INFLUENCE OF ANTIOXIDANT CAPACITY ON MICRONUCLEUS INDUCTION BY CIGARETTE SMOKE IN VARIOUS MAMMALIAN CELL LINES

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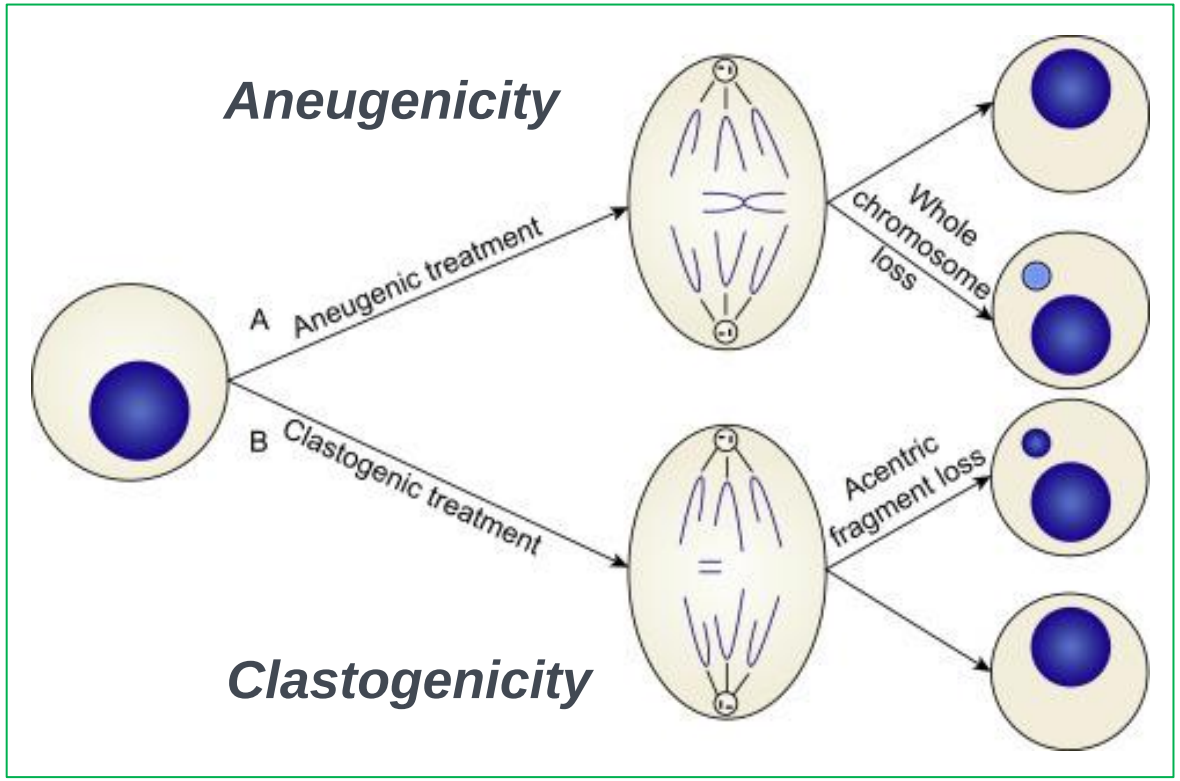
Scientific Product Assessment Center
Japan Tobacco Inc.

October 25, 2021

Outline

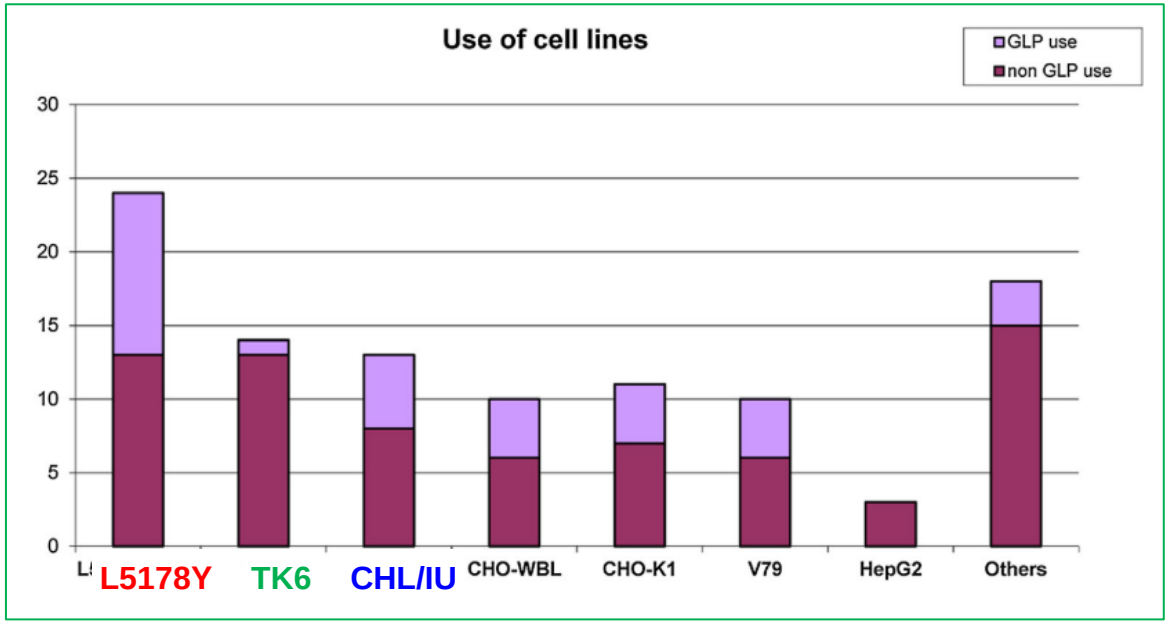
Introduction:
Cell Lines in *In Vitro* Micronucleus (MN) Assay



D'Costa A et al,: In: Advances in Biological Science Research 2019: 291-301.

Representative Cells in OECD Test Guideline 487

Primary	Human	Lymphocyte
Cell lines	Human	**TK6**
	Chinese hamster	**CHL/IU** CHO, V79
	Mouse	**L5178Y**



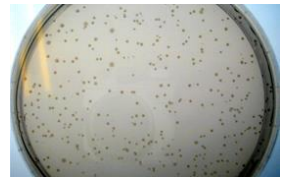
*Lorge E. et al, Mutat Res 2016, **809**:1-15.*

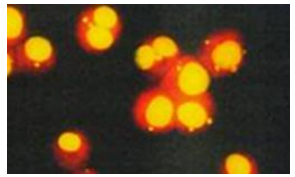
Introduction:
Genotoxic Assessment of Tobacco Products

