Utilizing ToxTracker to investigate the toxicological mode-of-action of METALs

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Annual Meeting of the GTA

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Exemplary metal(oid)s investigated with ToxTracker

Everyday Uses

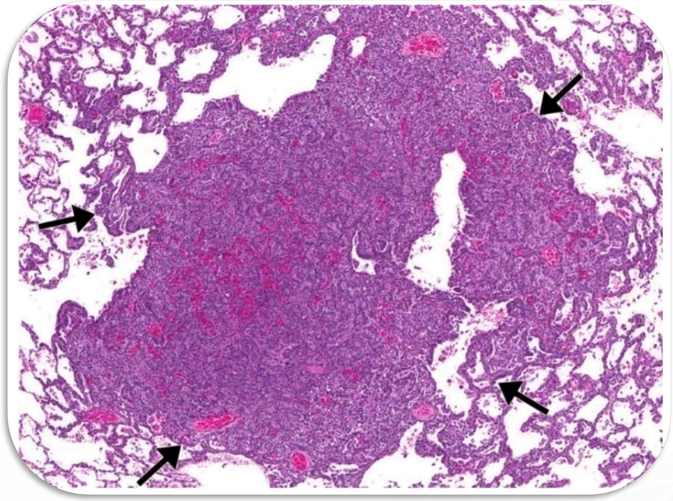
In Vitro Genetox

-ve mutation
+ve breaks
$Sb_2O_{3/5}$
$SbCl_{3/5}$

In Vivo Genetox

+ve for breaks

Primary rodent tumor site



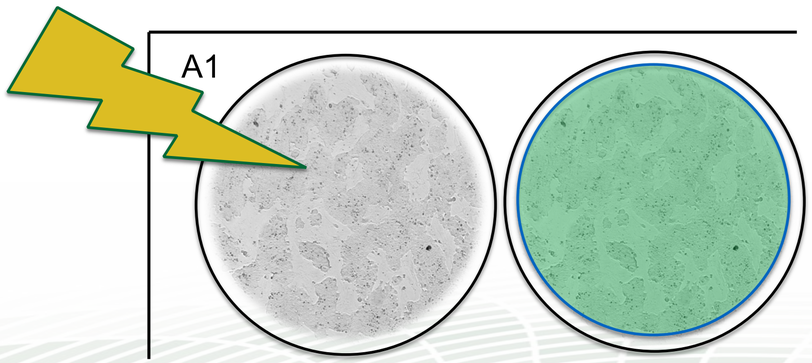
-ve mutation
+ve breaks
$CoCl_2$
$CoSO_4$

