Perinatal arsenic exposures

The long-term impact of perinatal exposures on the immune system and disease risk

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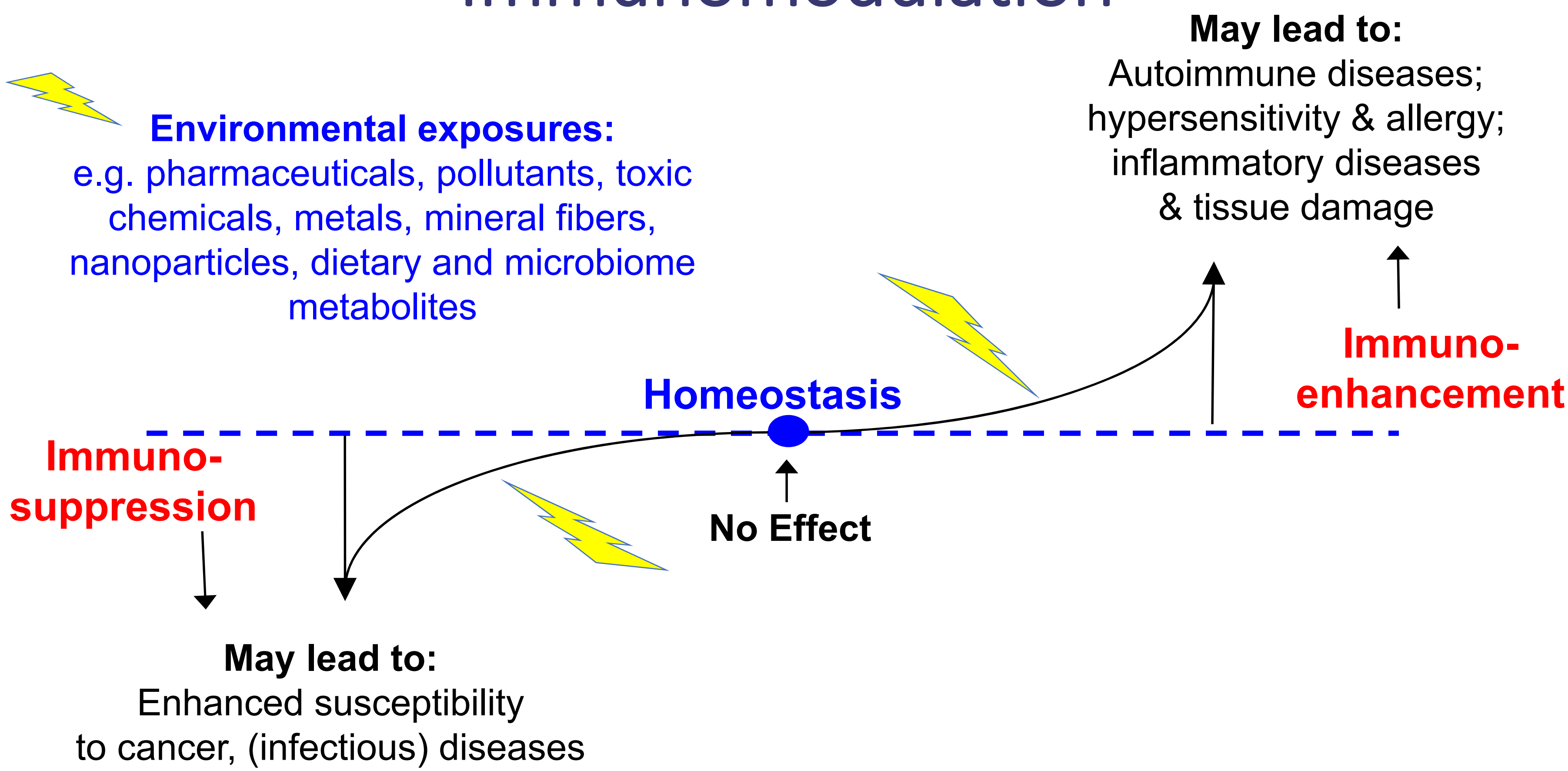
All human subject studies have been approved and conducted in accordance to both U.S. and Chile IRB
All animal procedures have been approved and conducted in accordance with the JHU institutional ACUC

No Conflict of Interest

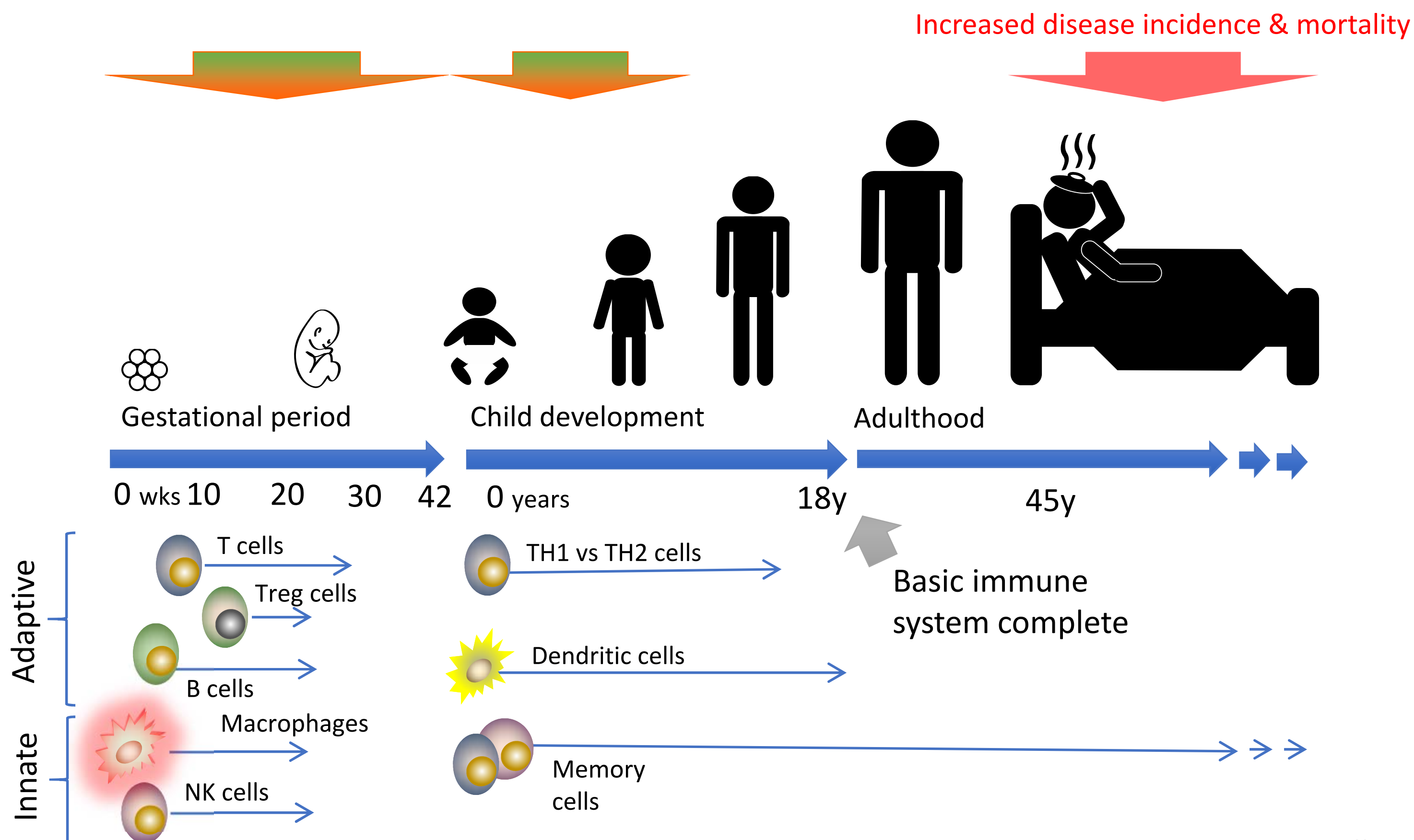
Immunomodulation



Adapted from: Casarett & Doull's Essentials of Toxicology, 2010. 2nd edition (Klaassen CD, Watkins JB, eds) New York: McGraw-Hill. ISBN – 978-0-07-162240-0

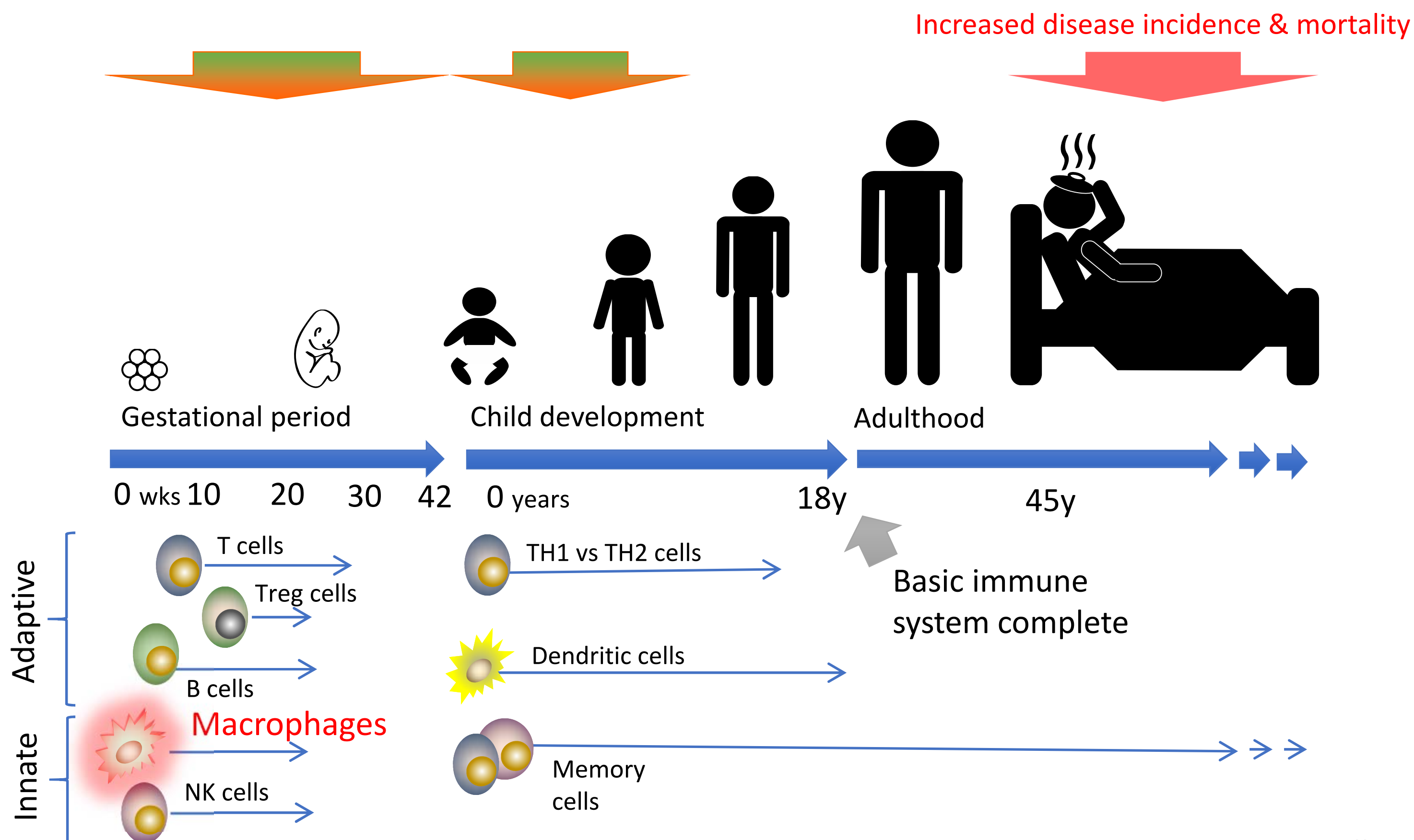
Perinatal windows of susceptibility

Early-life exposures to environmental factors



Environmental exposures during pregnancy: Mechanistic effects on immunity, Rychlik K., & Sillé, F. Birth Defects Research Vol. 111; 4: 178-196, 2019