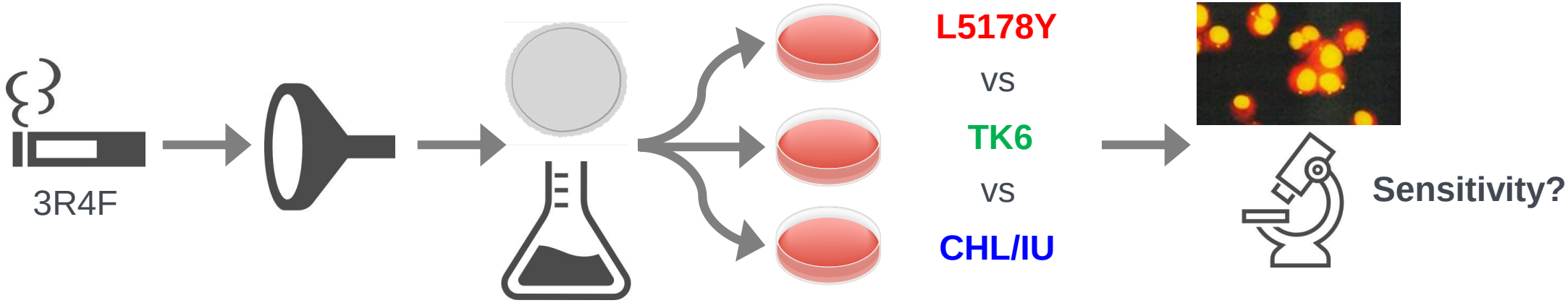
IVT - In Vitro Toxicity Testing - 2002

in vitro battery

- Bacterial mutation test (Ames test)
- *In vitro* mammalian cell test
  - MN assay
  - Chromosome aberration
  - Mouse lymphoma assay
- Neutral red uptake cytotoxicity







Genotoxic and Cytotoxic Responses of L5178Y, TK6, and CHL/IU to 3R4F

Test Items

3R4F

↓ ISO 3308

Total particulate matter (TPM)

↓ DMSO

Solution of 20-mg TPM/mL

MN assay in L5178Y, TK6, & CHL/IU

Cell culture

↓

3-h Treatment

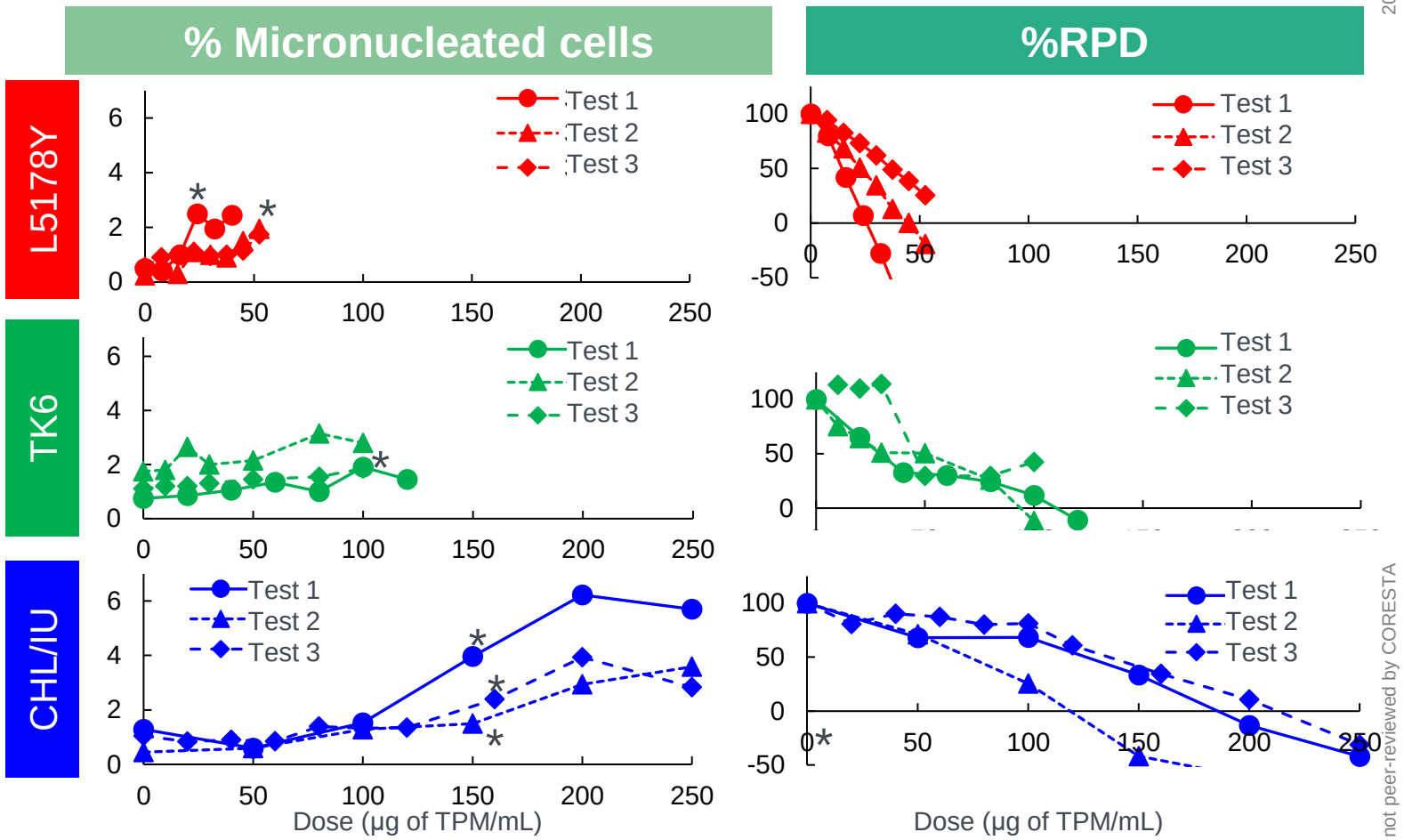
↓

21-h Recovery

↓

MN observation

Relative Population Doubling (RPD)



The most sensitive cell line to MN induction and cytotoxicity was L5178Y, followed by TK6, and the most resistant was CHL/IU cells.

** Significantly different from the DMSO control group (P < 0.05, Fishers' exact test).*

Comparison of the Total Antioxidant Capacity and Cellular GSH Between Each Cell Line

Cigarette Smoke

Reactive oxygen species (ROS)
Free radicals

Cellular Antioxidant Capacity

Non-enzymatic	*Enzymatic*
✓ **Glutathione**	✓ SOD
✓ Vitamin A	✓ Catalase
✓ Vitamin C	✓ GST
✓ Vitamin E	✓ GPx

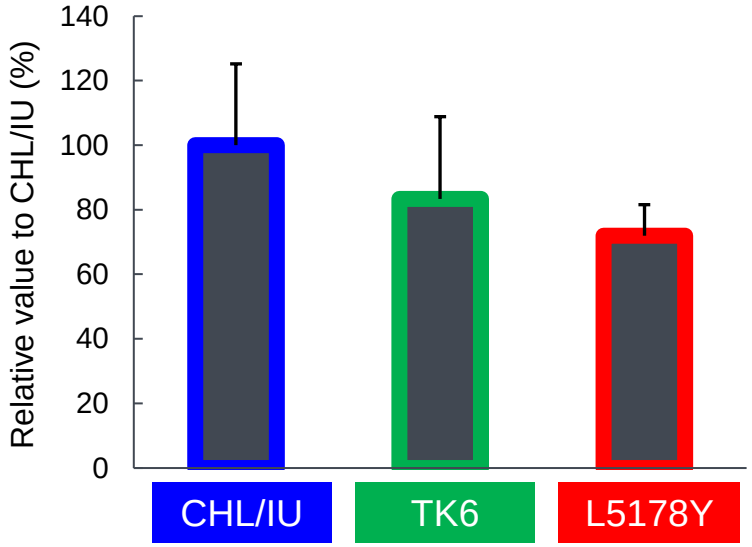
CHL/IU TK6 L5178Y

Total Antioxidant Capacity

Cell culture for 24 h at 37 °C under 5 % CO_2

Cell lysis → Total Antioxidant Capacity Colorimetric Assay Kit (BioVision)