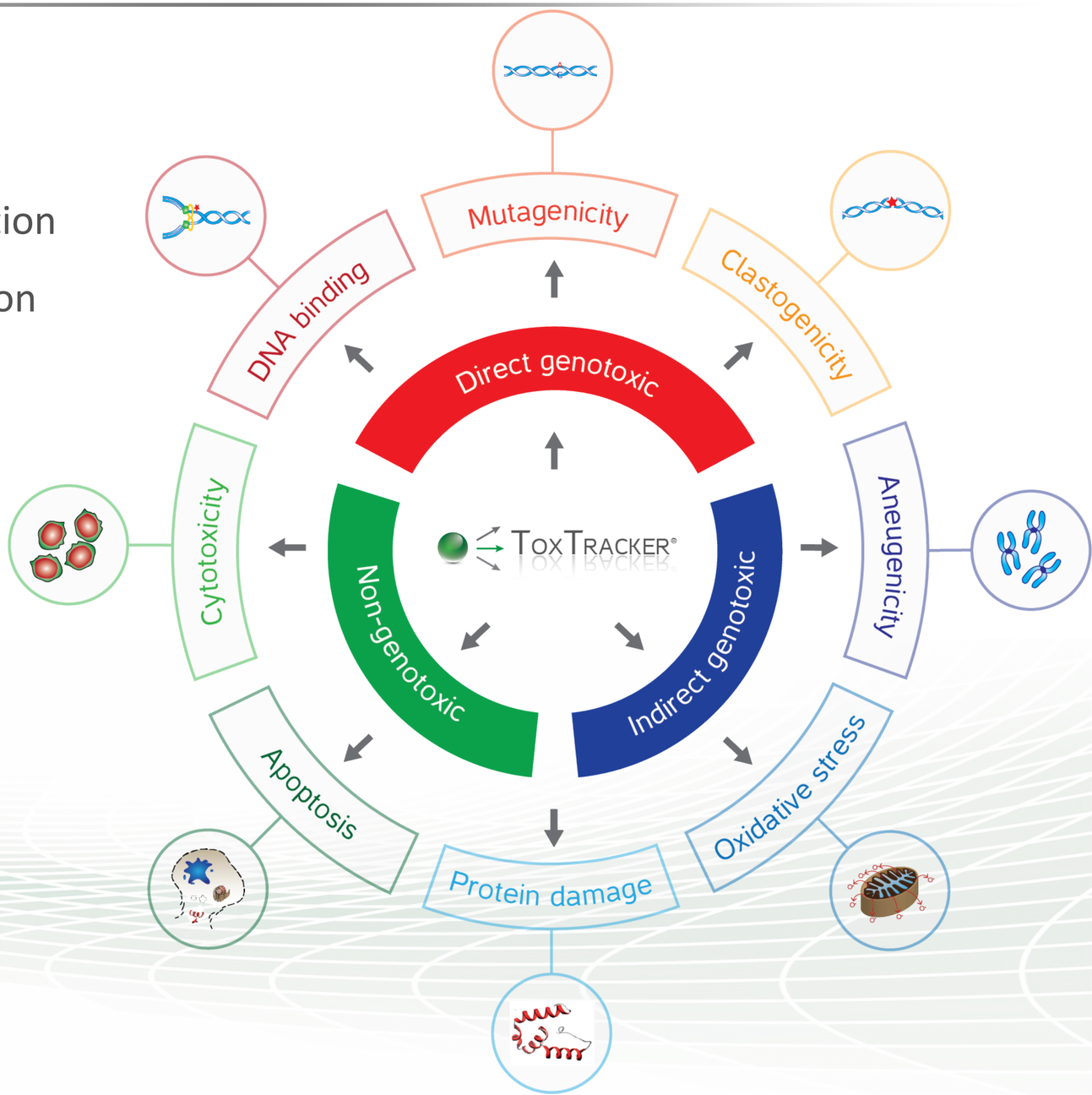
Conflicting
MN Studies

NTP TR581
Lung Adenoma
(5 mg/m^3 Cobalt)

What is ToxTracker?

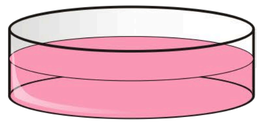
- A unique collection of *in vitro* genotoxicity assays
- Detects key carcinogenic events via GFP reporter activation
- Provides detailed insight into toxicological mode-of-action



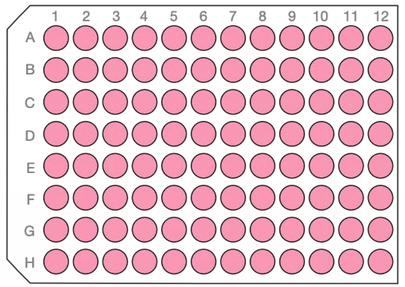


ToxTracker - Dose finding

Day 1

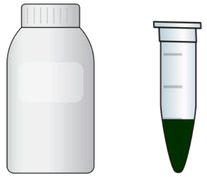


Wild type mouse stem cells

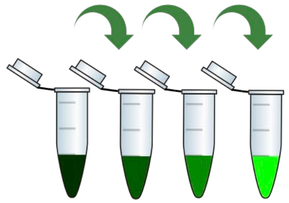


Seed cells in 96-wells plate

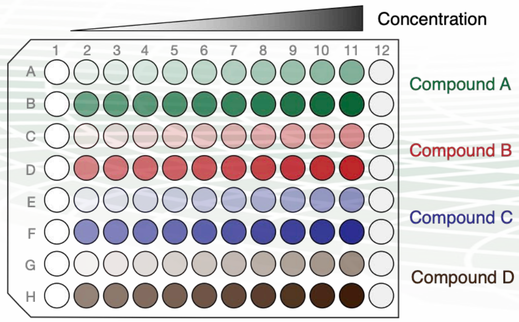
Day 2



Dissolve compound in DMSO or H_2O



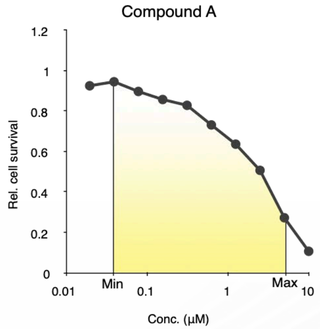
20 serial dilution (in 2-fold)



Expose cells to the compounds (24 h.)

Day 3

Cell count by flow cytometry

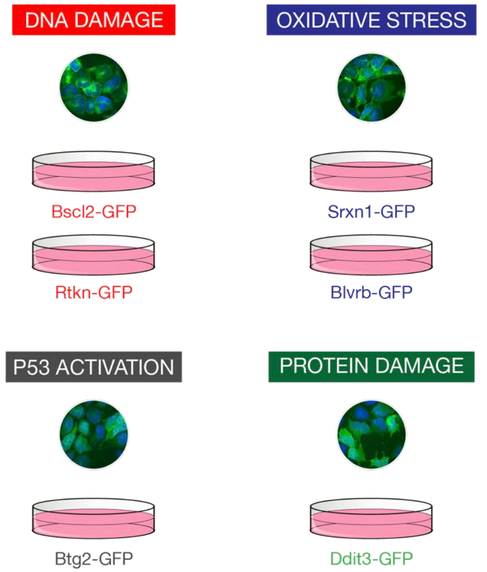


Dose range finding

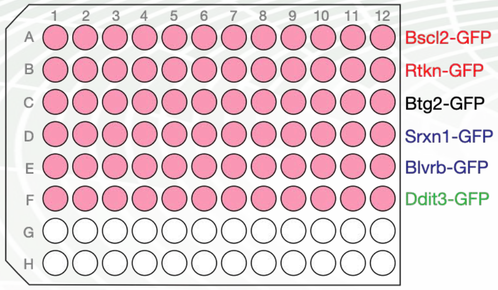
- Cytotoxicity
- Compound solubility
- Autofluorescence
- Metabolic activation (+S9)

ToxTracker - assay

Day 1



Six independent GFP reporter cell lines



Seed cells in 96-wells plate

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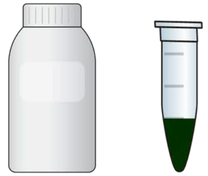
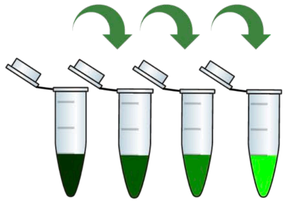
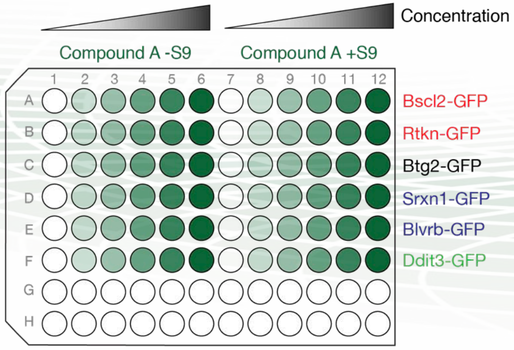


