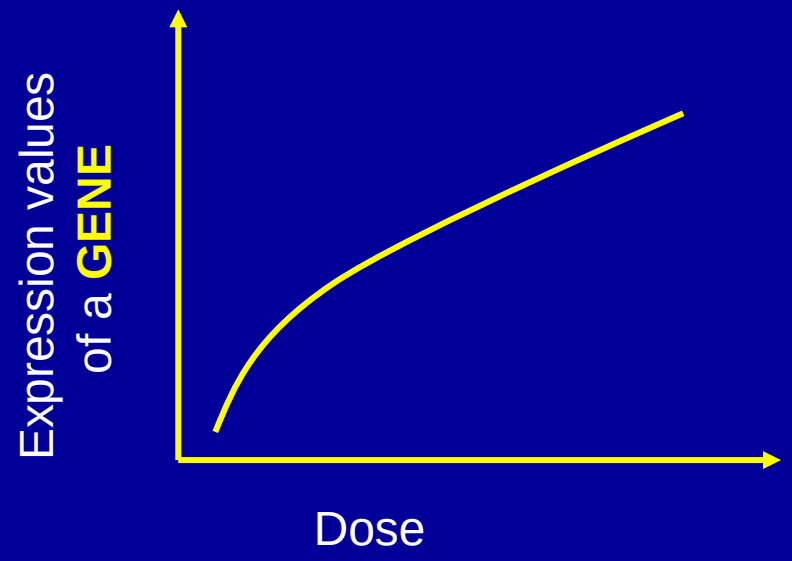


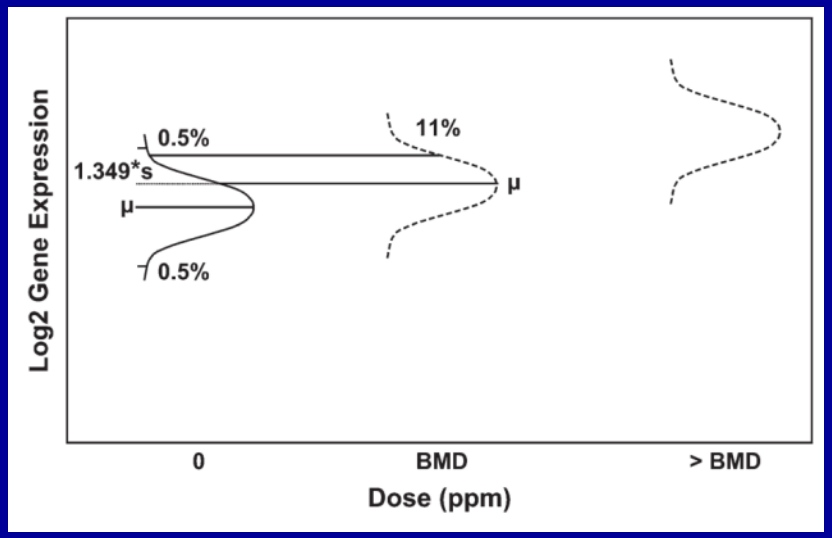
- Databases
  - DrugMatrix
  - ToxDB
  - ToxFx
  - Comparative Toxicogenomics Database
- Use in Risk assessment
  - Supporting MOA / Mechanism
  - Useful for qualitative assessment
  - Efforts to use it for quantitative risk assessment