Cell count

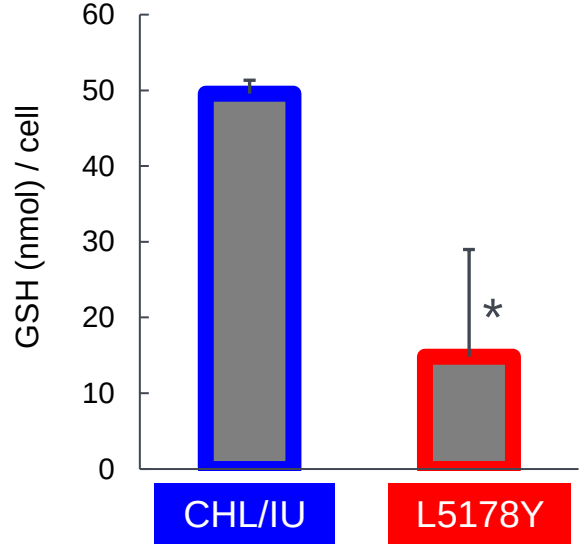


Reduced Glutathione Assay

Cell culture for 24 h at 37 °C under 5 % CO_2

Cell lysis → GSH-Glo Glutathione Assay Kit (Promega)

Cell count



Measuring the total antioxidant capacity and the total amount of glutathione per cell revealed that they were significantly higher in CHL/IU cells than in L5178Y cells.

** Significantly different from the CHL/IU ($P < 0.05$, paired t-test).*
Error bars indicate the standard error (N=3).

Effect of NAC and BSO on Genotoxicity and Cytotoxicity Induced by Each Sample in CHL/IU

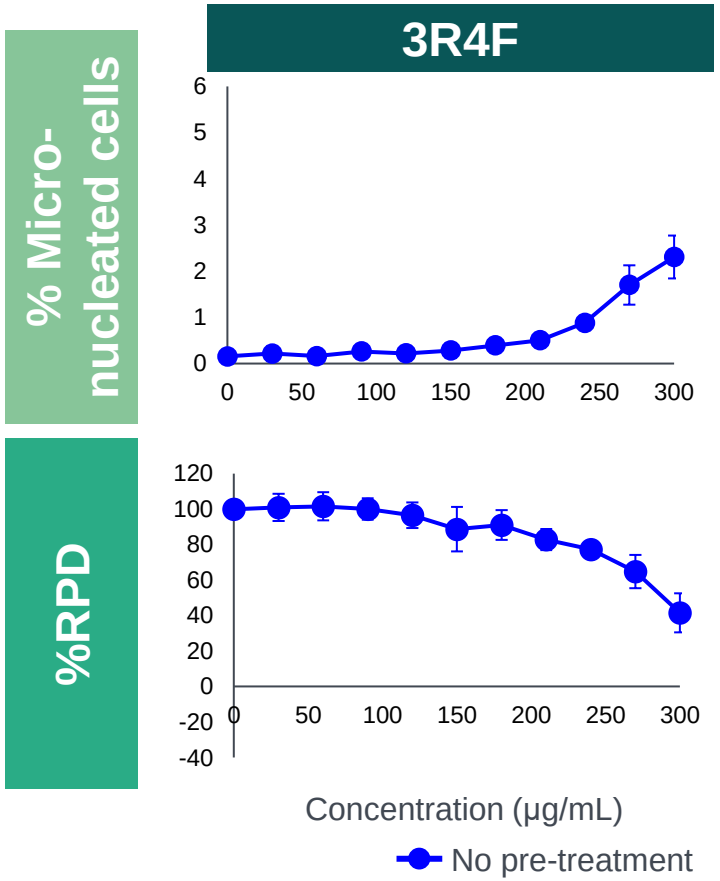
3R4F **Allyl isothiocyanate**

↓ *Indirect*

ROS, free radicals, etc.

↓

MN Induction (Cytotoxicity)



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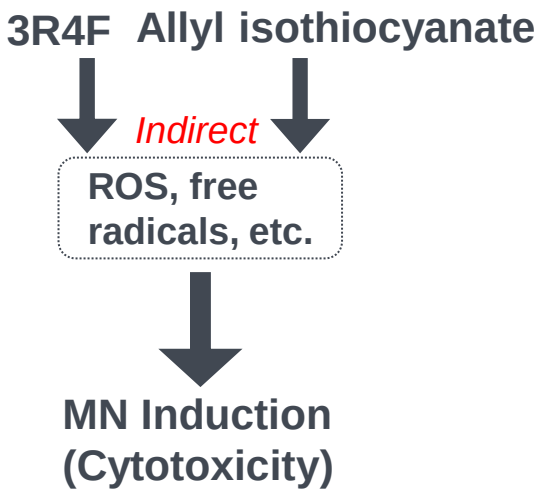