Dissolve compound in DMSO or H_2O



5 serial dilution (in 2-fold)



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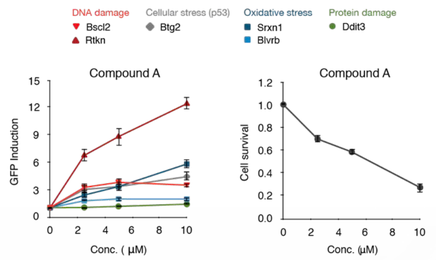
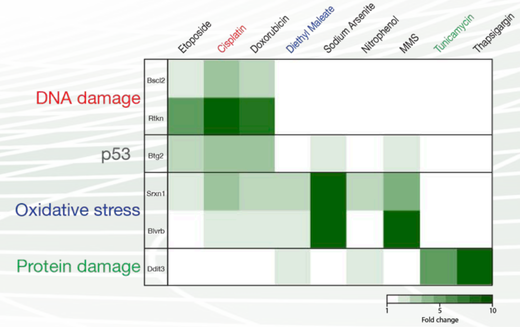
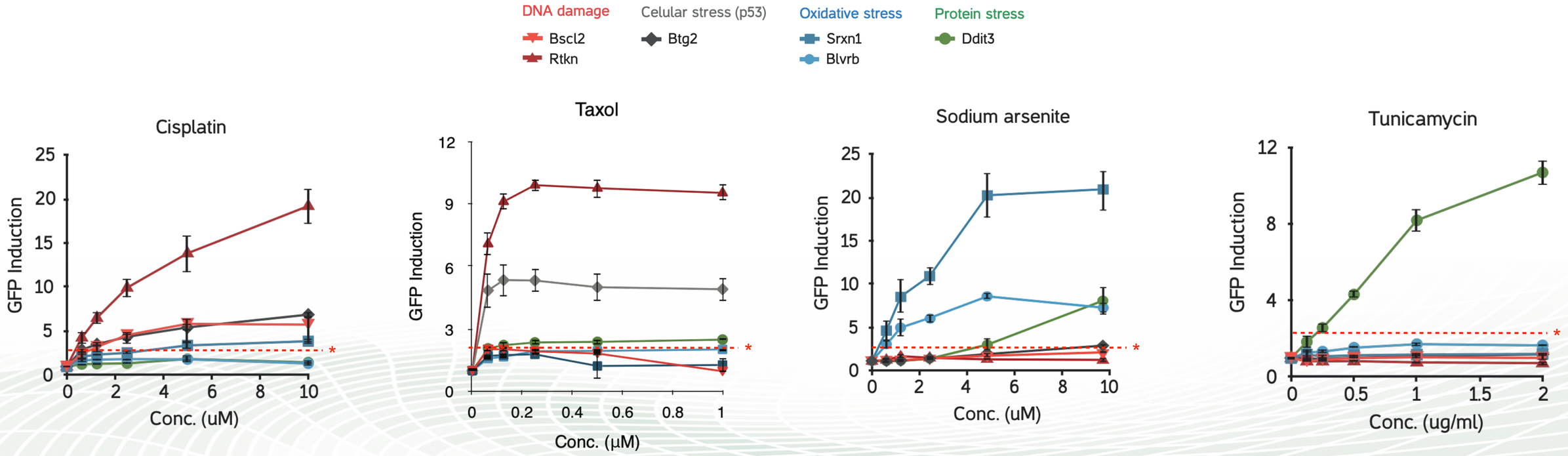




Data analysis using Toxplot software

Specificity of the ToxTracker reporters

- 6 unique report cell lines. Parental cells can be used for cell cycle and ploidy analysis (aneugenicity)
- 96/384-wells plates available, S9 compatable, express service optional.
- GFP induction and cell survival determined by flow cytometry



Ames pos.
MN pos.

Ames neg.
MN pos.

Ames neg.
MN pos.

Ames neg.
MN pos.

* A 2-fold GFP reporter induction is the threshold for a positive ToxTracker result.

Antimony – Stibnite the Egyptian mascara

REACH read across

Antimony Substances Evaluated in ToxTracker.

Name	Abbreviation	Formula	Valence
Antimony metal powder	Sb	Sb	0
Diantimony trioxide	ATO	Sb_2O_3	III
Diantimony trisulfide	ATS	Sb_2S_3	III
Antimony tris (ethylene) glycolate	ATEG	$Sb_2(C_2H_4O_2)_3$	III
Antimony triacetate	ATA	$SbC_6H_9O_6$	III
Antimony trichloride	ATC	$SbCl_3$	III
Antimony potassium tartrate	APT	$Sb_2K_2C_8H_4O_{12}$	III
Sodium hexahydroxoantimonate	SHHA	$NaSb(OH)_6$	V
Potassium hexahydroxoantimonate	PHHA	$KSb(OH)_6$	V
Sodium antimonate	SA	$NaSbO_3$	V
Diantimony pentoxide	APO	Sb_2O_5	V
Antimony pentachloride	APC	$SbCl_5$	V

Boreiko et al, Mutat Res Genet Toxicol Environ Mutagen 865:503333, 2021

i2a International Antimony Association

Generally, higher solubility in culture media and hence, more cytotoxic.