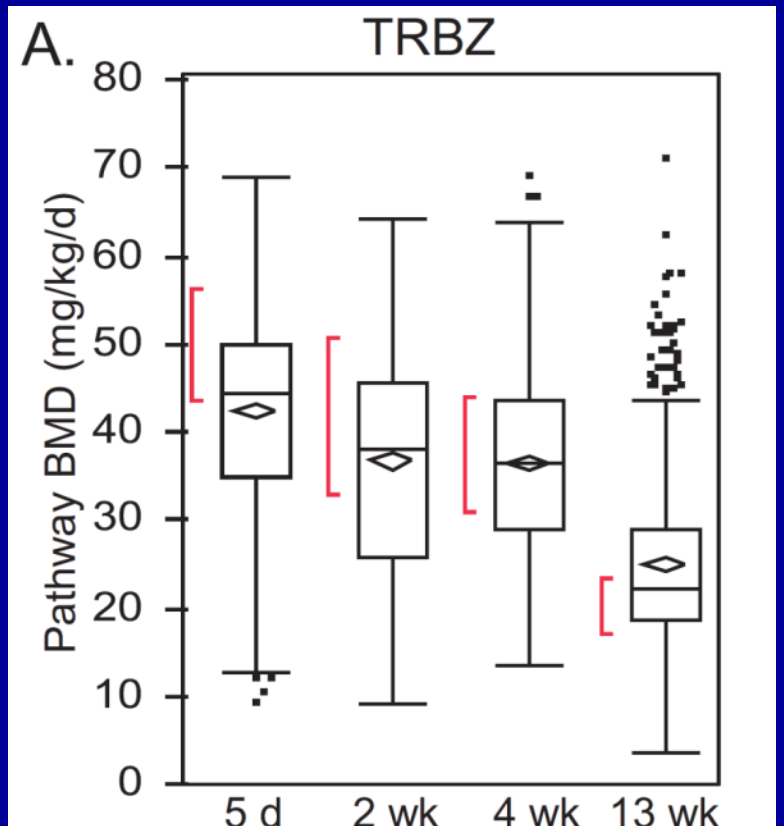
Sturla et al 2014

Thomas Method

(Thomas et al, 2007, 2011)







	5 d	2 wk	4 wk	13 wk
UQ	49.9	45.7	43.4	28.9
Median	44.5	37.8	36.4	22.0
LQ	34.8	25.8	28.8	18.8
Min	9.16	9.09	13.5	3.75

Kiyosawa method

Simple Scoring method

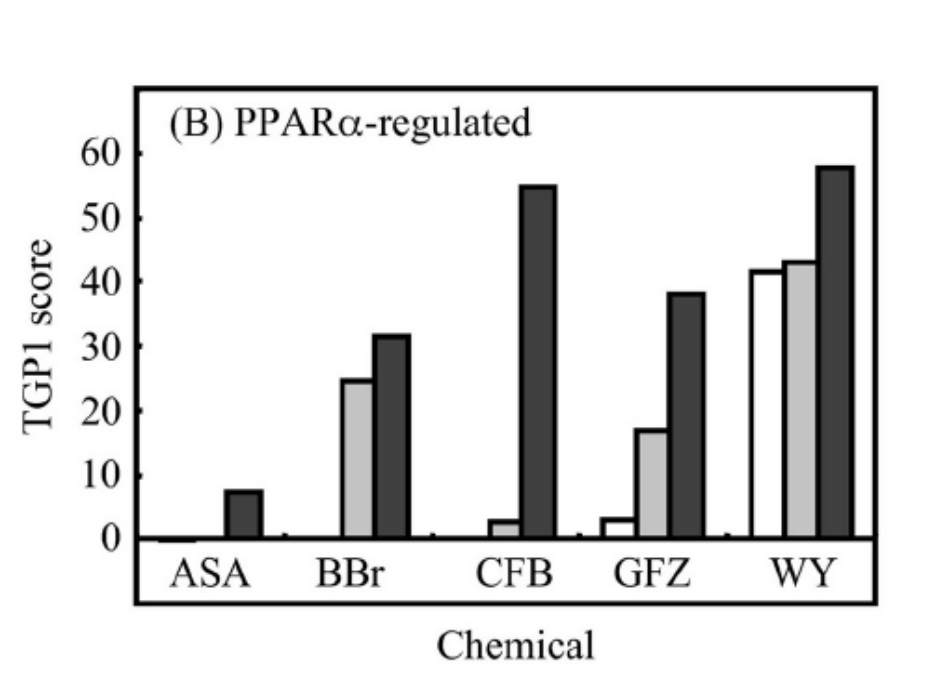
- Kiyosawa et al 2006
- Kiyosawa et al 2009