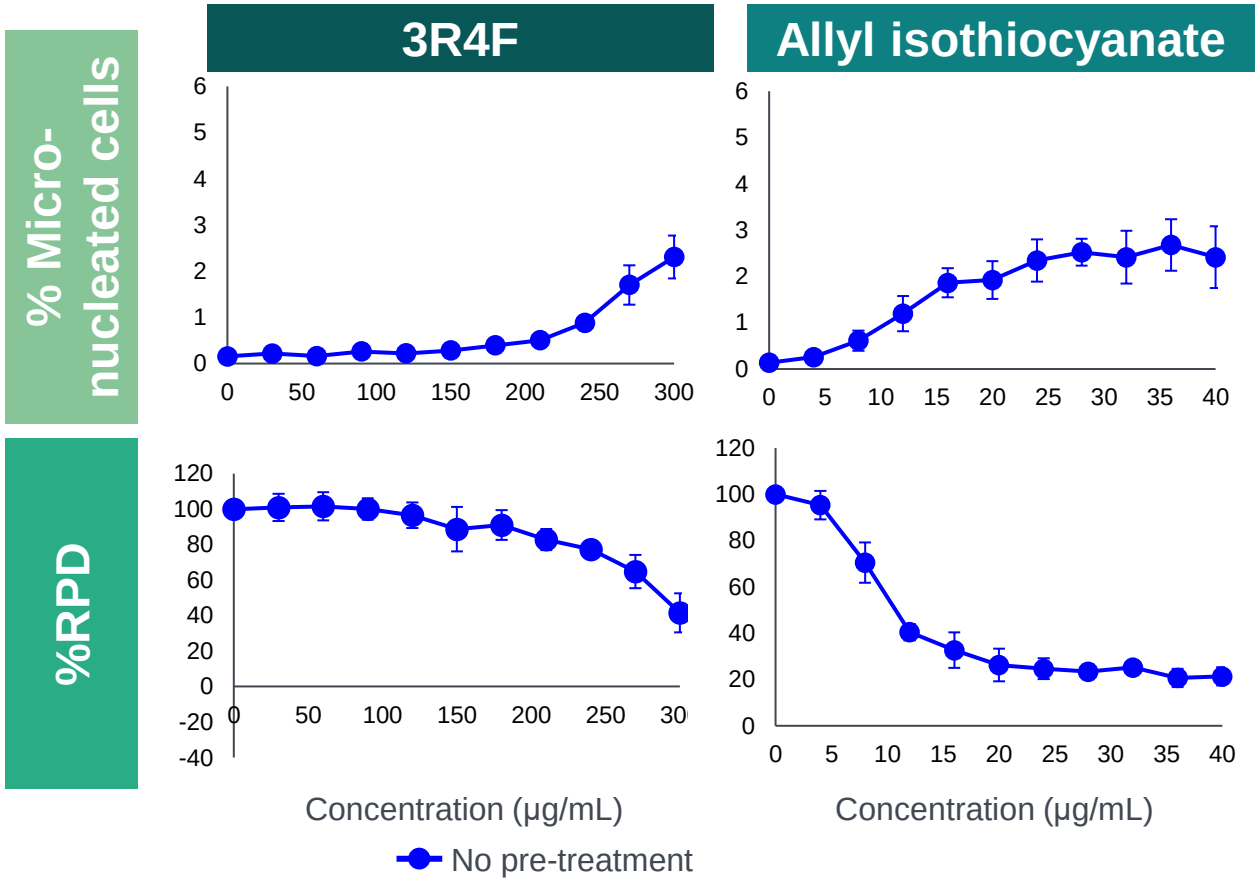

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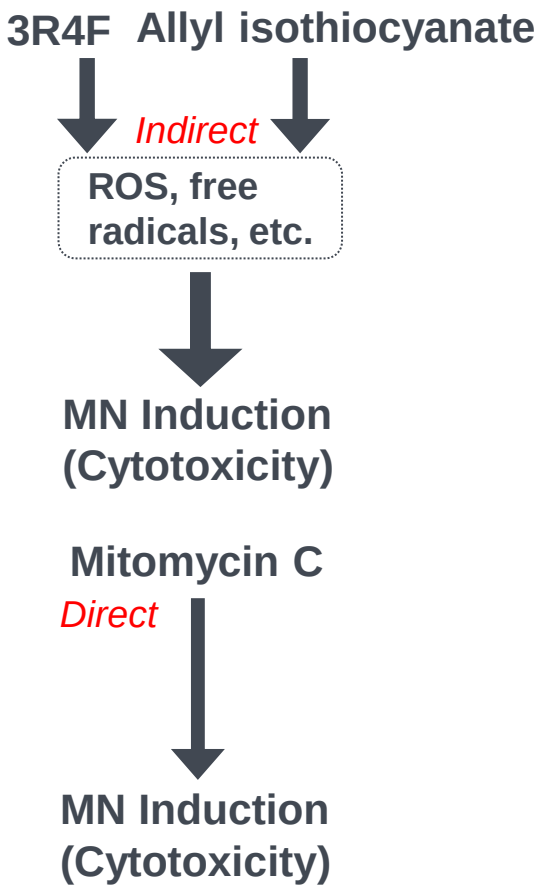