Generally, less soluble in culture media and hence, less cytotoxic.

Other ways to assess in vitro exposures?

Antimony – Stibnite the Egyptian mascara

Solubility and Toxicity of antimony compounds. ICP- MS according to ISO 17294-2

Substance	Formula Weight	Dissolved Sb (µg/mL)[1]		% Dissolved[2]	LC50[3]
Sb metal	122	54		54 %	0.54
ATO	292	1.5		1.8%	0.72
ATS	340	5.6	< 1.3 ug/mL tolerated in culture	7.8 %	1.0
ATEG	424	44		76 %	0.51
ATA	299	34		83 %	1.3
ATC	228	49		92 %	0.38
APT	614	32		81 %	0.46
SHHA	247	45		91%	>45
PHHA	263	38	> 2.4 ug/mL tolerated in culture	82%	>38
SA	193	3.0		4.7 %	>3.0
APO	324	5.8		7.7 %	>5.0
APC	299	30		74 %	2.4

[1] Dissolved Sb concentration resulting from overnight incubation of 100 µg/mL of test substance in cell culture medium.

[2] % Dissolved substance calculated from dissolved Sb concentration and formula weight.

[3] LC50 in µg/mL of Sb concentration.

Trivalent are (generally) more cytotoxic than pentavalent.

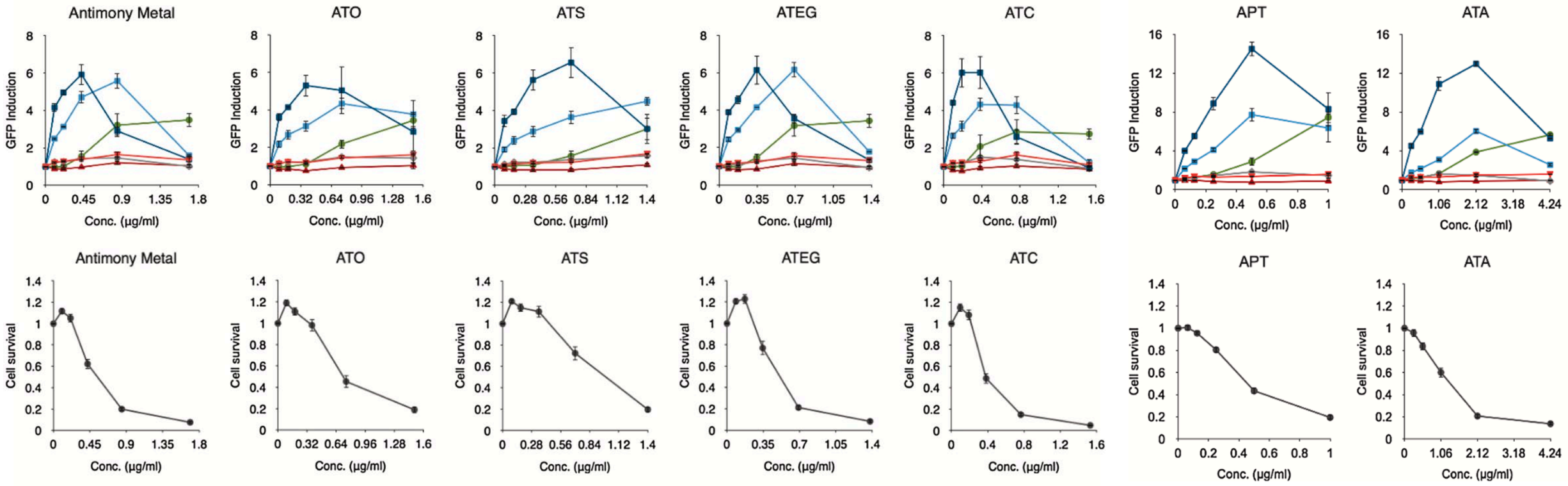
Antimony metal and Sb(III) ToxTracker Results