Early-life exposures to environmental factors



Environmental exposures during pregnancy: Mechanistic effects on immunity, Rychlik K., & Sillé, F. Birth Defects Research Vol. 111; 4: 178-196, 2019

Developmental immunotoxicity (DIT): windows of susceptibility

Gestation

	Immune Maturation Events		
Gestation Length	1. Initiation of Hema-topoiesis	2. Migration of Stem Cells and Expansion of Progenitor Cells	3. Colonization of Bone marrow and Thymus
Rat average: 22 days *Mouse average: 20 days*	Rat/Mouse: GD 7-9	Rat/Mouse: GD 9-16	Rat/Mouse: Birth- GD 11
Human average: 40 weeks	Human: GW 8-10	Human: GW 10-16	Human: Birth – GW 16

Lactation

	Immune Maturation Events	
Lactation Timing	4. Maturation to Immuno-competence	5. Establishment of Immune Memory
Rat/Mouse: Birth - PND21	Rat/Mouse: Birth – PND21	-
*Human: Birth to ~ 6 wk up to 3 yrs**	Human: Birth – 1 yr	Human: 1- 3 yrs

Pre-Pubescent

	Immune Maturation Events	
Pre-Pubescent Timing	4. Maturation to Immuno-competence	5. Establishment of Immune Memory
~6 weeks rat (~PND42) *~4-5 weeks mouse (varies by strain)*	Rat/Mouse: PND21 - PND30	Rat/Mouse: PND30 - PND60
~10-13 yr Human	Human: Birth – 1 yr	Human: 1 yr to ~10-13yr

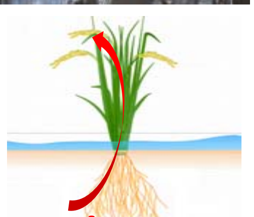
Post-Puberty

	Immune Maturation Events
Post-Puberty Timing	5. Establishment of Immune Memory
> ~ 6 weeks rat (~PND42) *> ~ 4-5 weeks mouse (varies by strain)*	Rat/Mouse: > PND60
> ~ 10-13 yr Human	Human: >10-13 yr

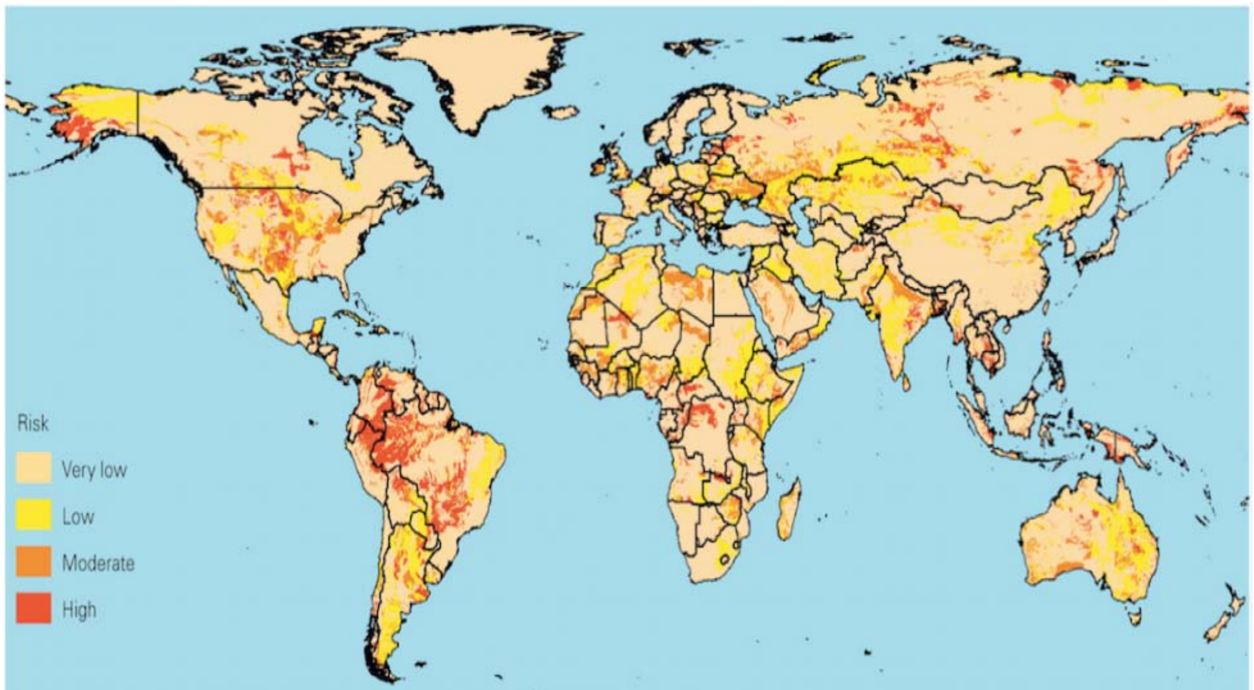
Current Status of Developmental Immunotoxicity: Early-Life Patterns and Testing, DeWitt, J., et al, Toxicologic Pathology, 40: 230-236, 2012

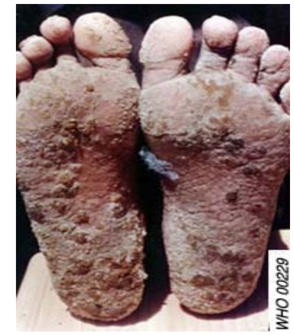
In utero and early life exposures to arsenic:
Later life disease

Arsenic prevalence, exposure & disease

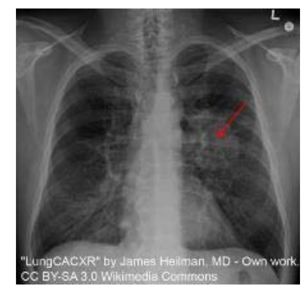


Arsenic





Immunotoxicant

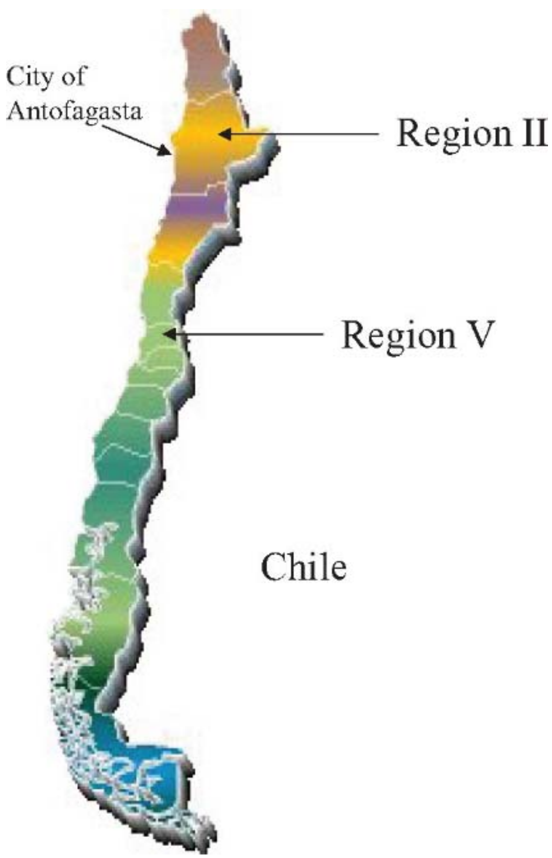


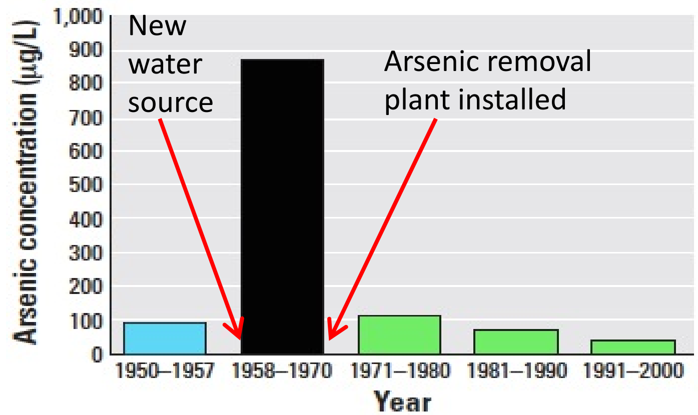
- US EPA & WHO drinking water standard = 10 µg/L (10 ppb)

Google Images, Wikimedia Commons

Schwarzenbach et al. (2010) Annual Review of Environment and Resources Vol. 35:109-136

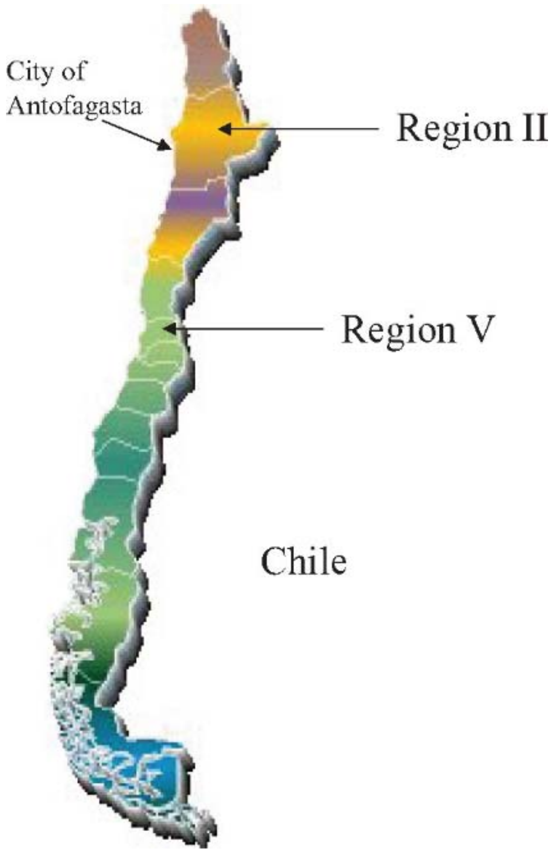
Early-life exposure to arsenic in Chile

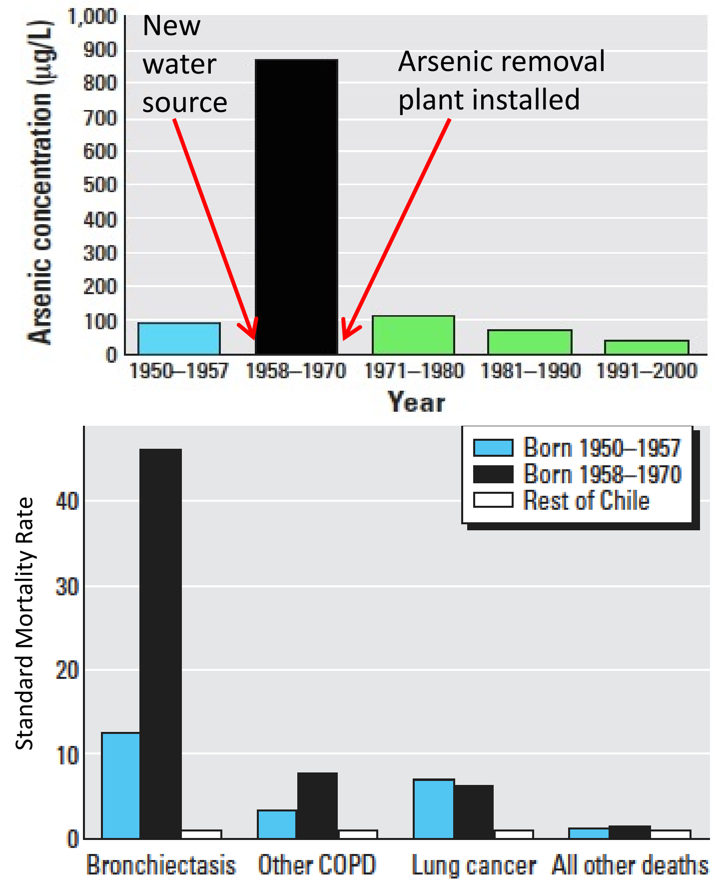




Ferreccio, C., *et al.* Epidemiology 2000; Smith, A., *et al.* EHP 2006 ; Yuan, Y., *et al.* Epidemiology 2010; Steinmaus, C., *et al.* CEBP 2013