Pros –

- Simple method
- Based on pathway of toxicity

Cons-

- Good for qualitative comparison
- Artificially inflate/deflate the effect of change in expression
- Output is an index, without Stdev/confidence intervals



Kiyosawa Method

$$\text{Signal log ratio } (i) = \log_2 \left\{ \text{Average Signal } i \text{ (treated) / Average Signal } i \text{ (control)} \right\}$$

$$\text{Index 1} = \frac{\sum_{i=1}^{N} \text{Signal log ratio } (i)}{\text{Number of probe sets}}$$

To capture general tendency of expression changes

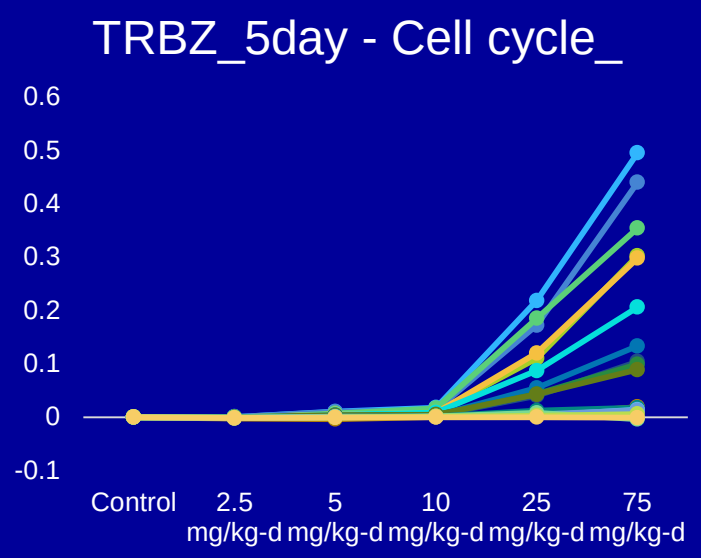
$$\text{Index 2} = \frac{\sum_{i=1}^{N} \left\{ \text{Signal log ratio } (i) \right\}^2}{\text{Number of probe sets}}$$

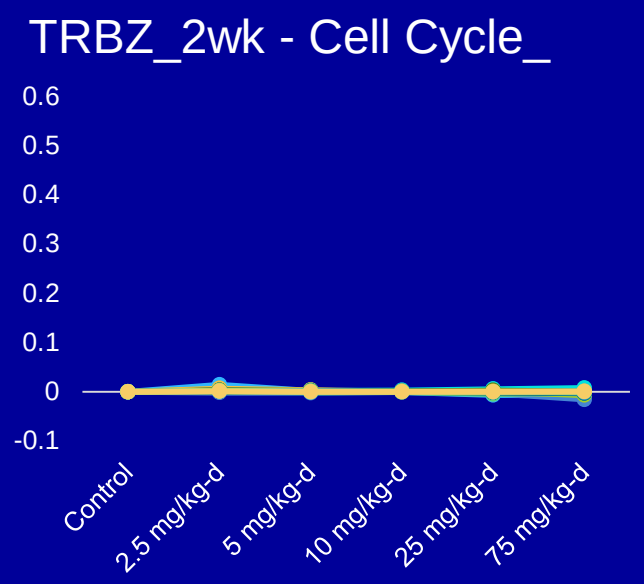
To capture general size of the expression changes