DNA damage
- Bscl2
- Rtkn

Cellular stress (p53)
- Btg2

Oxidative stress
- Srxn1 1
- Blvrb 2

Protein damage
- Ddit3 3



Antimony metal and Sb(V) ToxTracker Results

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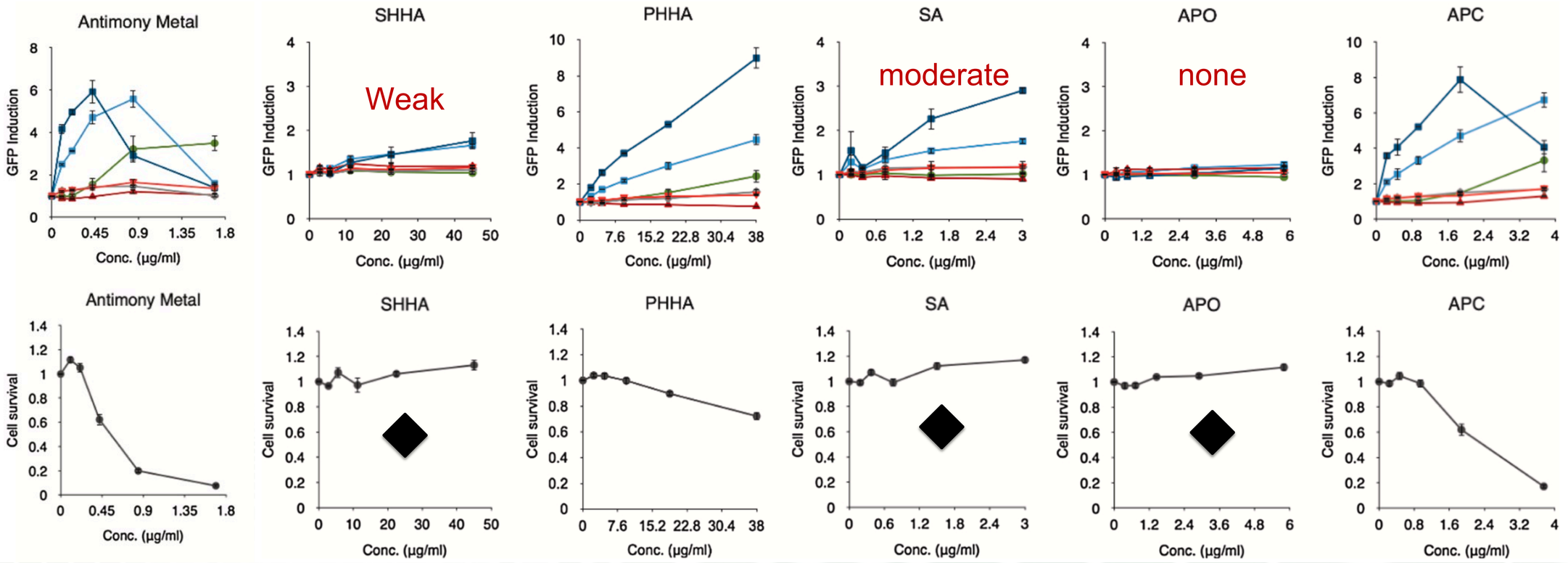
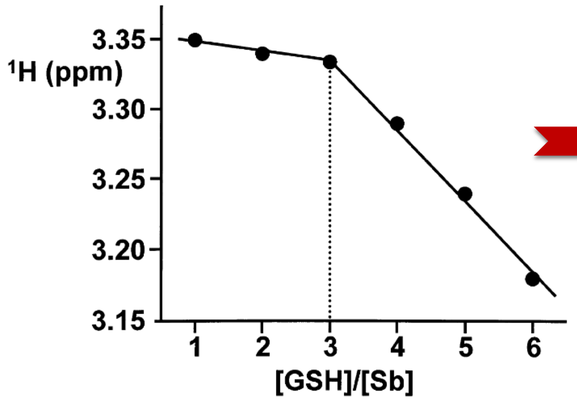


◆ Limited exposures

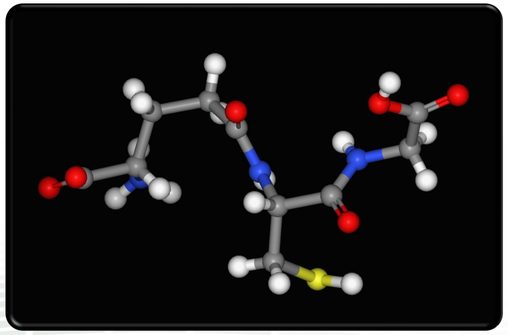
Antimony – Mechanism(s) of Carcinogenesis

- Srxn1, Blvrb and Ddit3 inhibition suggest oxidative eustress followed by oxidative distress.
  - Sulfiredoxin (Nrf2 driven), biliverdin reductase (oxidoreductase activity), and unfolded protein response (respectively)
- Layer in other details from peer reviewed literature – AOPish.
- Process!

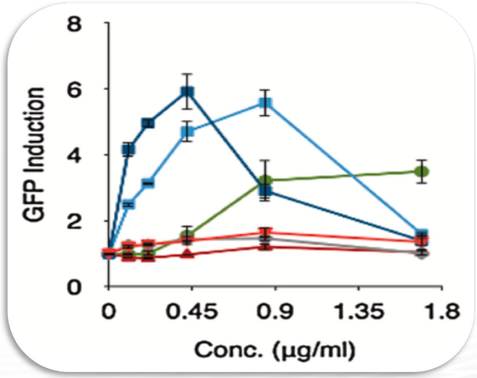
Sb displaces SH
(NMR)



Eur. J Biochem 267, 5450-5457, 2000



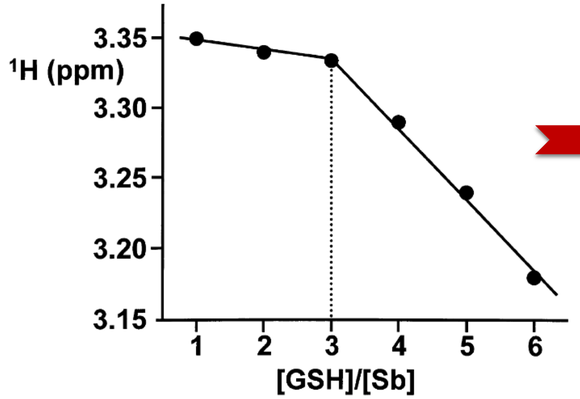
Glutithione
Thiol Displacement



↑ Oxidative Stress
↑ Protein Damage

Once a critical threshold is reached, oxidative DNA lesions occur

Antimony – Mechanism(s) of Carcinogenesis (Draft)



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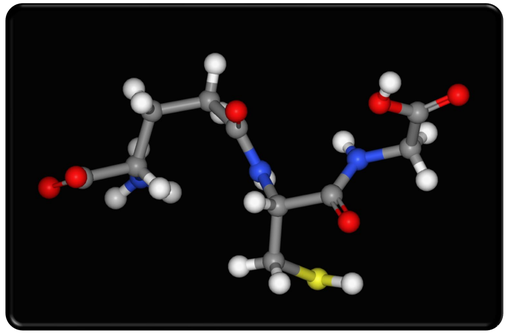