Early-life exposure to arsenic in Chile – Later life disease

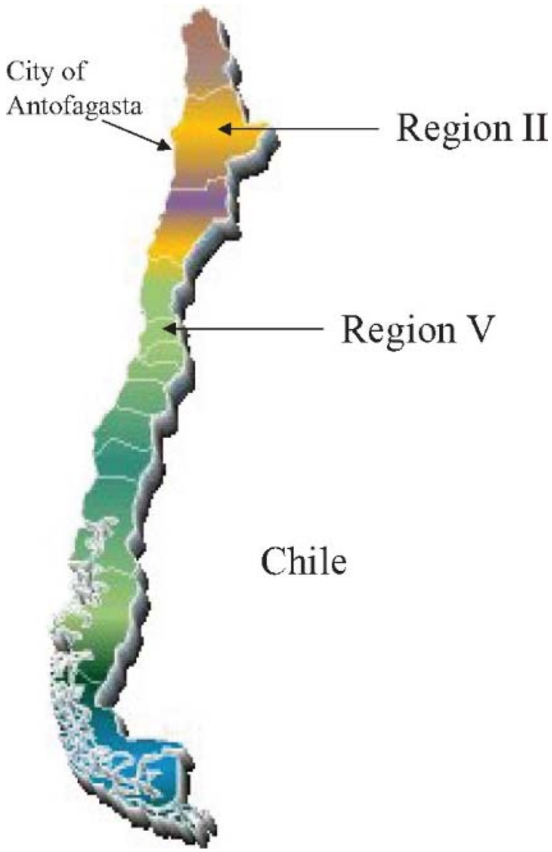




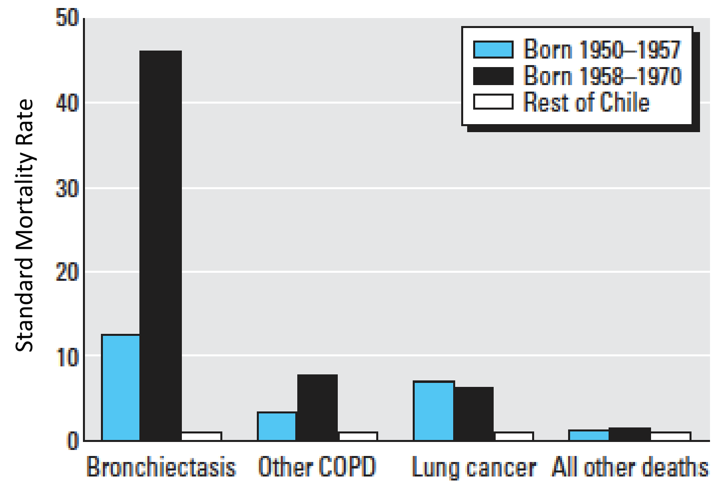
> 40 years later

Ferreccio, C., *et al.* Epidemiology 2000; Smith, A., *et al.* EHP 2006 ; Yuan, Y., *et al.* Epidemiology 2010; Steinmaus, C., *et al.* CEBP 2013

Early-life exposure to arsenic in Chile – Later life disease



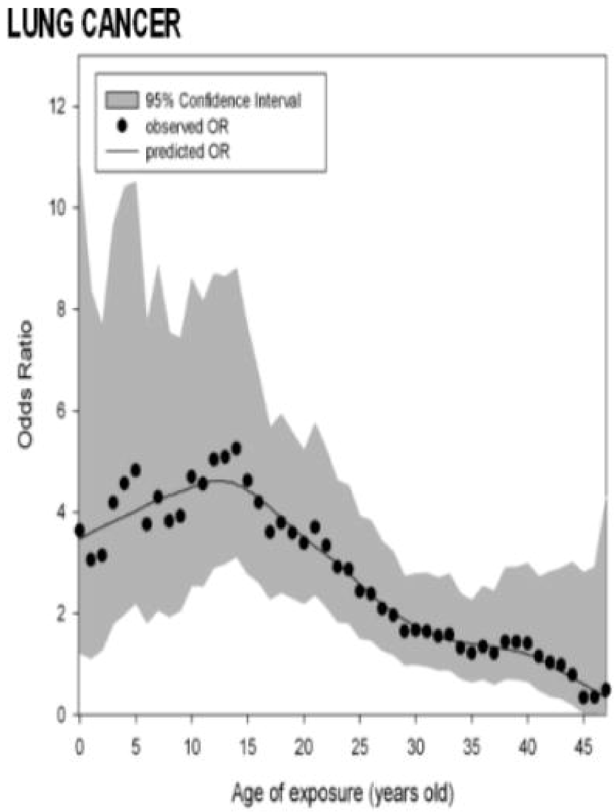
Rare evidence supporting the "**Developmental Origins of Health and Disease**" hypothesis.

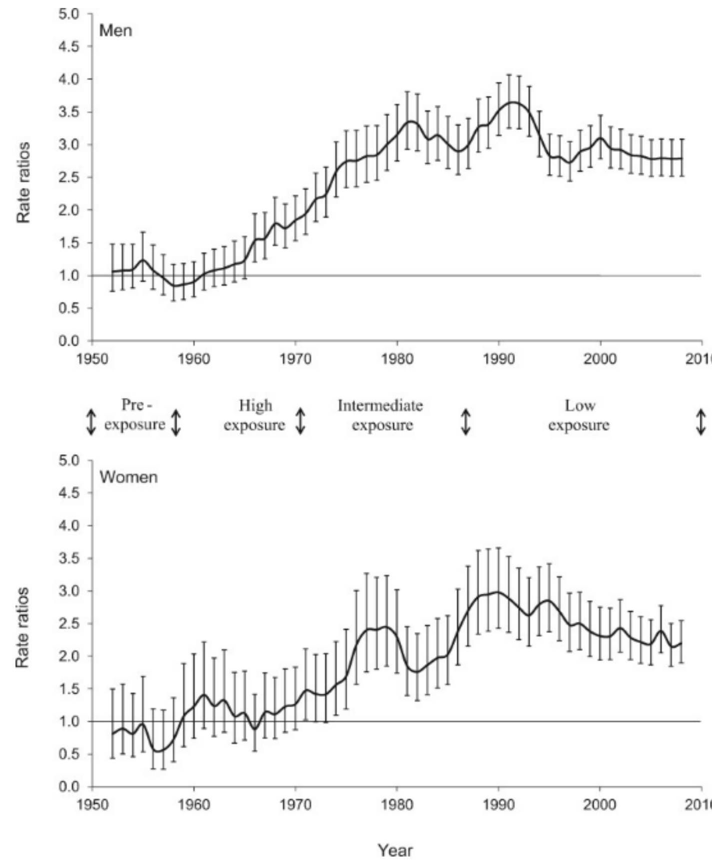


> 40 years later

Ferreccio, C., *et al.* Epidemiology 2000; Smith, A., *et al.* EHP 2006 ; Yuan, Y., *et al.* Epidemiology 2010; Steinmaus, C., *et al.* CEBP 2013

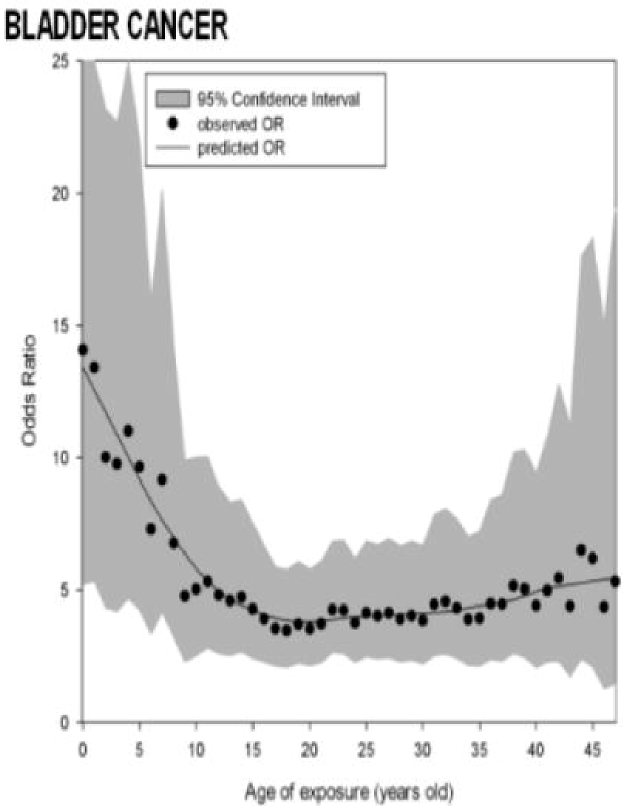
Early-life exposure to arsenic in Chile – Lung cancer

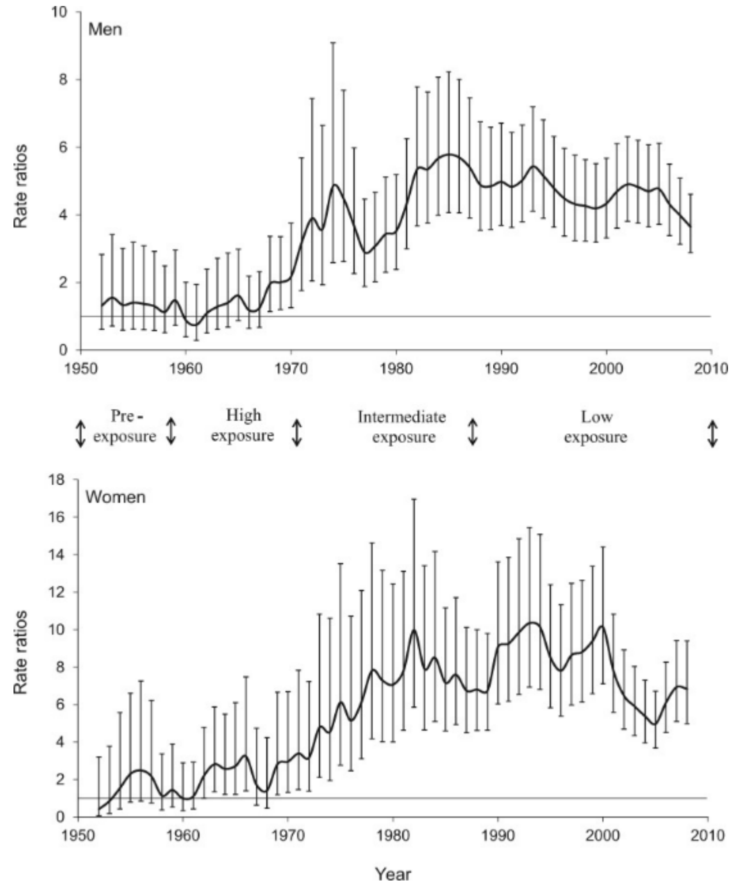




Steinmaus, C. et al Cancer Epidemiol Biomarkers Prev. 2014 Aug;23(8):1529-38. and Smith, A., et al. J Natl Cancer Inst. 2018 Mar 1;110(3):241-249.