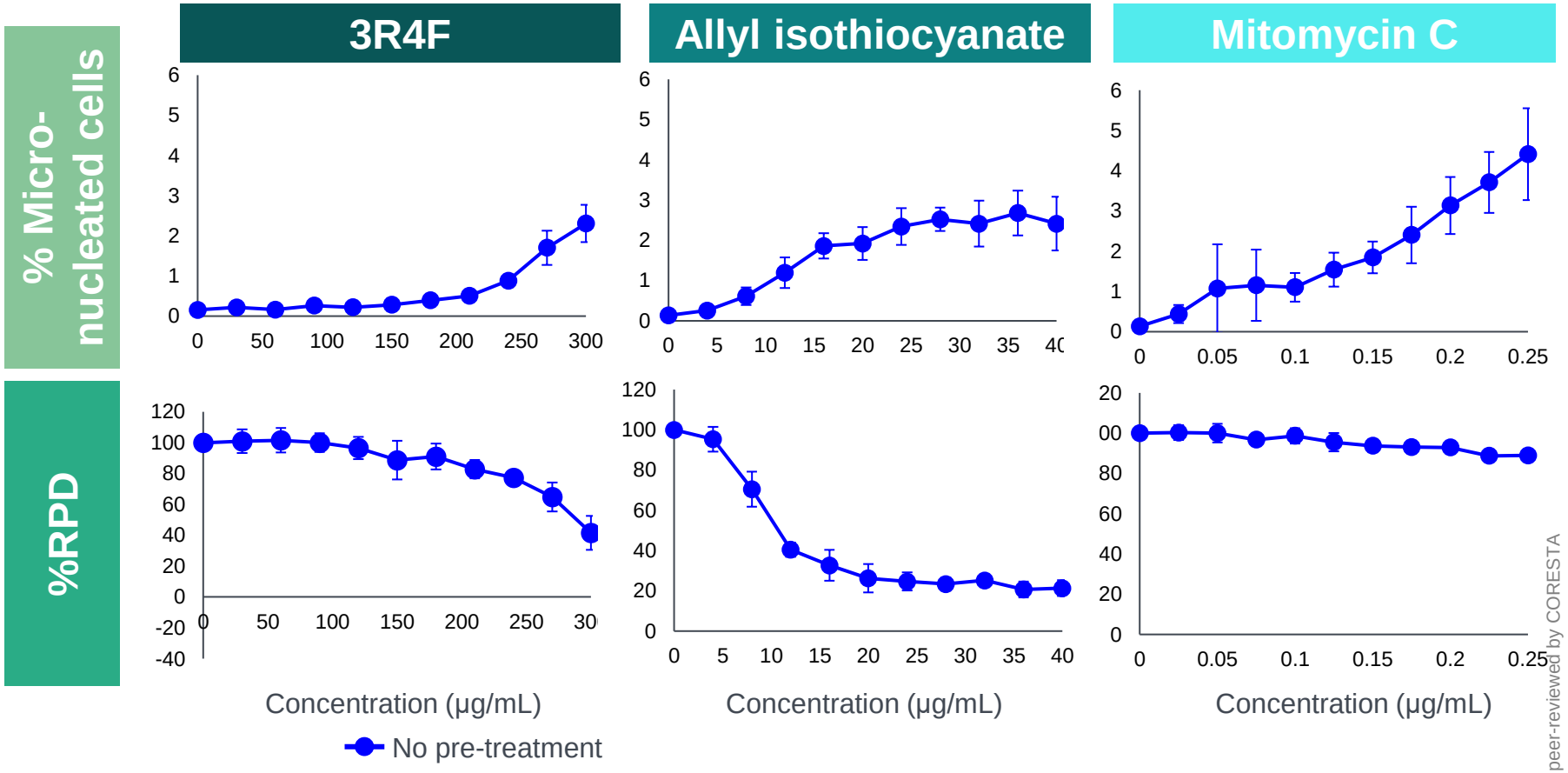

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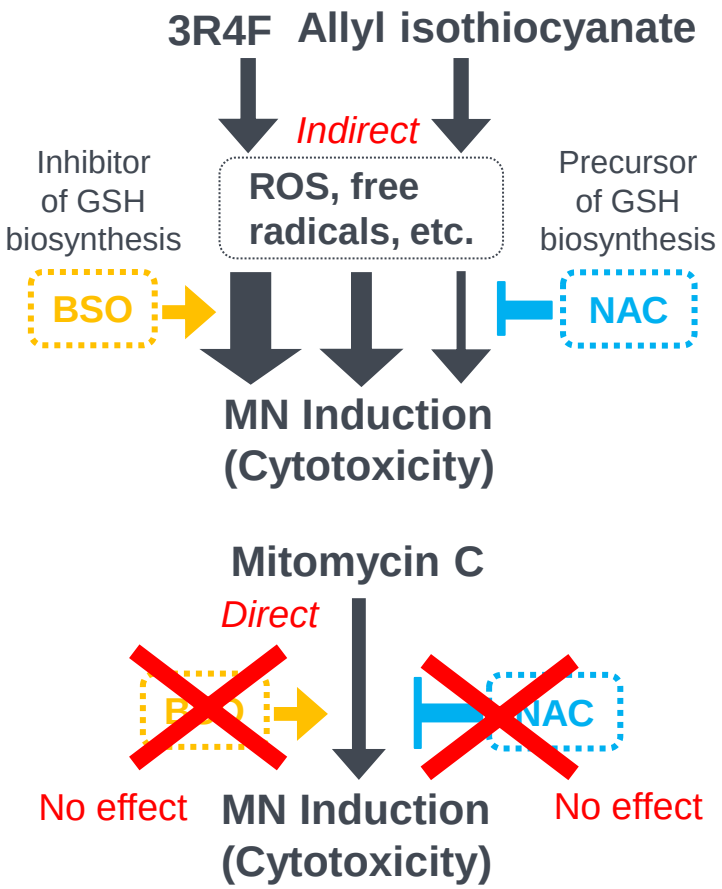

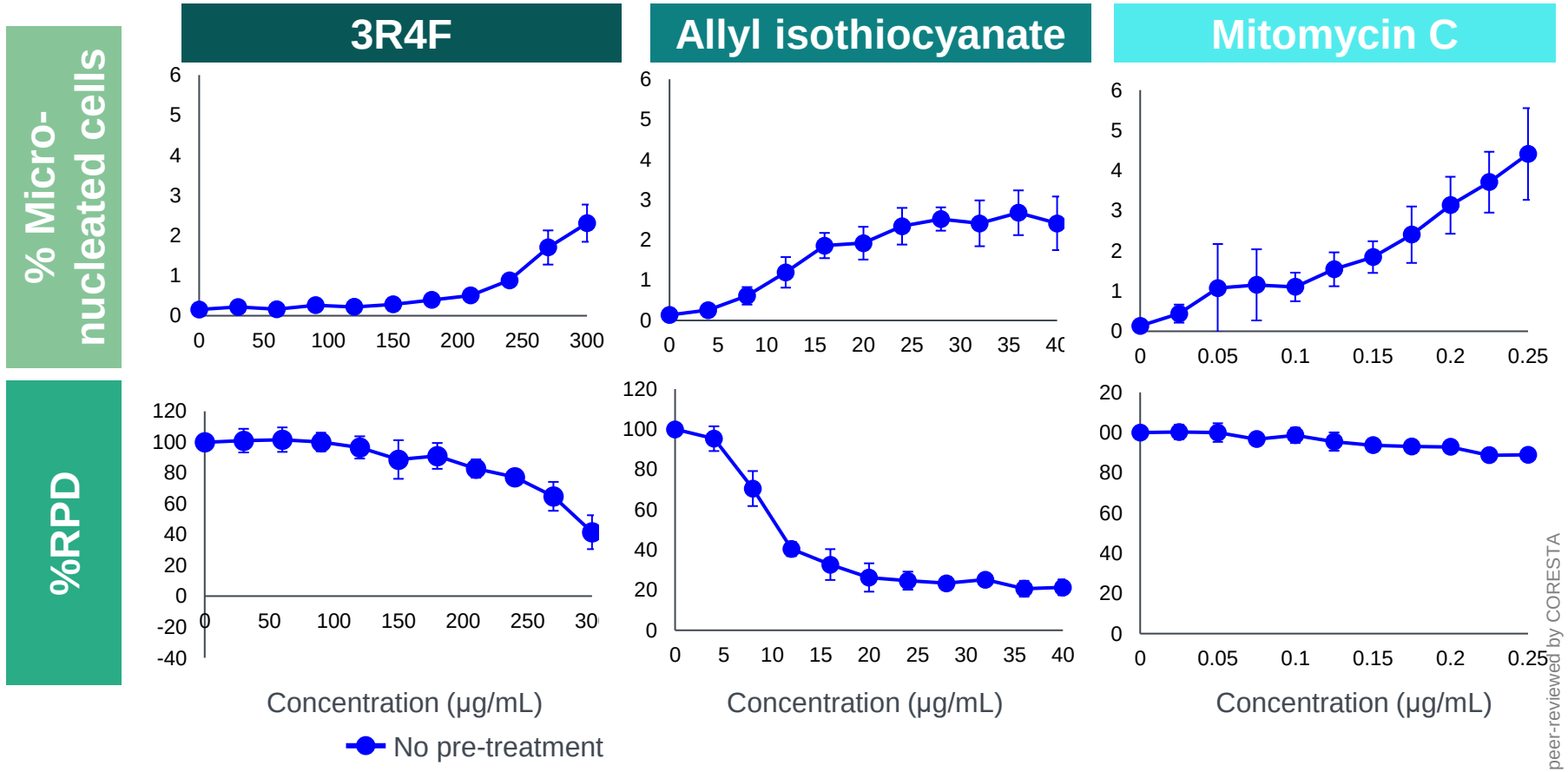

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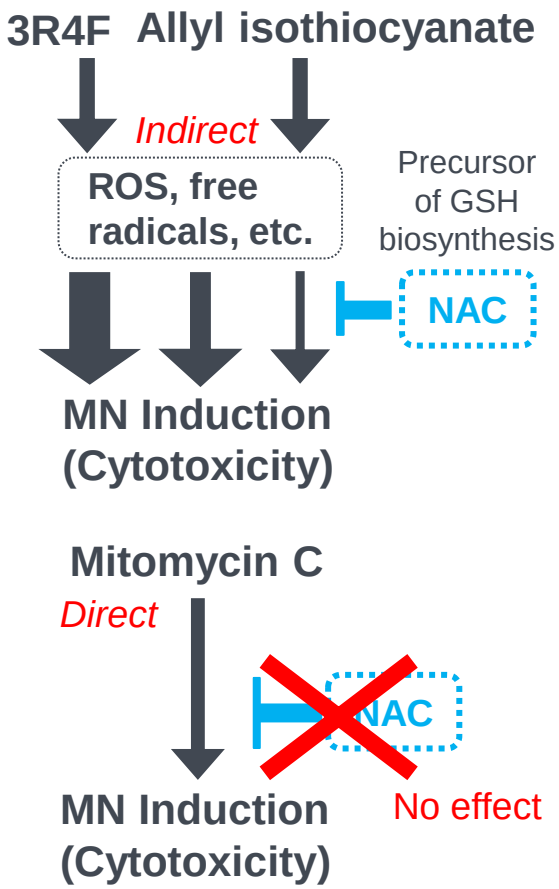

