

# Genotoxicity assessment of Antimony compounds using the ToxTracker assay

# International Antimony Association

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#### Introduction

DNA lesions strand breaks

\*\*Kuroda 1991, SCE = Sister Chromatid Exchange

The metalloid Antimony (Sb) is commonly used as a compound in flame retardants and as hardener for lead. There is still a lot of discussion on the genotoxicity of antimony compounds. Both trivalent and pentavalent compounds are generally negative in non-mammalian genotoxicity assays, but in mammalian test systems trivalent compounds have generated some positive results, while pentavalent compounds remained negative.

#### Objective

To investigate the genotoxic potential of Antimony compounds and how this correlates with the valency of the Antimony or the solubility of the compound, we tested the genotoxic potential and cytotoxicity of a group of 9 antimony compounds (Sb-metal, 4 trivalent and 4 pentavalent) in the ToxTracker assay.

#### Conclusions

Tunicamycin

Aflatoxin B1\*

\* only in presence of S9

- None of the tested antimony compounds showed genotoxic properties in the ToxTracker assay

ToxTracker results

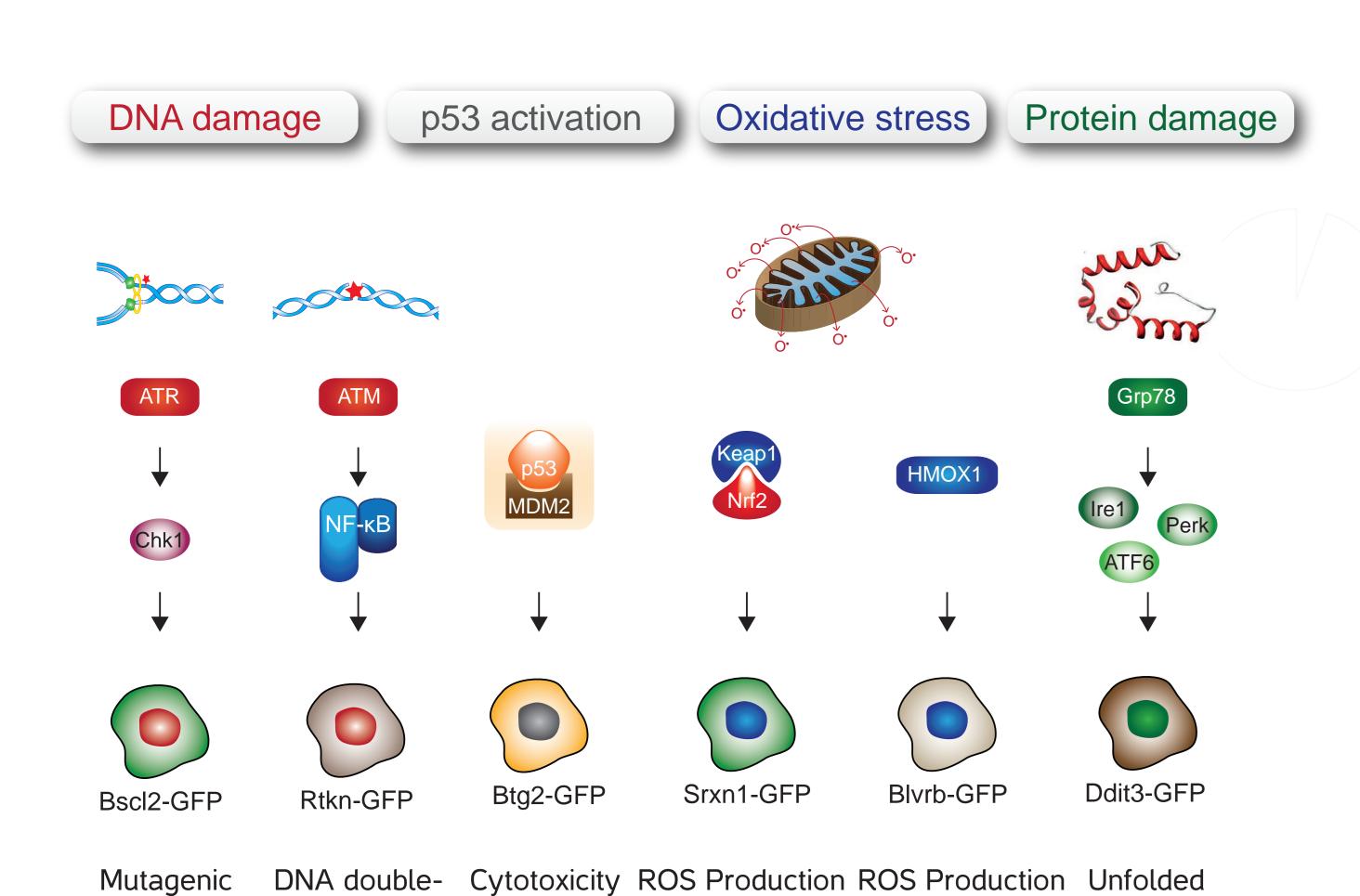
- Seven out of nine compounds induced significant levels of oxidative stress
- Valency correlates with the cytotoxicity of Antimony compounds

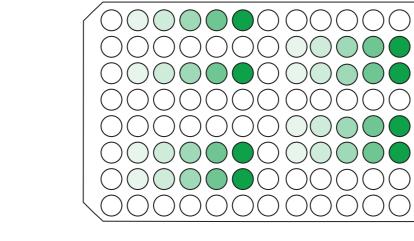
### The ToxTracker reporter assay

Stem cell-based reporter assay consisting of 6 GFP reporter cell lines

Developed for *in vitro* carcinogenicty hazard screening

Provides insight into mechanisms of genotoxicity





Exposure of reporter cell lines in 96-wells plates, in absence or presence of a bioactivation system (S9 liver extract)

Cytotoxicity and GFP reporter detection by flow cytometry

Quantitiavite data analysis using Toxplot software

# Solubility 5+ Cytotoxicity 3+ Valency (3+ or 5+) of Antimony compounds correlates with cytotoxicity, while solubility does not. Valency (3+ or 5+) of Antimony compounds correlates with cytotoxicity, while solubility does not. A) Selected Antimony compounds were tested in the ToxTracker assay and the LC50 values were calculated based on the cytotoxicity data (panel B below) Solubility Solubility

trivalent agents are more cytotoxic than the pentavalent agents, as has been observed previously.