Early-life exposure to arsenic in Chile – Bladder cancer





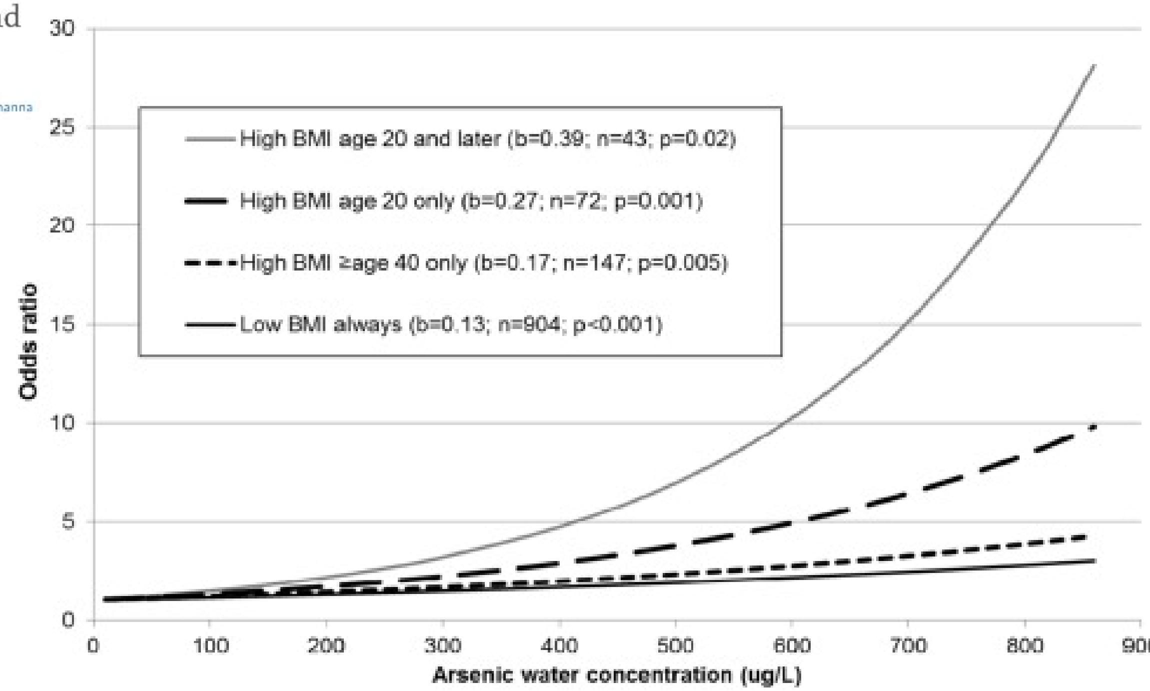
Steinmaus , C. et al Cancer Epidemiol Biomarkers Prev. 2014 Aug;23(8):1529-38. and Smith, A., et al. J Natl Cancer Inst. 2018 Mar 1;110(3):241-249.

Early-life exposure to arsenic in Chile + Obesity = high cancer risk

Environmental Research
Volume 142, October 2015, Pages 594-601

Obesity and excess weight in early adulthood and high risks of arsenic-related cancer in later life

Craig Steinmaus [a, b], Felicia Castriota [c], Catterina Ferreccio [d], Allan H. Smith [a], Yan Yuan [a], Jane Liaw [a], Johanna Acevedo [d], Liliana Pérez [d], Rodrigo Meza [e], Sergio Calcagno [f], Ricardo Uauy [g, h], Martyn T. Smith [i]



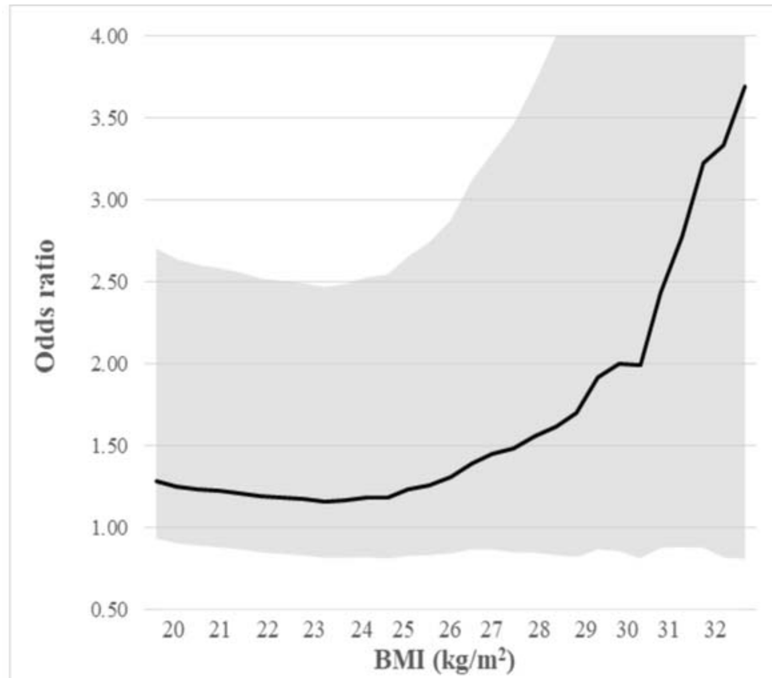
Steinmaus et al, Environ Res. 2015 Oct;142:594-601.

Early-life exposure to arsenic in Chile – T2D

Environmental Research
Volume 167, November 2018, Pages 248-254

Obesity and increased susceptibility to arsenic-related type 2 diabetes in Northern Chile

Felicia Castriota [a], Johanna Acevedo [b], Catterina Ferreccio [b], Allan H. Smith [c], Jane Liaw [c], Martyn T. Smith [a], Craig Steinmaus [c, d]



Dark line represents the odds ratios. Shaded area represents the 95% confidence intervals. 10,000 μg/L is approximately the range between lower and upper tertile groups of arsenic exposure.

Castriota et al, Environ Res. 2018 Nov;167:248-254

Early-life exposure to arsenic in Chile – T2D

ELSEVIER

Environmental Research
Volume 172, May 2019, Pages 578-585

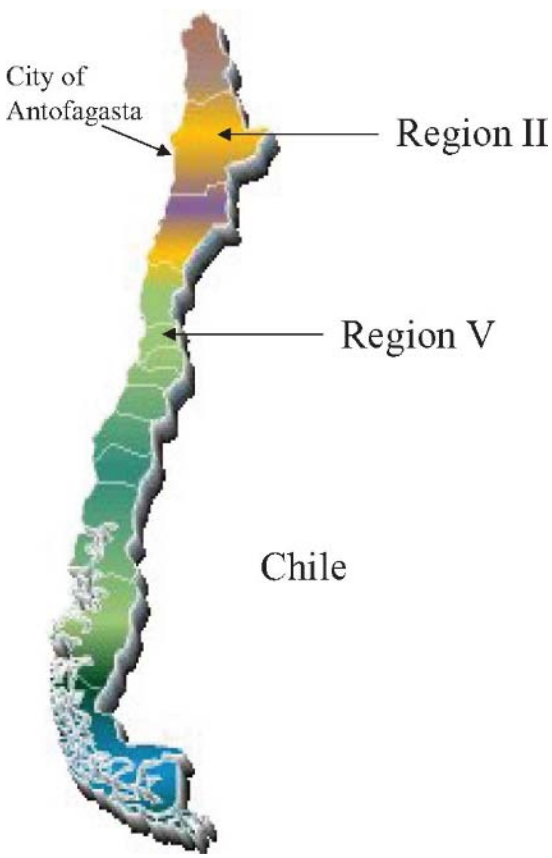
Socioeconomic status and the association between arsenic exposure and type 2 diabetes

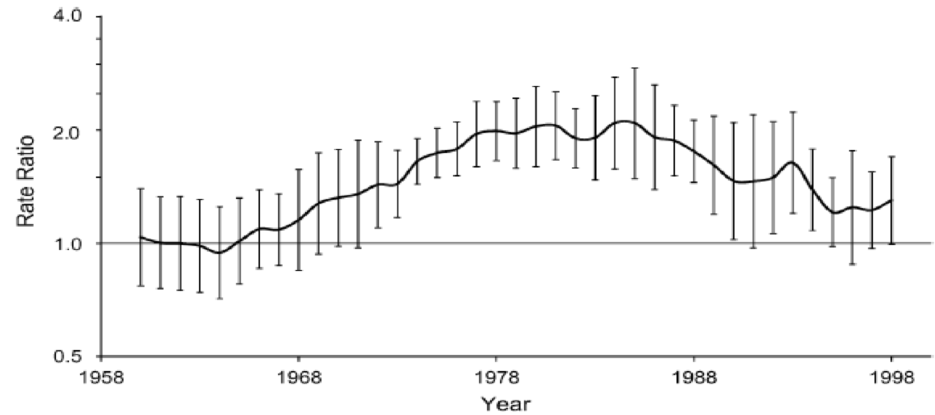
Stephanie M. Eick [a], Catterina Ferreccio [b], Johanna Acevedo [b], Felicia Castriota [c], José F. Cordero [a], Taehyun Roh [d], Allan H. Smith [d], Martyn T. Smith [c], Craig Steinmaus [d, e]

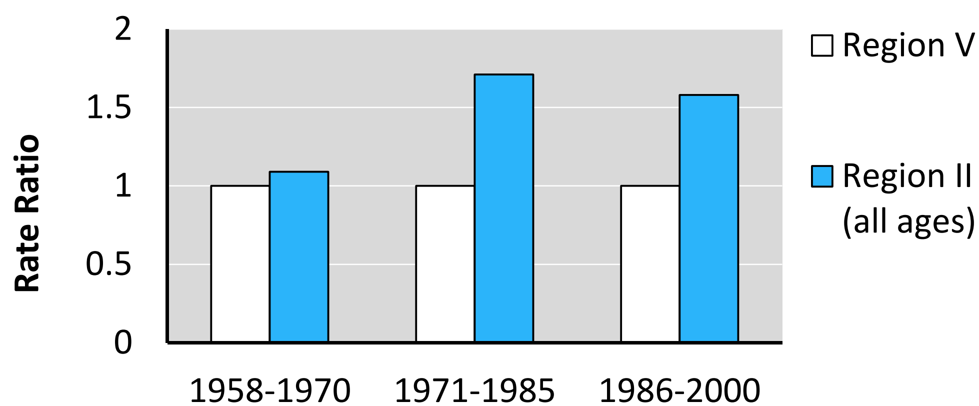
Low SES

Eick et al, Environ Res. 2019 May;172:578-585.