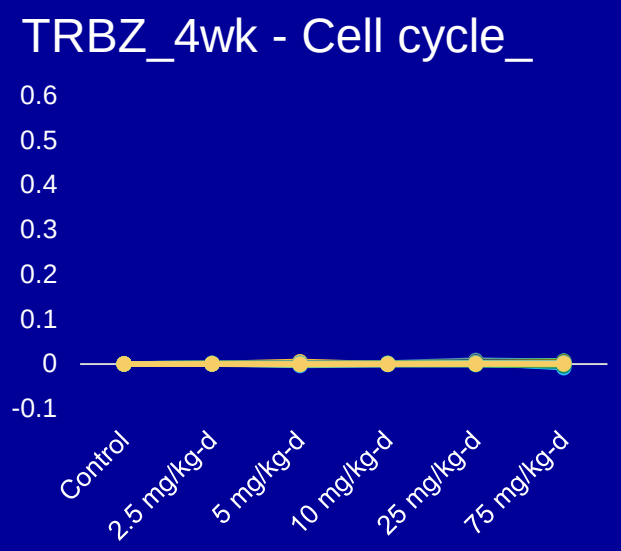
$$\text{TGP score 1} = (\text{Index 1}) \text{ X } (\text{Index 2})$$

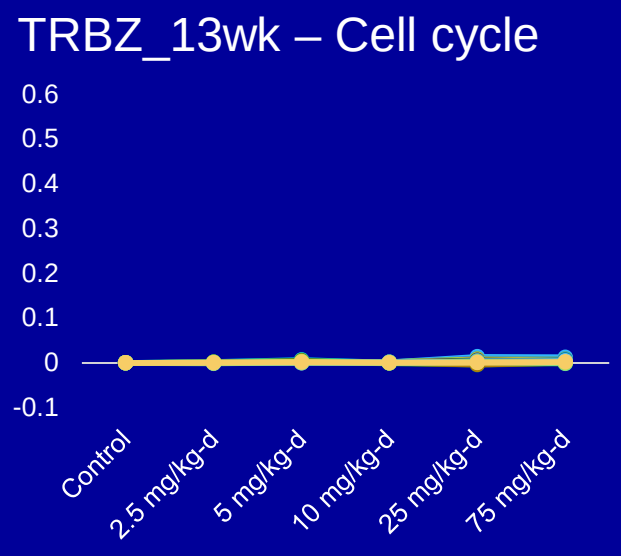
Dose Response: Cell Cycle_Pathways

1,2,4-Tribromobenzene





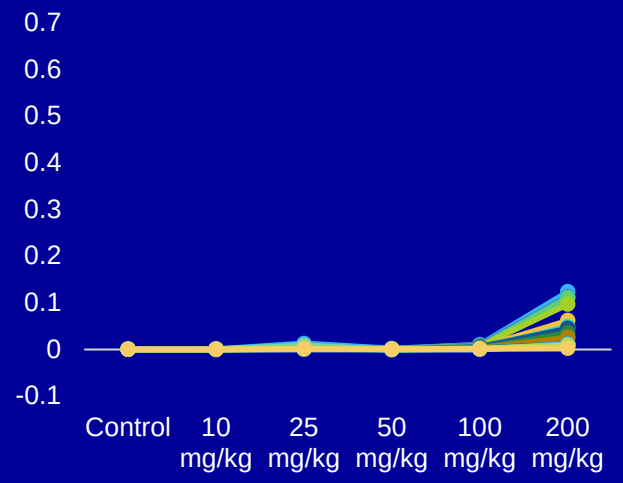




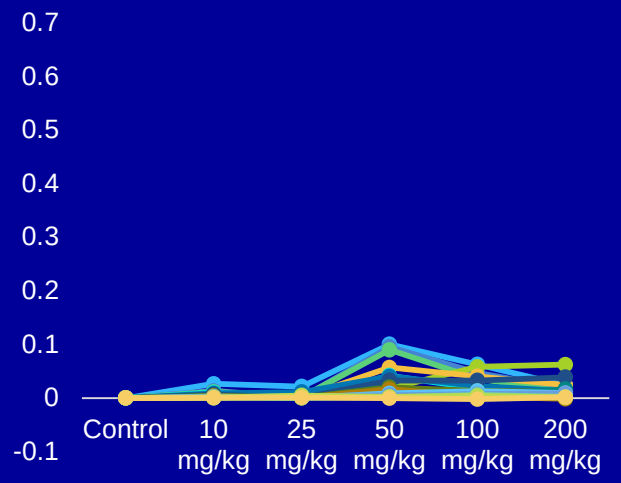
Dose Response: Cell Cycle_Pathways

2,3,4,6-tetrachlorophenol

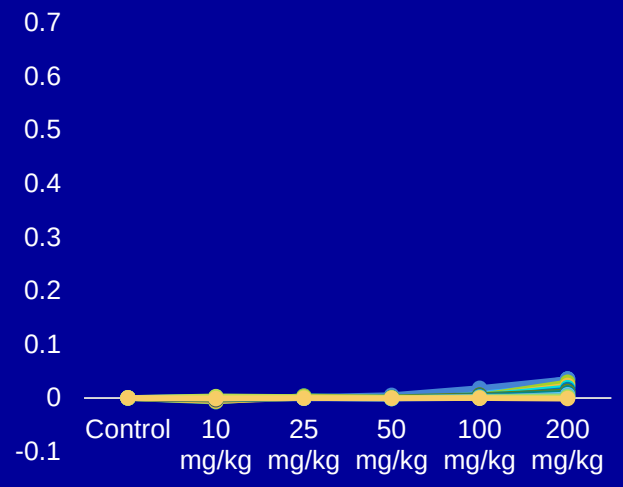
TTCP_5day - Cell Cycle



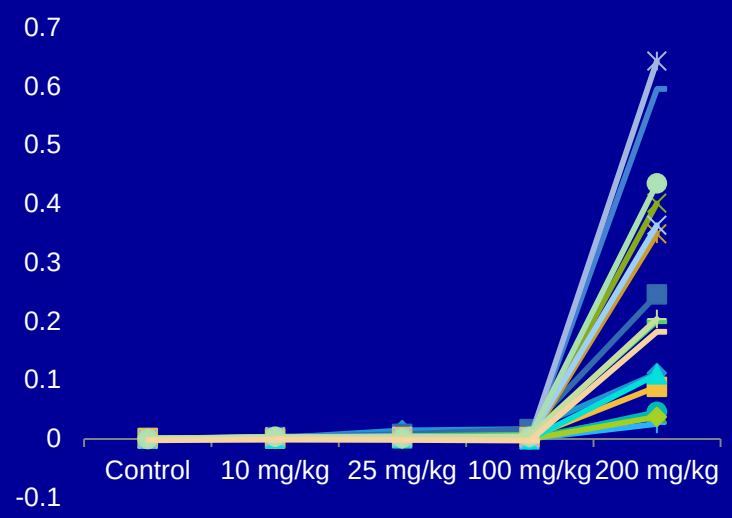
TTCP_2wk - Cell Cycle



TTCP_4wk - Cell Cycle



TTCP_13wk - Cell Cycle



Conclusion

- Toxicogenomics data is useful for mechanistic information
- Useful for relative comparison between different agents, doses and time points
- Useful for prioritization
- Need to define which pathways/gene-sets are adverse to use them for deriving POD

Acknowledgements

Cohen lab at U of Nebraska Medical Center, Omaha NE

- Samuel Cohen
- Lora Arnold
- Karen Pennington
- Mitscheli DaRocha

U.S. EPA National Center for Environmental Assessment, Cincinnati

- Jason Lambert
- Scott Wesselkamper
- Jay Zhao
- J. Philip Kaiser
- Glenn Rice
- Lucina Lizarraga-Zazueta

Collaborators

- X. Chris Le - University of Alberta
- J. L.V. De Camargo - São Paulo State University