B) The cytotoxicity of the compounds does not correlate with their solubility. The solubility of the compounds in medium was determined using HPLC in duplicate

Test compounds
oxicity, while solubility

Antimony
Sodium antimonate
Diantimony pentoxide
Diantimony trioxide
Na-hexahydroxoantimonate
Antimony-3-sulfide
Antimony-3-sulfide
Antimony-3-chloride
Antimony-5-chloride
Controls
Cisplatin
Diethyl maleate

Positive (>2-fold induction)
Weak positive (1.5 to 2-fold induction)
Negative (<1.5-fold induction)
Not tested in this study

The validity of the ToxTracker assay results was confirmed by exposure to various reference compounds and assessing the specificity of the different reporter cell lines.

None of the 9 antimony compounds induced the Bscl2-GFP genotoxicity reporter that is associated with generation of pro-mutagenic DNA lesions or the Rtkn-GFP genotoxicity reporter for DNA strand breaks.

Antimony, Sodium antimonate, Diantimony trioxide, Antimony-3-sulfide, Antimony glycolate, Antimony-3-chloride and Antimony-5-chloride all induced the Srxn1-GFP and Blvrb-GFP oxidative stress markers significantly.

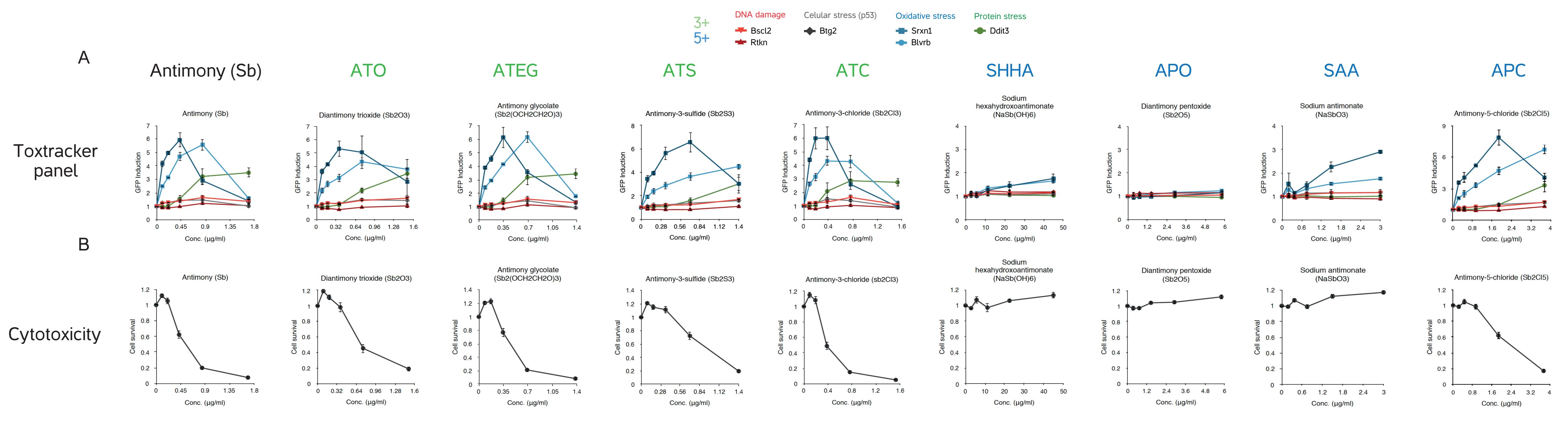
The unfolded protein response was activated by Diantimony trioxide, Antimony-3-sulfide, Antimony glycolate, Antimony-3-chloride and Antimony-5-chloride at cytotoxicity levels <75%.

There was no indication in ToxTracker for metabolic activation of any of the antimony compounds.

## Antimony compounds in this study

Valency 0	Acronym Sb/Sb metal		Physical form Powder	Ames test*  Equivocal positive	SCE in V79**
3+	ATO	Diantimony trioxide	Powder	Negative	Weak positive
	ATEG	Antimony tris(ethylene glycolate	) Powder	_	
	ATS	Antimony sulfide	Powder	_	
	ATC	Antimony trichloride	Powder	Negative	Weak positive
5+	SHHA	Sodium hexahydroxoantimonate	Powder	_	_
	APO	Diantimony pentoxide	Powder	Negative	Negative
	SAA	Sodium antimonate	powder	_	_
	APC	Antimony pentachloride	liquid	Negative	Negative

# Cytotoxicity of Antimony compounds correlates with oxidative stress and protein damage



Trivalent antimony compounds, sodium antimonate and antimony-5-chloride activate the markers for oxidative damage. The trivalent antimony compounds and Antimony compounds. As a positive control, GFP reporter cells were exposed to increasing concentrations of the DNA damaging agent cisplatin, the oxidative stress-inducing agent diethyl maleate and the UPR-activating compound tunicamycin (data shown in results summary). GFP inductions above 2-fold for the exposure using flow cytometry.