Early-life exposure to arsenic in Chile – Later life TB







Smith, A., *et al.* EHP 2006; Smith, A. *et al.* Am. J. Epi. 2011

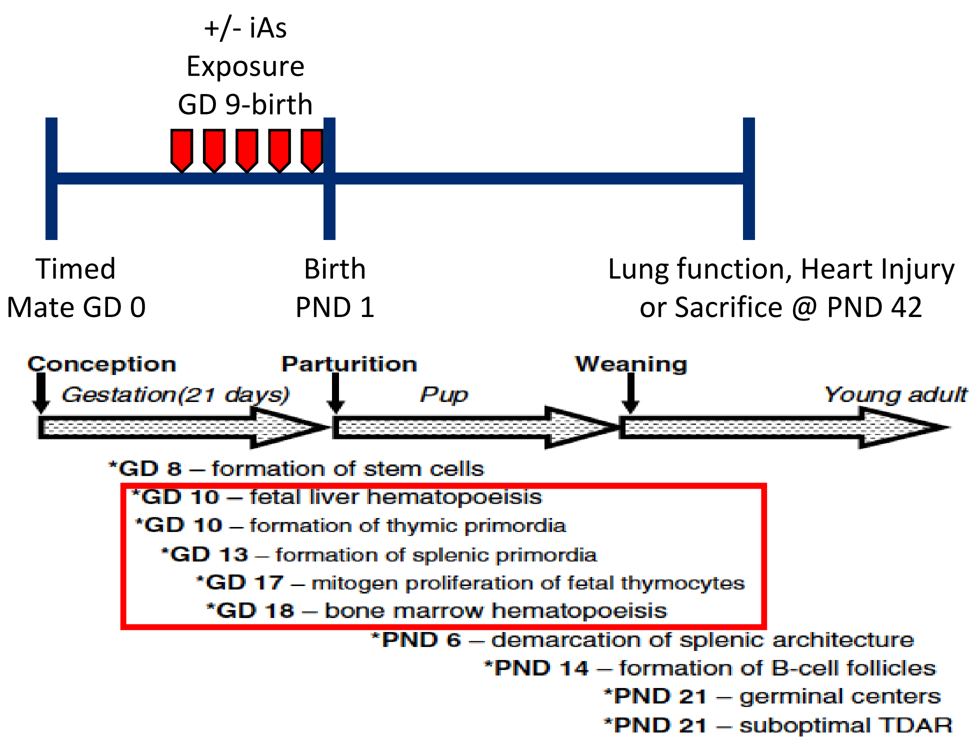
Early-life exposure to arsenic in Chile – Later life cytokine profiles

*External Exposure at Birth (ug/L)									
		Continuous Scaled per 200 ug/L				Categorical Low: <860 ug/L; High: 860 ug/L			
Cytokine**	**% detec table**	**Unadjusted OR (95% CI)**	**p-value**	**Adjusted OR*** (95% CI)**	**p-value**	**Unadjusted OR (95% CI)**	**p-value**	**Adjusted OR*** (95% CI)**	**p-value**
MCP-1	100	0.06 (0.00, 0.11)	0.036	0.05 (-0.01, 0.10)	0.082	0.22 (-0.00, 0.44)	0.052	0.19 (-0.04, 0.42)	0.097
IP-10	100	0.02 (-0.04, 0.08)	0.499	0.00 (-0.06, 0.06)	0.941	0.04 (-0.20, 0.28)	0.727	-0.03 (-0.27, 0.22)	0.830
MIP-1-β	99	0.05 (-0.01, 0.11)	0.094	0.05 (-0.01, 0.11)	0.083	0.20 (-0.04, 0.45)	0.096	0.22 (-0.03, 0.46)	0.083
Eotaxin-CCL-11	98	0.10 (-0.00, 0.21)	0.060	0.10 (-0.01, 0.21)	0.074	0.43 (0.00, 0.86)	0.049	0.44 (-0.01, 0.88)	0.056
EGF	88	0.10 (-0.04, 0.25)	0.171	0.10 (-0.05, 0.25)	0.188	0.40 (-0.19, 0.99)	0.180	0.38 (-0.22, 0.98)	0.208
IL-1Ra	85	0.06 (-0.06, 0.19)	0.329	0.09 (-0.03, 0.21)	0.150	0.27 (-0.24, 0.79)	0.296	0.40 (-0.10, 0.90)	0.116
TNF-α	73	0.01 (-0.11, 0.12)	0.906	0.02 (-0.10, 0.14)	0.704	0.02 (-0.45, 0.48)	0.941	0.09 (-0.39, 0.57)	0.718
IL-8	69	0.11 (-0.00, 0.23)	0.056	0.12 (0.00, 0.24)	0.047	0.40 (-0.07, 0.88)	0.097	0.45 (-0.04, 0.94)	0.072
VEGF	61	-0.18 (-0.44, 0.08)	0.183	-0.17 (-0.44, 0.10)	0.223	-0.70 (-1.76, 0.37)	0.196	-0.65 (-1.77, 0.46)	0.248
IL-15	59	0.05 (-0.09, 0.19)	0.503	0.08 (-0.06, 0.21)	0.265	0.22 (-0.35, 0.79)	0.446	0.33 (-0.22, 0.87)	0.235
MIP-1-α	56	0.10 (0.01, 0.19)	0.039	0.12 (0.02, 0.21)	0.016	0.38 (-0.00, 0.76)	0.052	0.45 (0.07, 0.84)	0.022
IL-5	46	0.08 (-0.05, 0.22)	0.218	0.11 (-0.02, 0.23)	0.093	0.34 (-0.20, 0.89)	0.213	0.44 (-0.07, 0.95)	0.088
IL-12p40	44	0.04 (-0.11, 0.20)	0.600	0.08 (-0.08, 0.24)	0.316	0.23 (-0.40, 0.86)	0.470	0.40 (-0.24, 1.03)	0.216
GM-CSF	42	0.05 (-0.06, 0.15)	0.375	0.06 (-0.05, 0.15)	0.279	0.26 (-0.15, 0.67)	0.209	0.30 (-0.10, 0.71)	0.140
TNF-β	42	0.14 (-0.01, 0.28)	0.062	0.19 (0.05, 0.33)	0.010	0.54 (-0.04, 1.12)	0.066	0.78 (0.21, 1.35)	0.008
IL-10	38	0.13 (-0.05, 0.30)	0.150	0.13 (-0.04, 0.31)	0.129	0.44 (-0.27, 1.14)	0.221	0.48 (-0.23, 1.18)	0.184
IL-1-β	27	0.02 (-0.02, 0.07)	0.323	0.02 (-0.02, 0.07)	0.319	0.12 (-0.09, 0.32)	0.256	0.12 (-0.08, 0.31)	0.250
IFN-α-2	23	0.09 (-0.03, 0.21)	0.122	0.08 (-0.04, 0.21)	0.191	0.39 (-0.09, 0.87)	0.113	0.34 (-0.17, 0.84)	0.187
IL-6	21	0.05 (-0.02, 0.12)	0.126	0.06 (-0.01, 0.13)	0.094	0.24 (-0.04, 0.53)	0.094	0.26 (-0.02, 0.55)	0.072
IL-2	19	0.02 (-0.05, 0.09)	0.571	0.01 (-0.06, 0.08)	0.784	0.09 (-0.18, 0.37)	0.497	0.06 (-0.23, 0.35)	0.672
IL-12p70	18	0.01 (-0.03, 0.06)	0.527	0.02 (-0.03, 0.06)	0.509	0.08 (-0.11, 0.27)	0.402	0.09 (-0.11, 0.29)	0.369
IL-13	17	0.07 (-0.03, 0.16)	0.152	0.08 (-0.01, 0.17)	0.084	0.28 (-0.10, 0.66)	0.152	0.33 (-0.05, 0.70)	0.088
IFN-γ	13	-0.00 (-0.09, 0.08)	0.918	0.00 (-0.08, 0.08)	0.986	-0.02 (-0.36, 0.31)	0.891	-0.01 (-0.35, 0.34)	0.971
G-CSF	6	0.09 (-0.03, 0.21)	0.149	0.11 (-0.01, 0.23)	0.073	0.40 (-0.08, 0.88)	0.103	0.49 (0.00, 0.98)	0.048
IL-4	5	-0.00 (-0.07, 0.07)	0.971	-0.00 (-0.08, 0.08)	0.965	0.03 (-0.27, 0.32)	0.864	0.02 (-0.29, 0.33)	0.889
IL-17a	5	-0.02 (-0.08, 0.04)	0.610	-0.02 (-0.08, 0.05)	0.582	-0.06 (-0.30, 0.19)	0.651	-0.07 (-0.33, 0.19)	0.598
IL-7	4	-0.01 (-0.04, 0.03)	0.606	-0.00 (-0.04, 0.03)	0.929	-0.02 (-0.16, 0.12)	0.748	0.01 (-0.14, 0.15)	0.916