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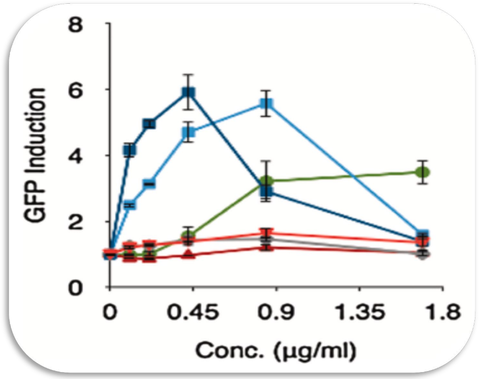

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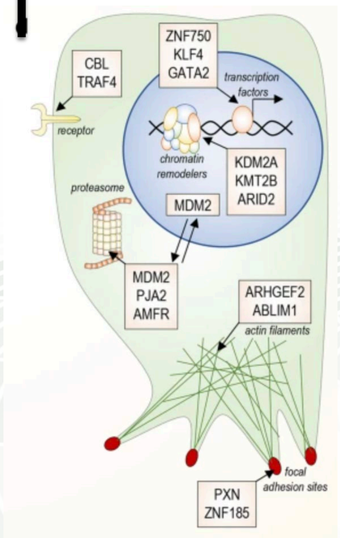
↑ Protein Damage

Once a critical threshold is reached, oxidative DNA lesions occur

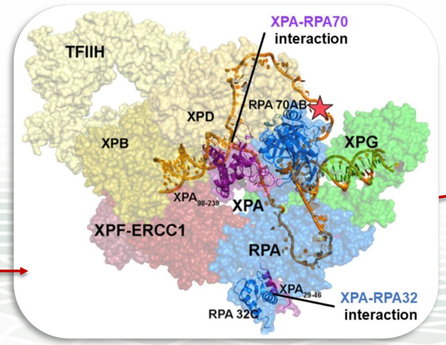
Increasing Concentration



Disruption of zinc finger proteins

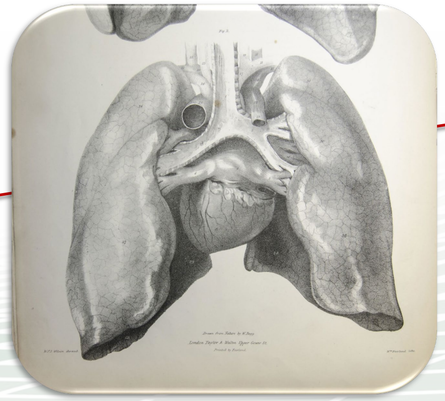
XPA

NER Impacted



Chem Res tox 23(7)1175-83, 2010.