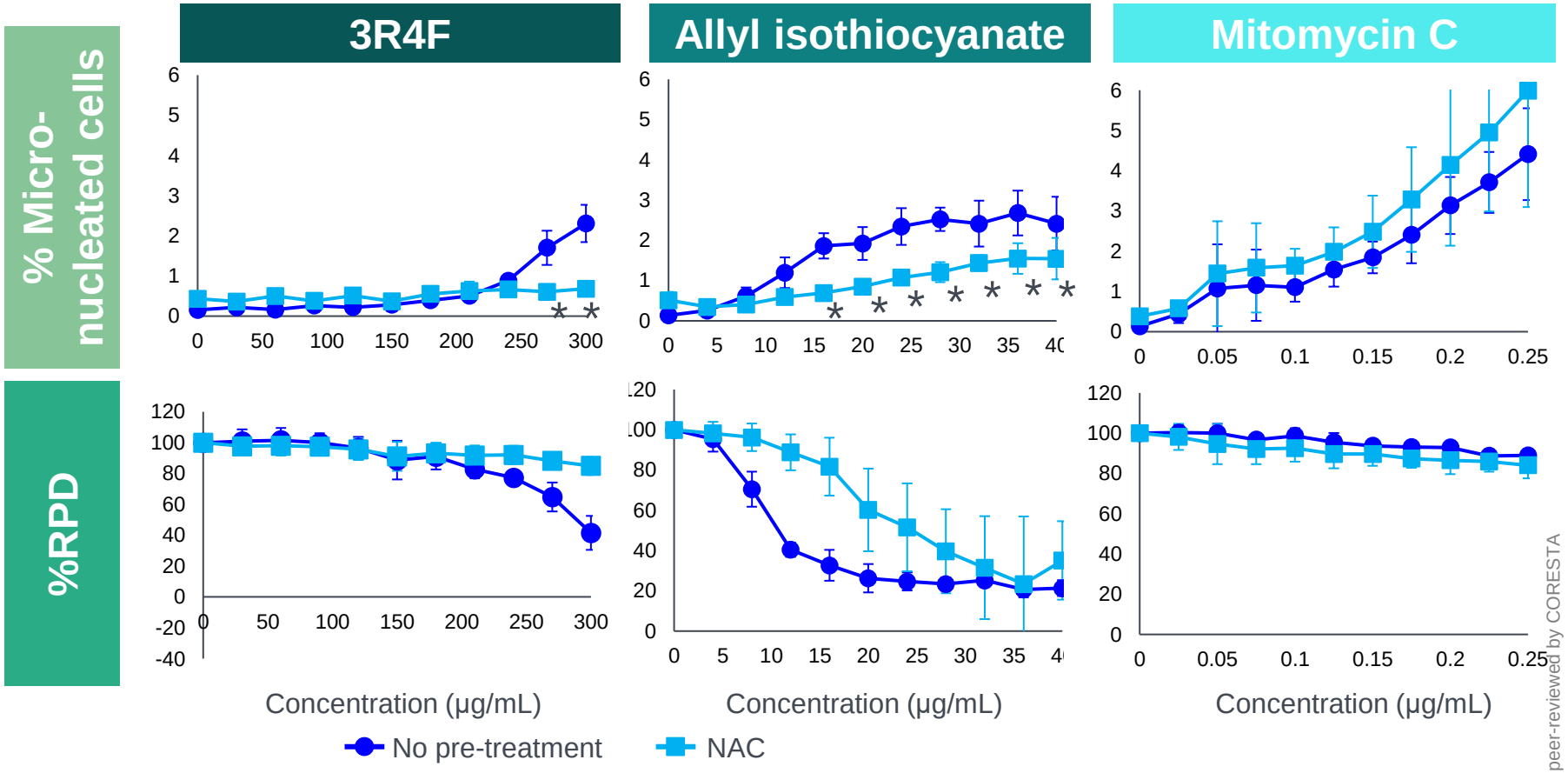

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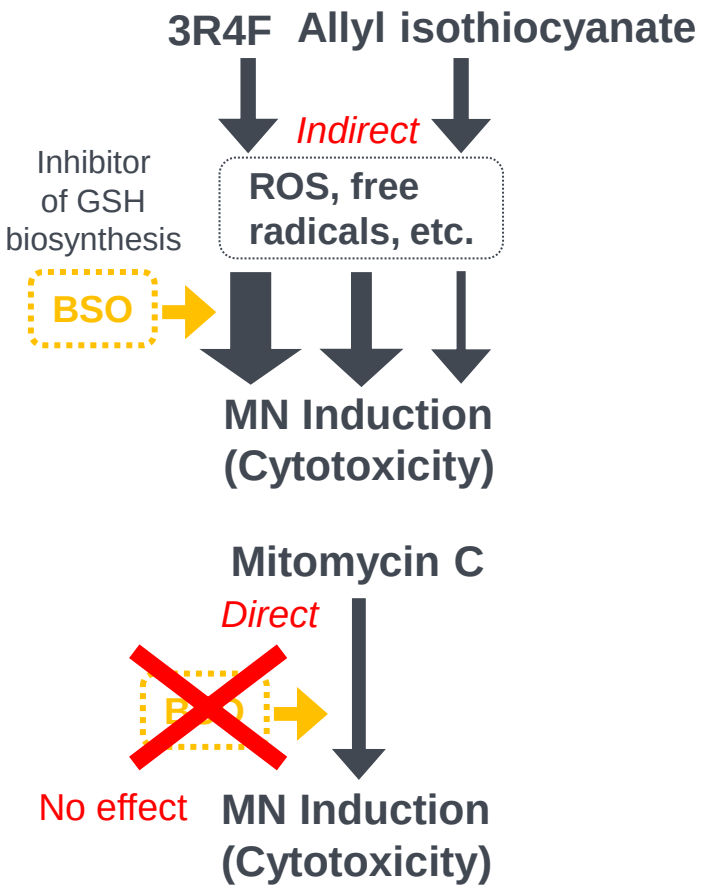