Grant-Alfieri , A. , Zhang, H., et al, unpublished

In utero arsenic exposure model

In utero Arsenic Exposure Model

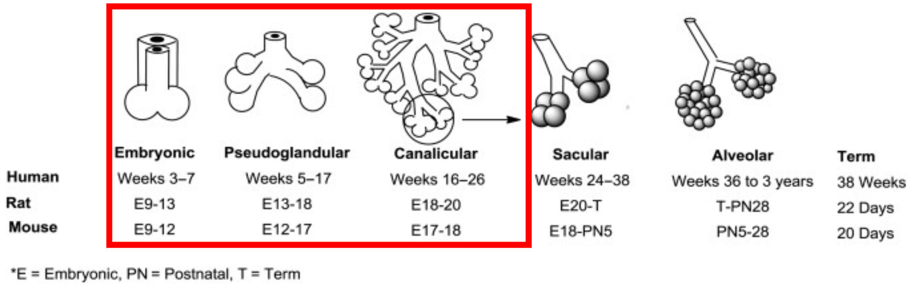


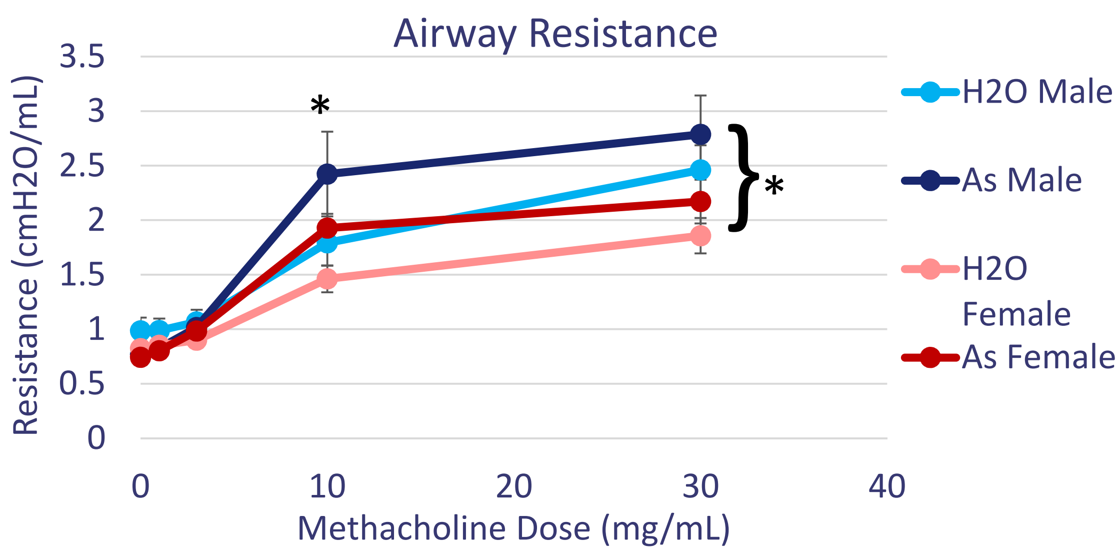
Kristal Rychlik, PhD

Rychlik & Sillé et al, unpublished

* $P < 0.05$

In utero Arsenic Exposure Model & Lung Function



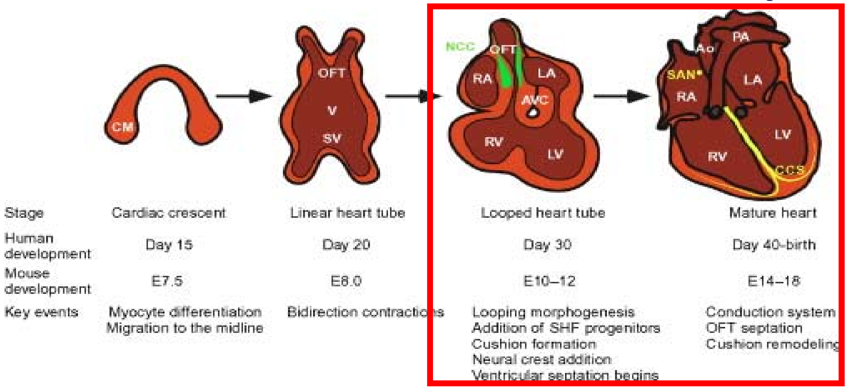


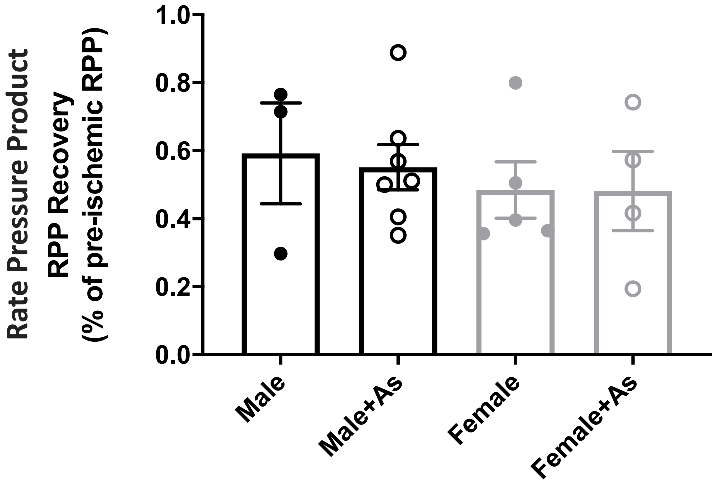
* *P* < 0.05

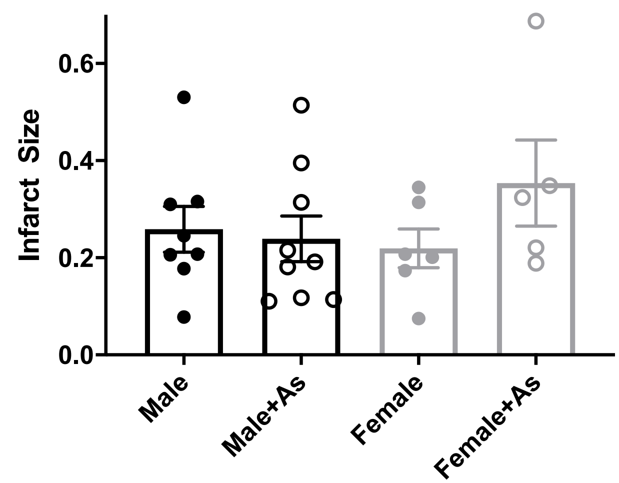
Rychlik, Mitzner & Sillé et al, unpublished

Chapter 15 - Lung Development. Lin Liu et al. MicroRNA in Regenerative Medicine; 381-399; 2015

In utero Arsenic Exposure Model & Heart Injury

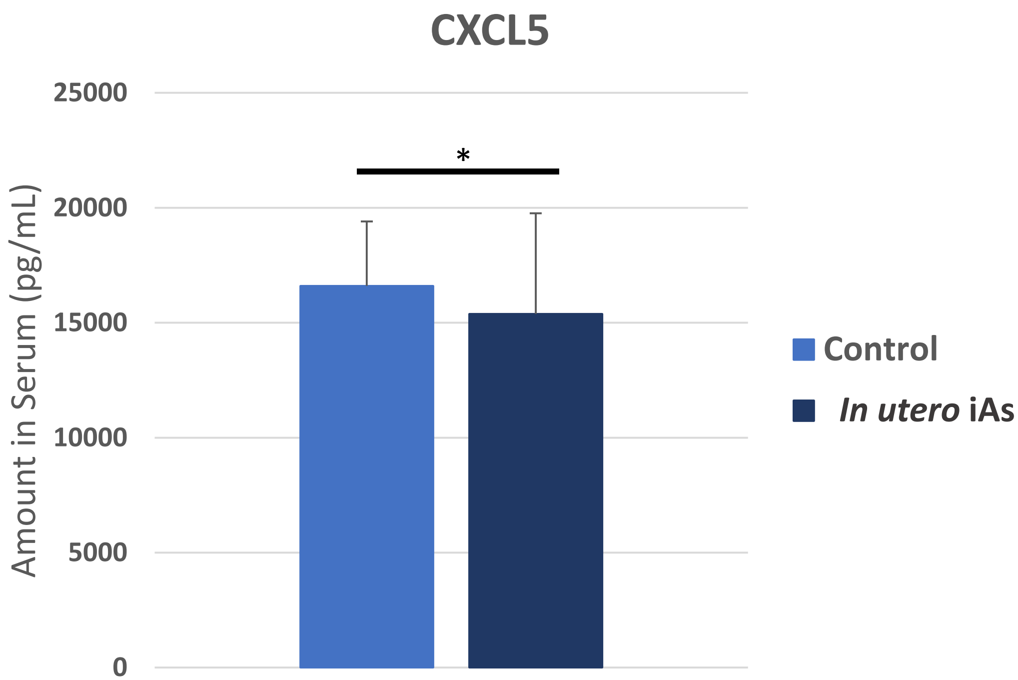






Rychlik, Kohr & Sillé et al, unpublished Heart Development. David J. McCulley, Brian L. Black, Current Topics in Developmental Biology, 2012

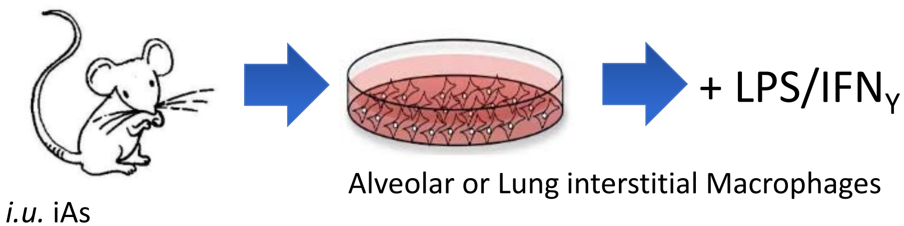
In utero Arsenic Exposure Model: serum cytokine changes

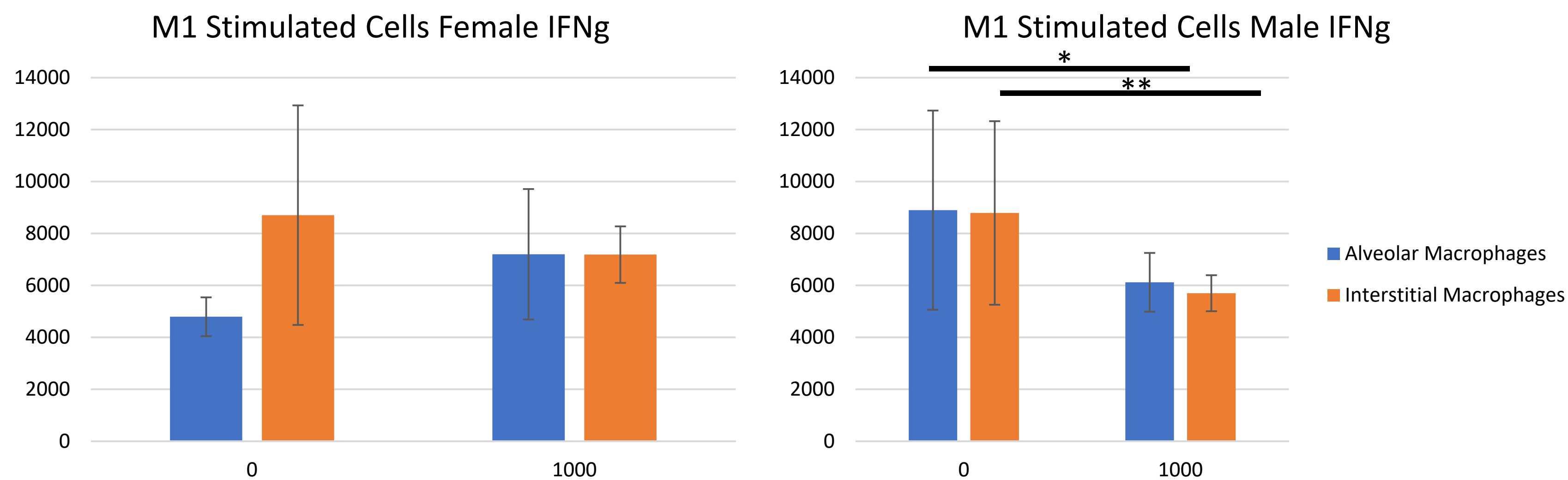


Age: 4 wks
N=4-8.
*p<0.05

Rychlik & Sillé et al, unpublished

In utero Arsenic Exposure Model: Macrophage cytokines





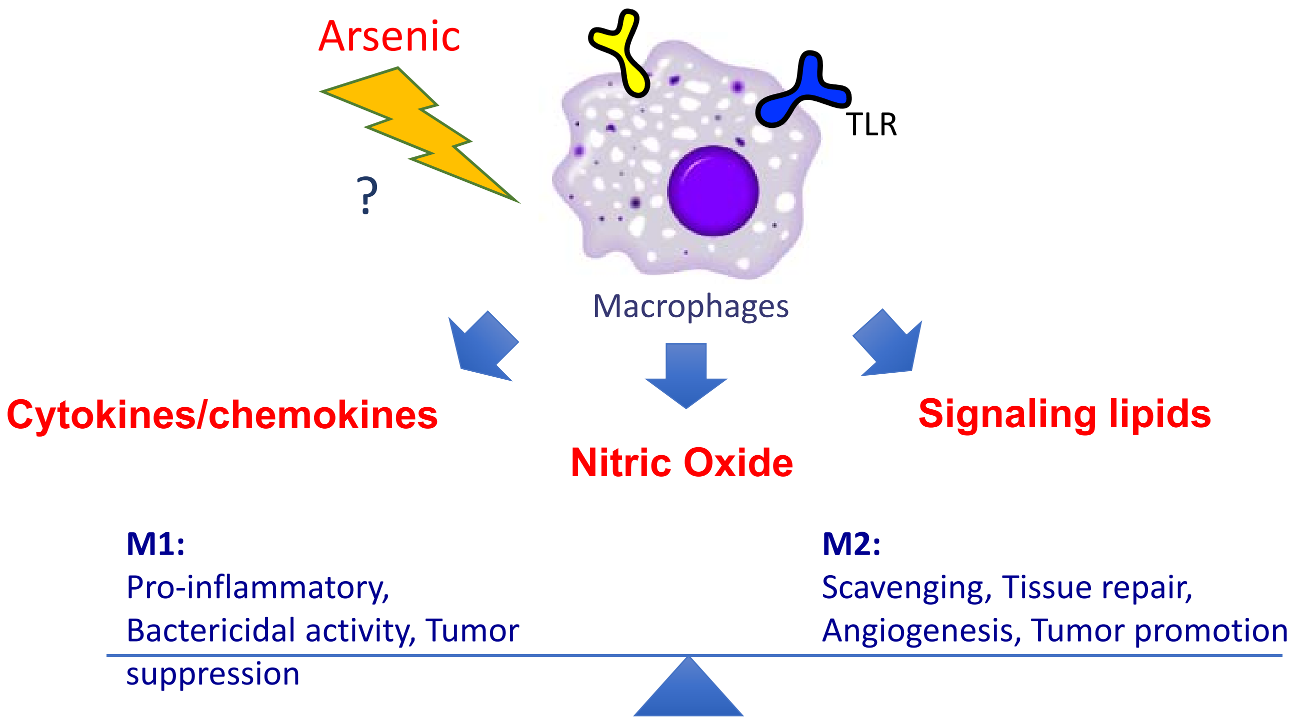
Rychlik & Sillé et al, unpublished

Two-way ANOVA with Tukey's Multiple Comparisons Test; N=3; P<0.03

In vitro models for *in utero* exposures to arsenic: Macrophages

Arsenic & macrophages

Hypothesis:
Early-life exposure to arsenic alters macrophage development & function causing increased disease later in life.