Tumorigenesis



Oxidative stress higher in organs with higher O_2

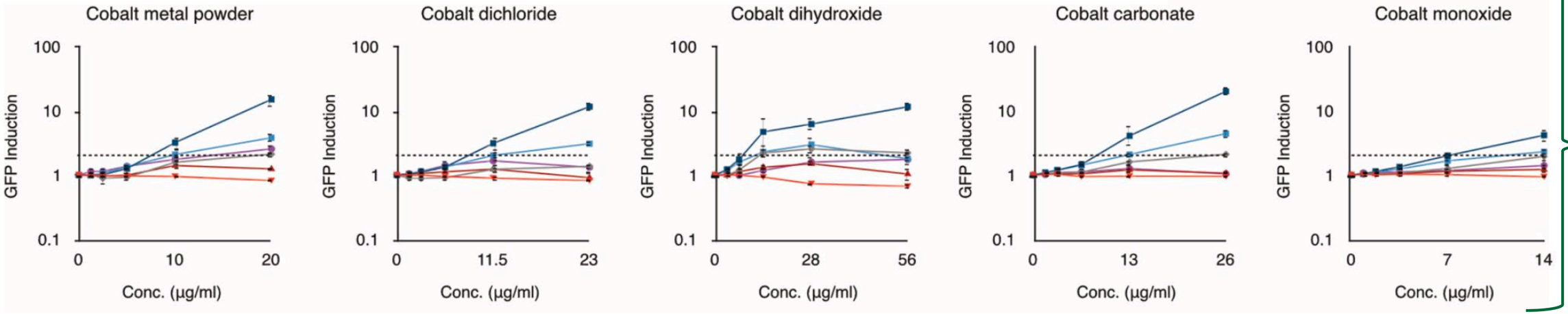
Cobalt – when beer wasn't foamy

DNA damage
- Bscl2
- Rtkn

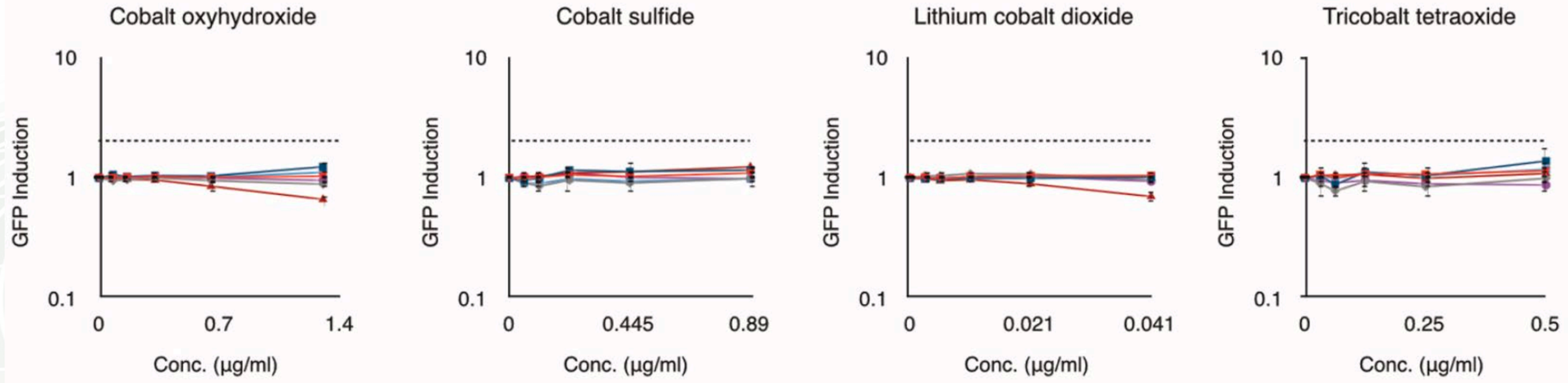
Cellular stress (p53)
- Btg2

Oxidative stress
- Srxn1
- Blvrb

Protein damage
- Ddit3

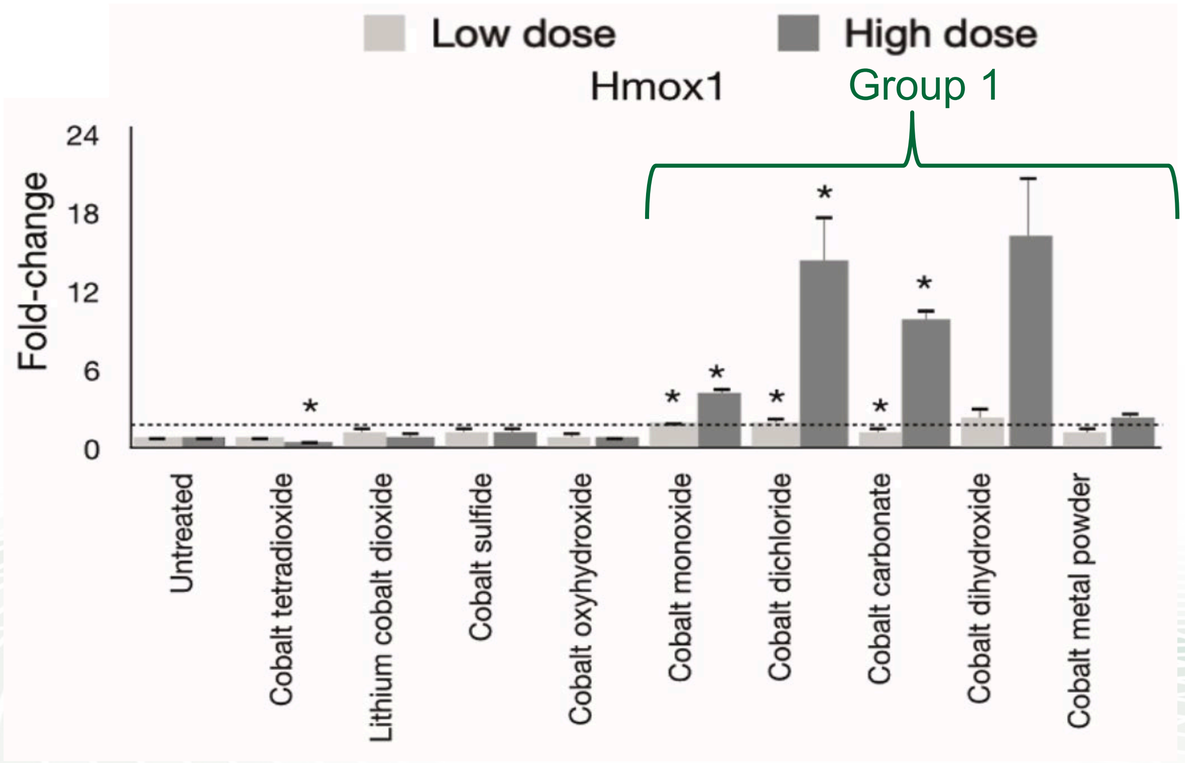


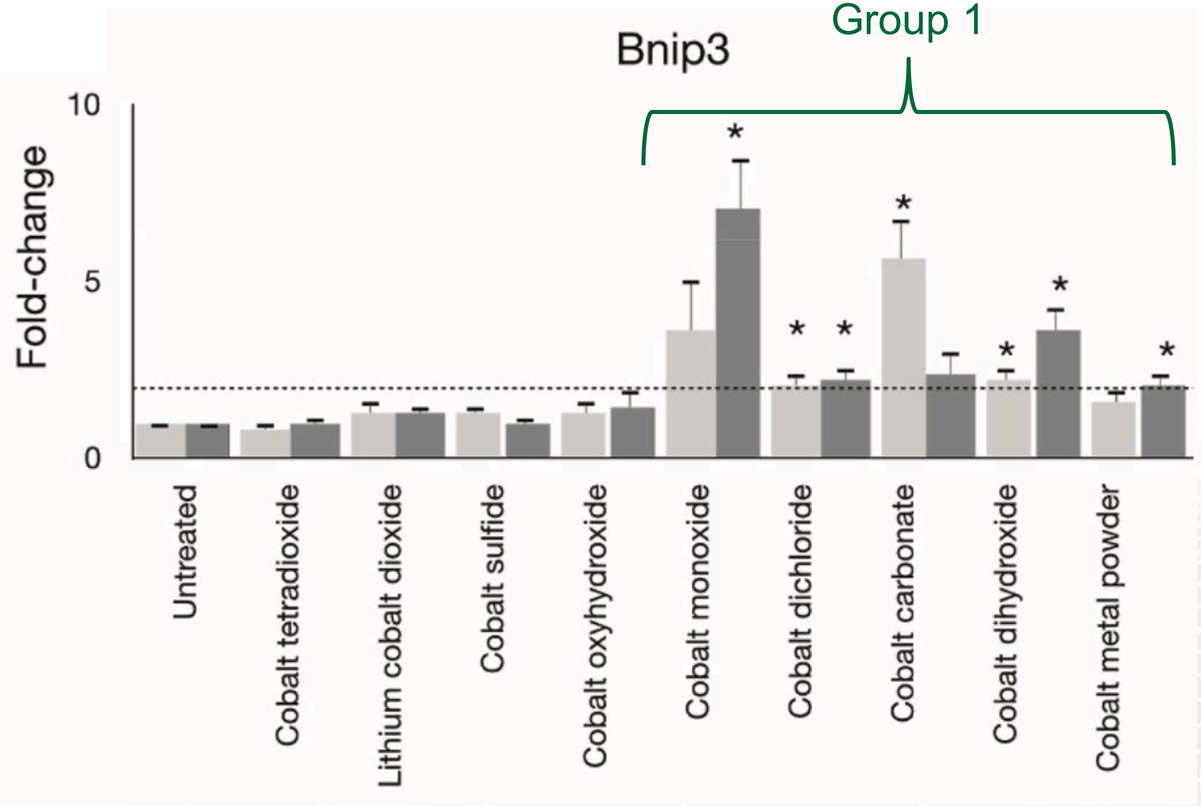
Group 1



Cobalt – “Group 1” Mechanism(s) of Carcinogenicity

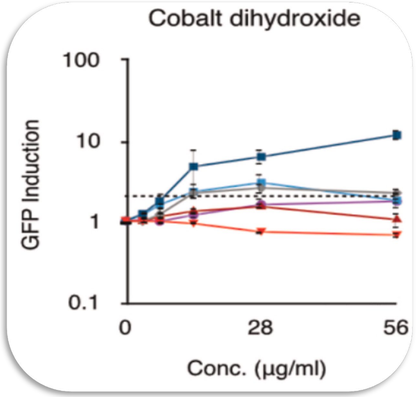
- Antioxidant response is again part of a process. .
- HIF stabilization and hypoxia promotion has also been implicated and correlates with this group of Co materials.
- qPCR on Hmox1, Ddit4, Slc2A1, and Bnip3.





Cobalt – Mechanism(s) of Carcinogenicity (Draft)

Intracellular Co dissociates



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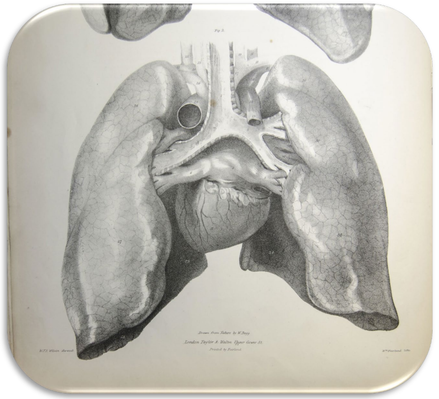