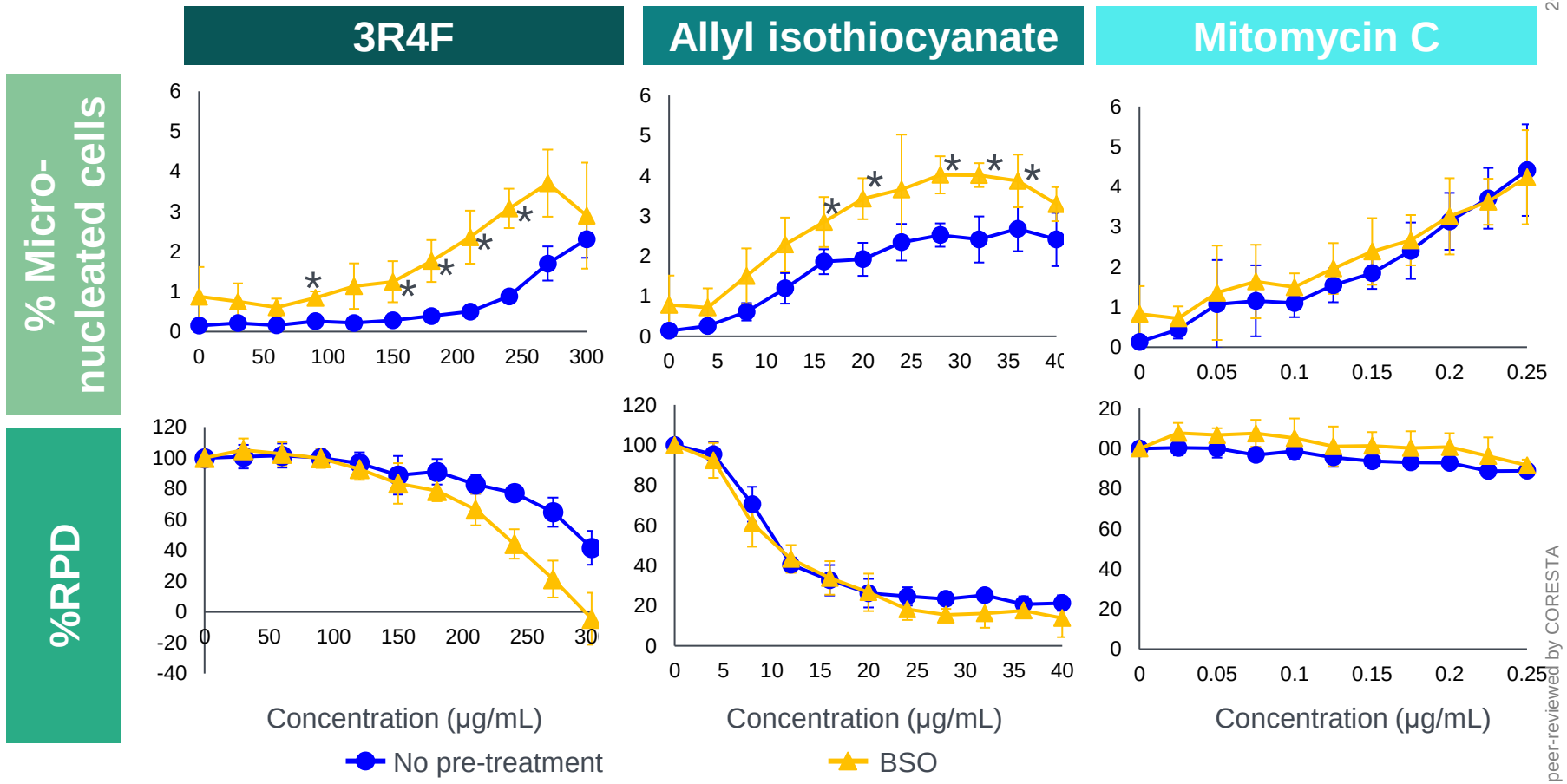

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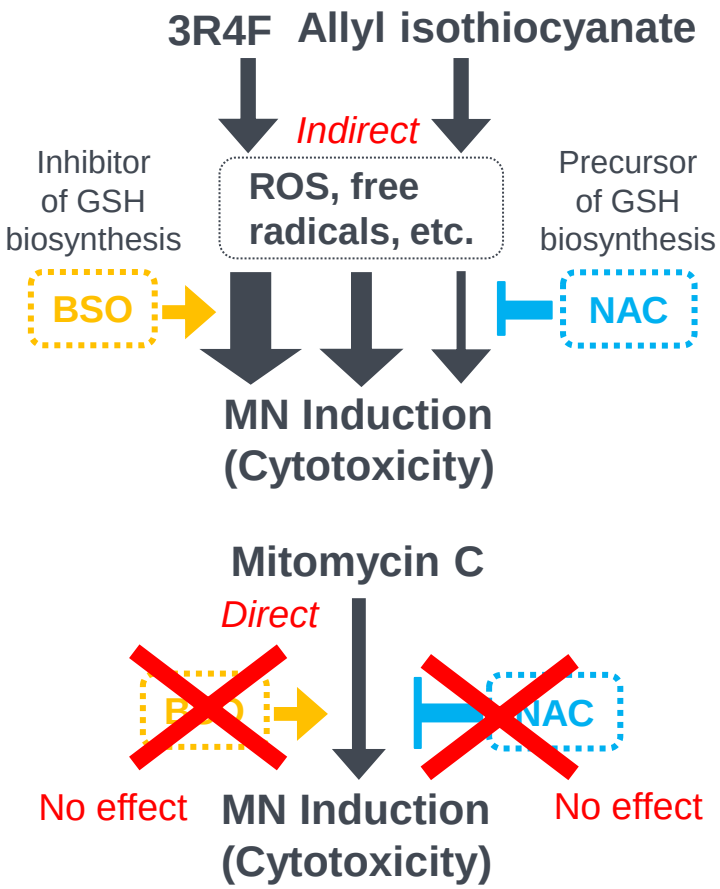

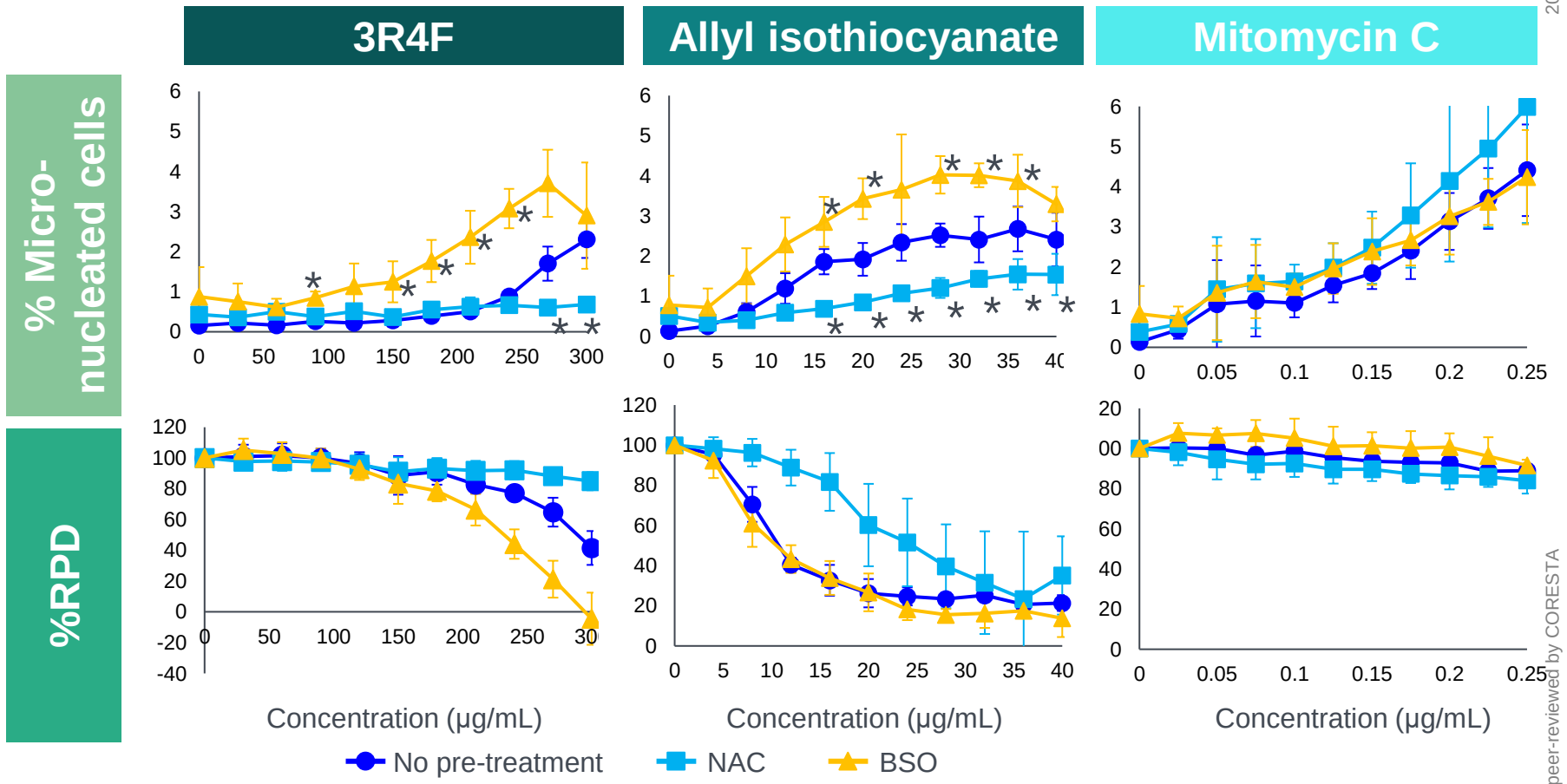

These results supposed that NAC or BSO pretreatment increased or decreased the cellular antioxidant capacity, resulting in suppression or acceleration of genotoxicity and cytotoxicity caused by 3R4F TPM.