Evaluate function and polarization states of arsenic-exposed macrophages

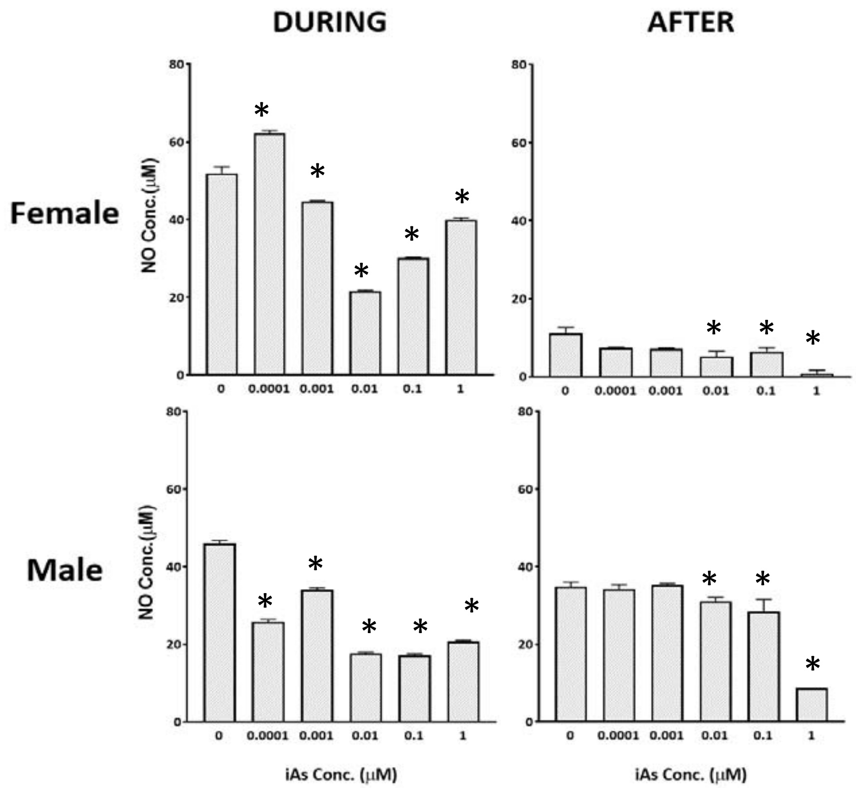
Emily Illingworth

*Arsenic was added to culture either during or after differentiation in doses: 0, 0.01, 0.1, 1 μM

Arsenic alters macrophage function

Developmental model vs. Mature model:

Griess Assay > Nitric Oxide

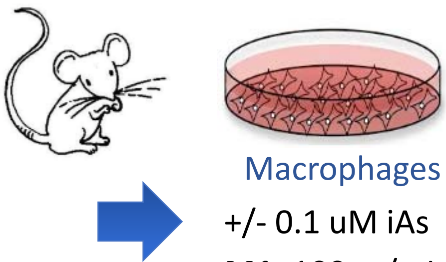


Illingworth & Sillé *et al*, unpublished

* $P < 0.05$

Arsenic alters cytokine/chemokine expression

Homeostasis



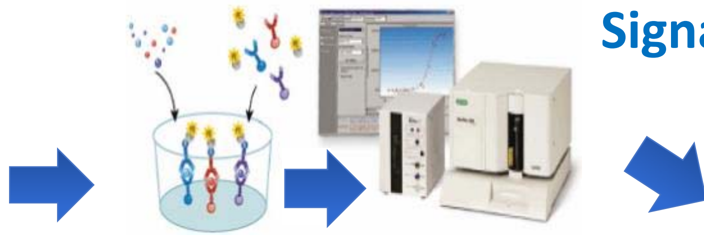
Mouse bone marrow

Macrophages

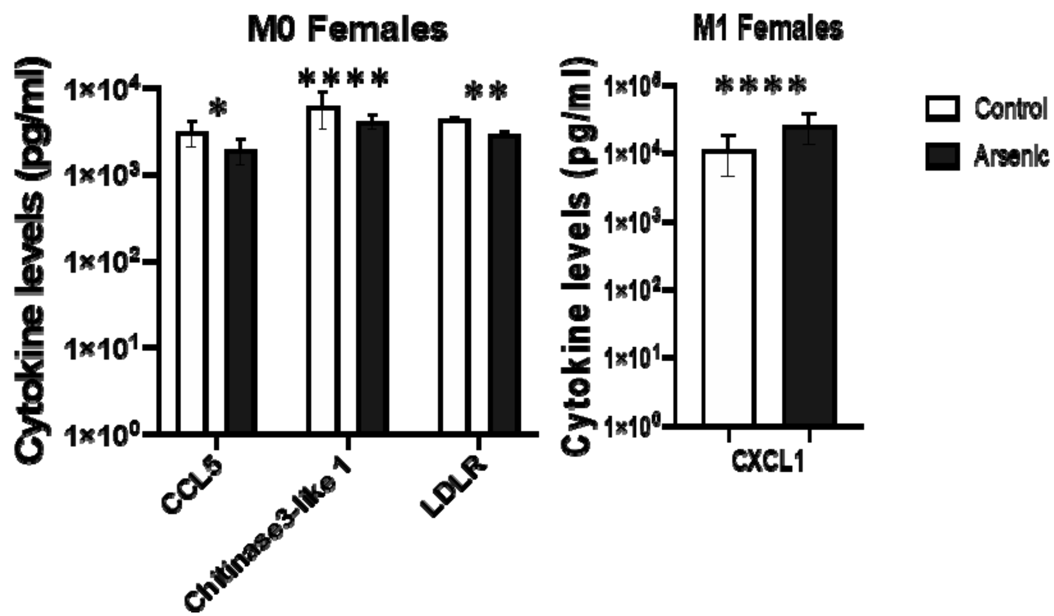
+/- 0.1 uM iAs

M1: 100ng/mL LPS + 6.25 ng/mL IFNg

M2: 20ng/mL IL-4 and IL-13



Signaling protein analysis



Sillé et al, unpublished

Arsenic alters cytokine/chemokine expression

Homeostasis

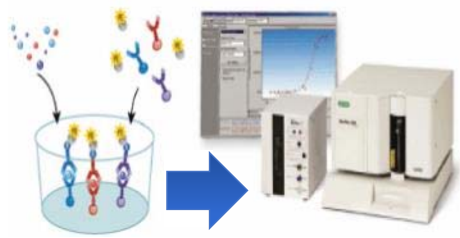
Mouse bone marrow

Macrophages

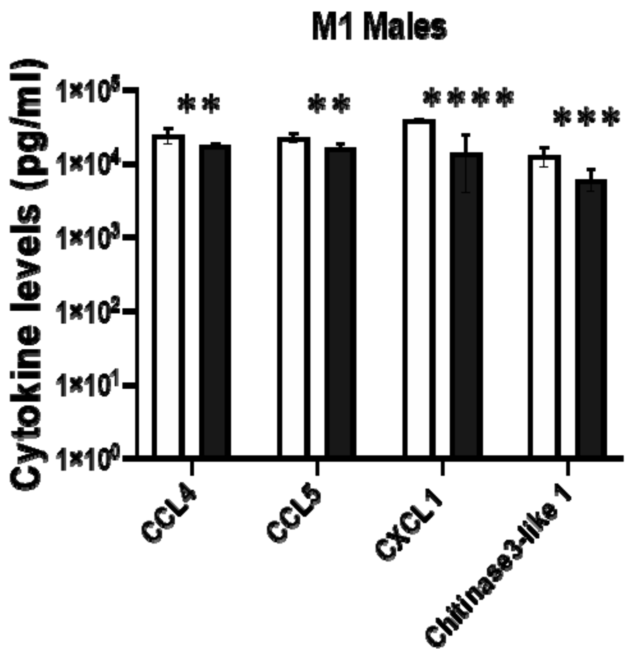
+/- 0.1 uM iAs

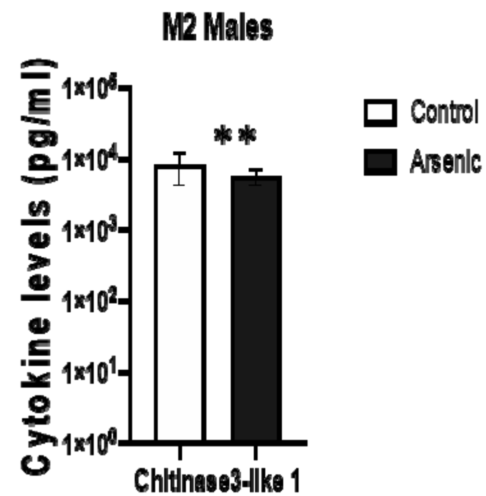
M1: 100ng/mL LPS + 6.25 ng/mL IFNg

M2: 20ng/mL IL-4 and IL-13



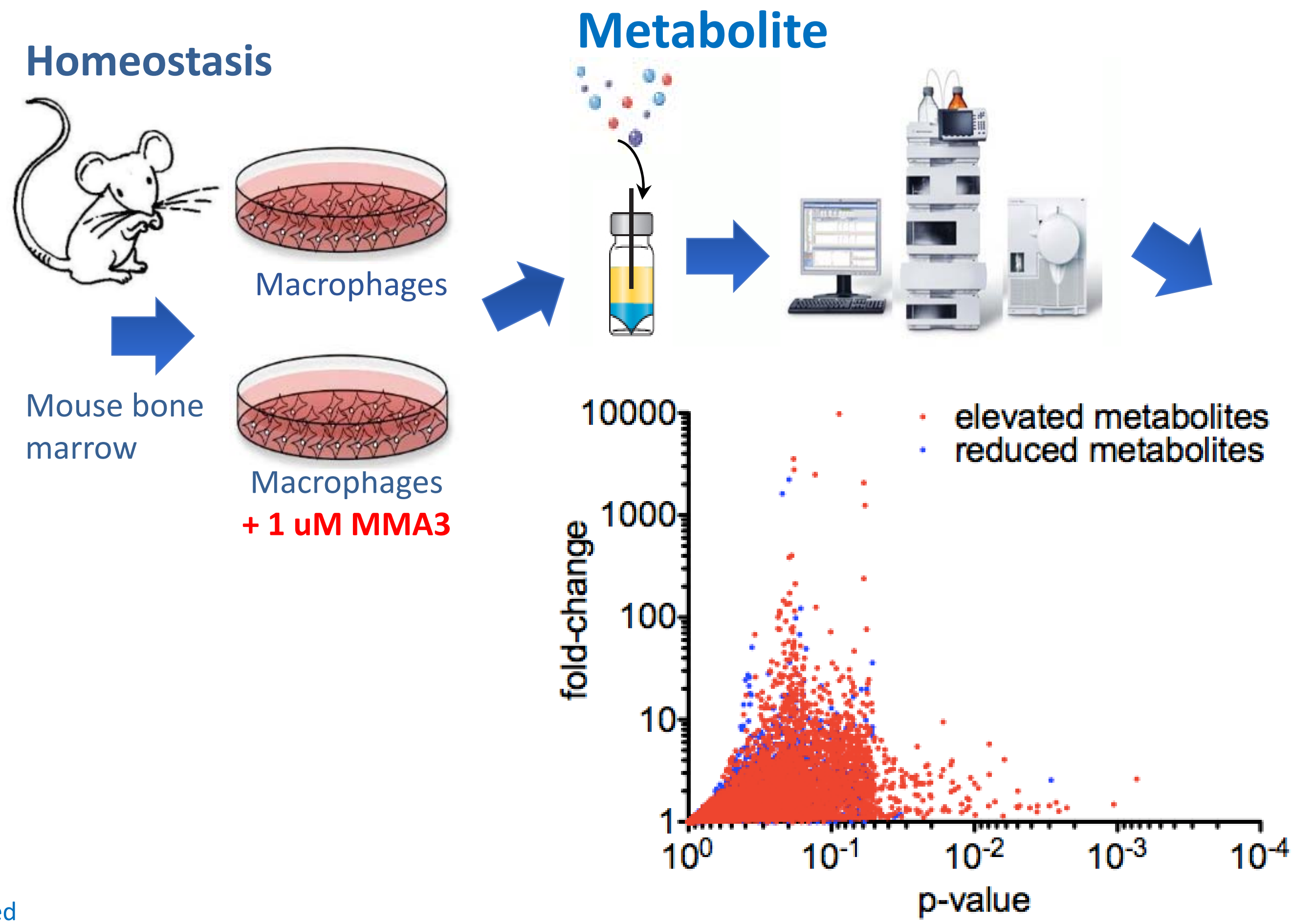
Signaling protein analysis





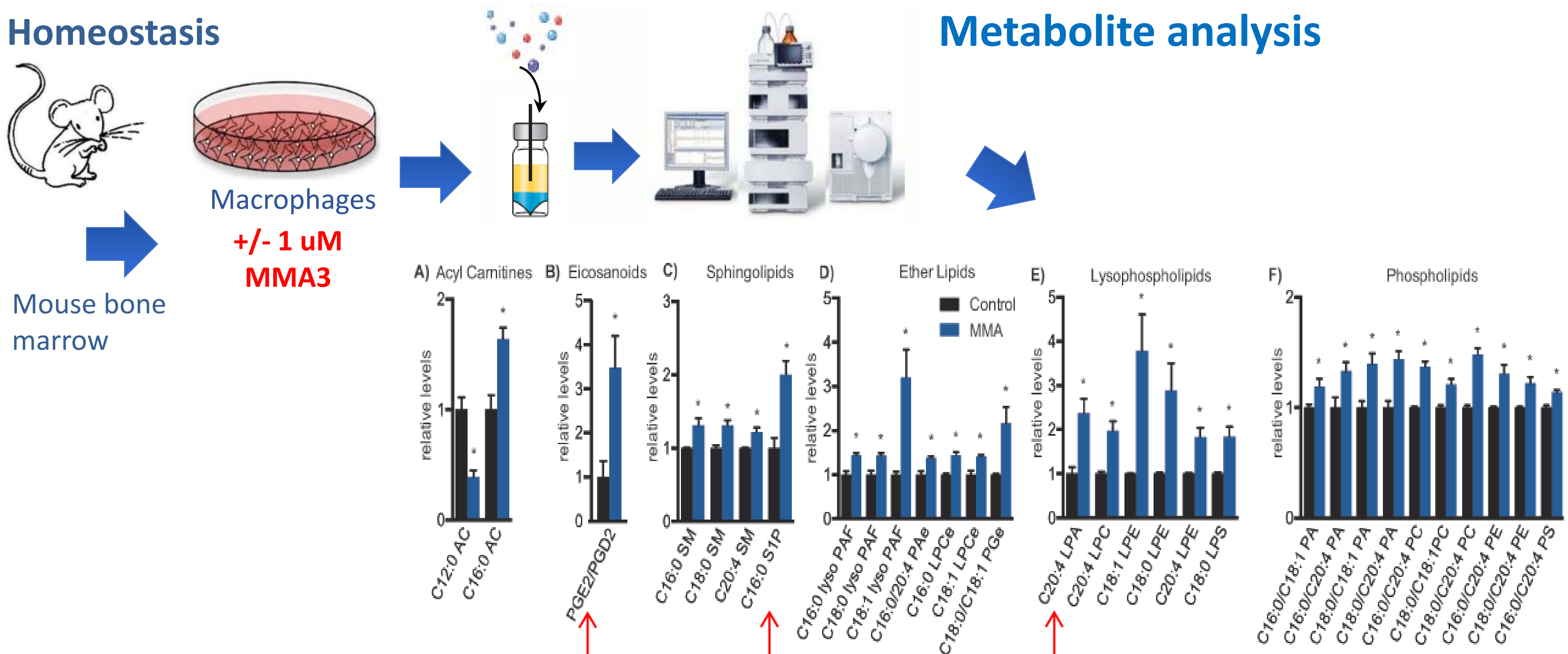
Sillé et al, unpublished

Arsenic alters signaling lipids expression



Sillé et al, unpublished

Arsenic alters signaling lipids expression

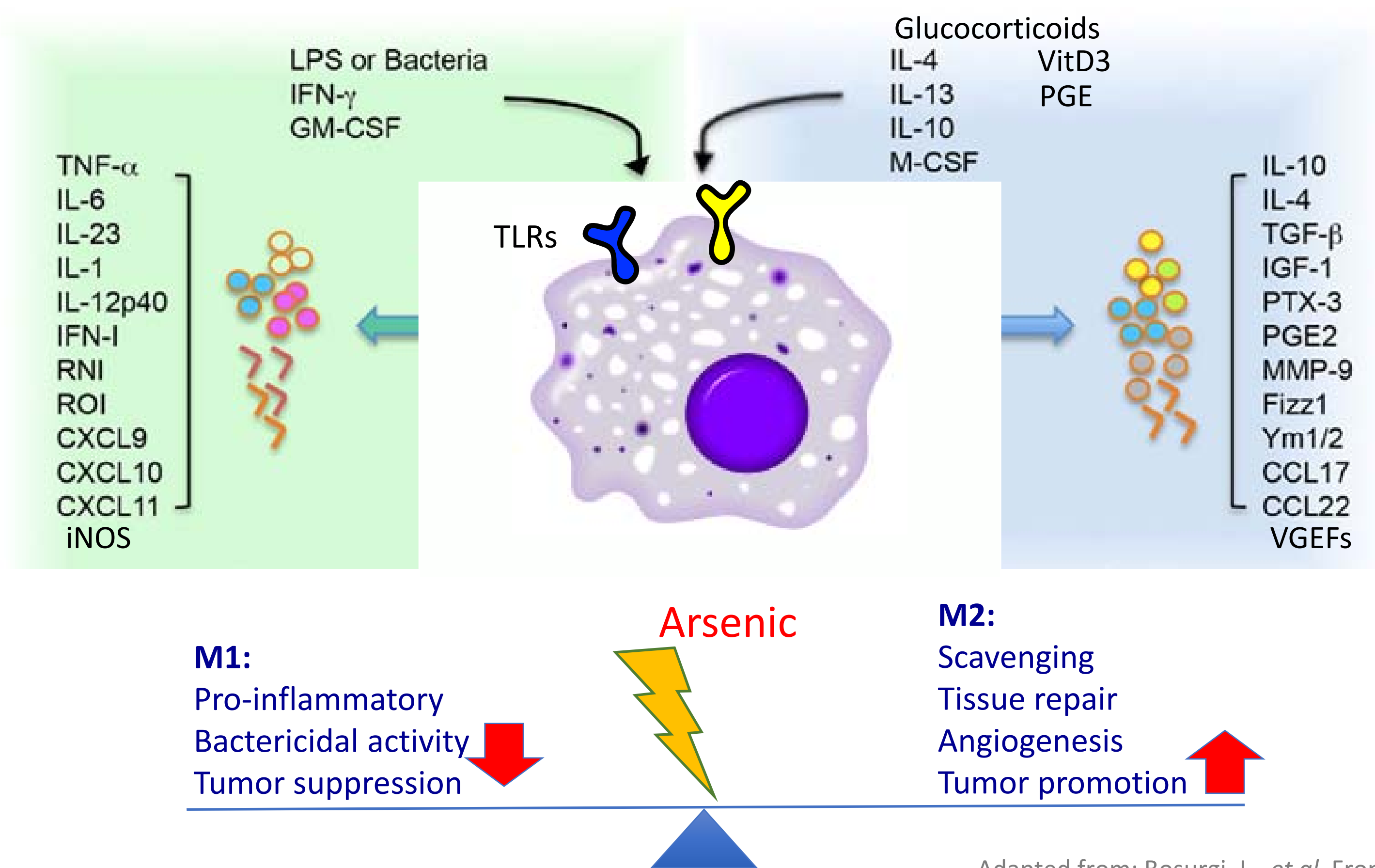


Pro-inflammatory and pro-tumorigenic signaling lipids

Sillé et al, unpublished

PGE2/PGD2 = Prostaglandins; C16:0 S1P = sphingosine-1-phosphate; LPA= lysophosphatidic acid.

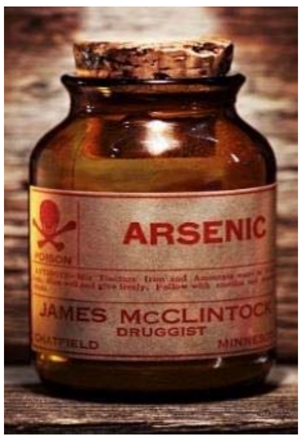
Arsenic & macrophages



Adapted from: Bosurgi, L., *et al*. Front. Immunol. 2011

Conclusions

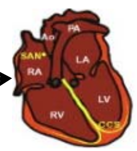
Environmental exposures:



Arsenic

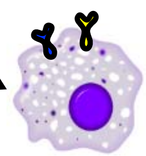
In utero & early life arsenic: increased cytokine profiles, and increased mortality from immune-related diseases even >40 years later.

In utero (*P9-birth)* >> Reduced pro-inflammatory cytokines

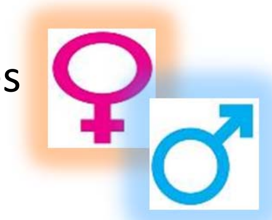


In utero (P9-birth), no effect on ischemia

In utero (P9-birth), no effect on airway resistance



iAs-exposed during differentiation vs mature macrophages >> M1/M2 skewing
>> Reduced pro-inflammatory cytokines
>> Increased pro-inflammatory lipids



Google Images, Wikimedia Commons

Thank you!

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