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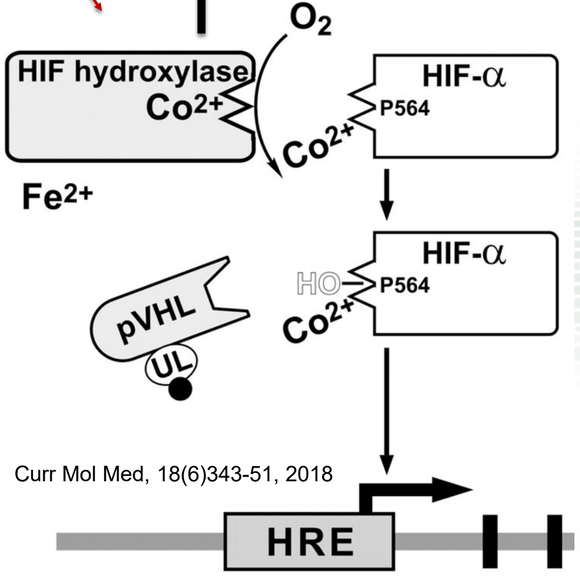
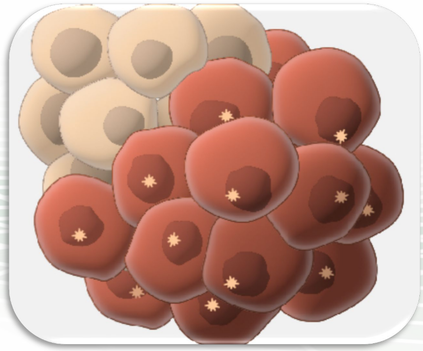
Increasing Concentration



Curr Mol Med, 18(6)343-51, 2018

HIFalpha is stabilized



Oncogene Activation
-VEGF
-Met
-HGF

Tumorigenesis

Summary

- Sb and Co induce oxidative stress but likely initiate and progress carcinogenesis via multiple pathways.
  - Unique In vivo considerations - Inflammation, hormonal effects, hypoxia, etc….
- Mode of action is never (IMO) as simple as one thing. It may scream the loudest, but perhaps we are all victim of the WYSIATI fallacy.
- NAMs that can detect hallmarks of carcinogenicity are useful when understanding toxicological mechanisms of action and can help build AOPs with temporal and dosimetry information ;)

- And a very big **THANK YOU** to the Toxys team and collaborators for generating the data, and for your kind attention! It’s great to be back at the GTA in person!!