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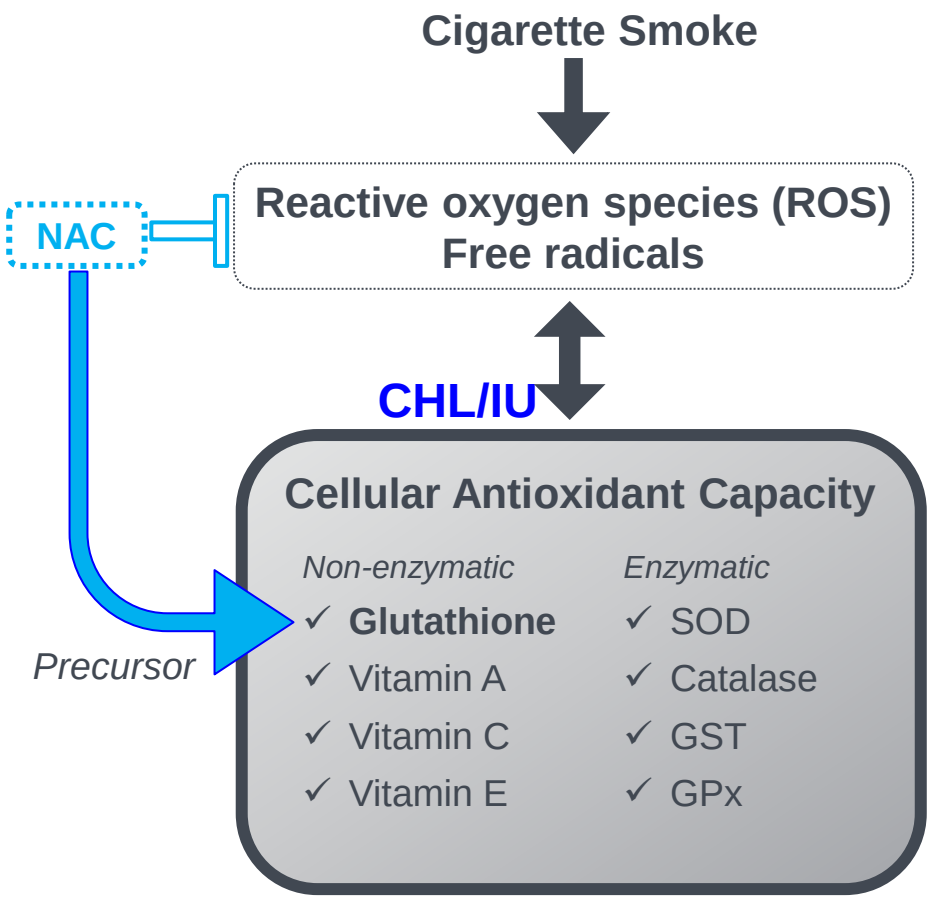
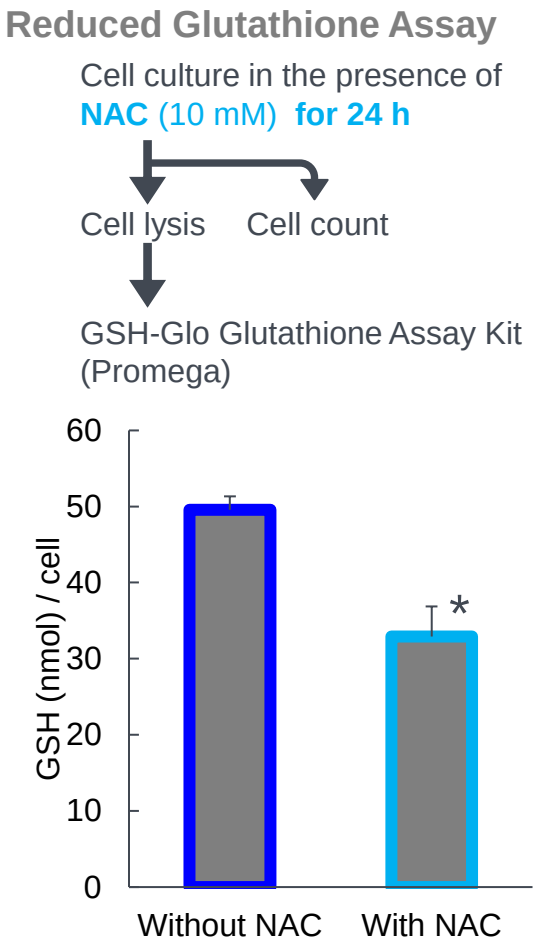
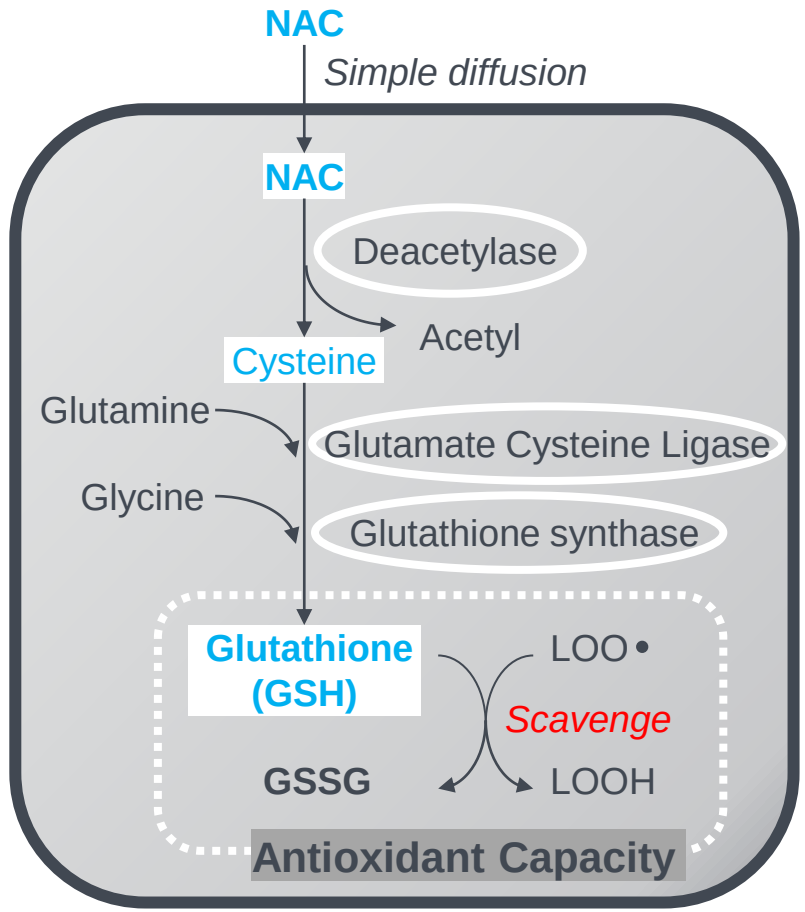
Effect of NAC on Cellular GSH Level per Cell



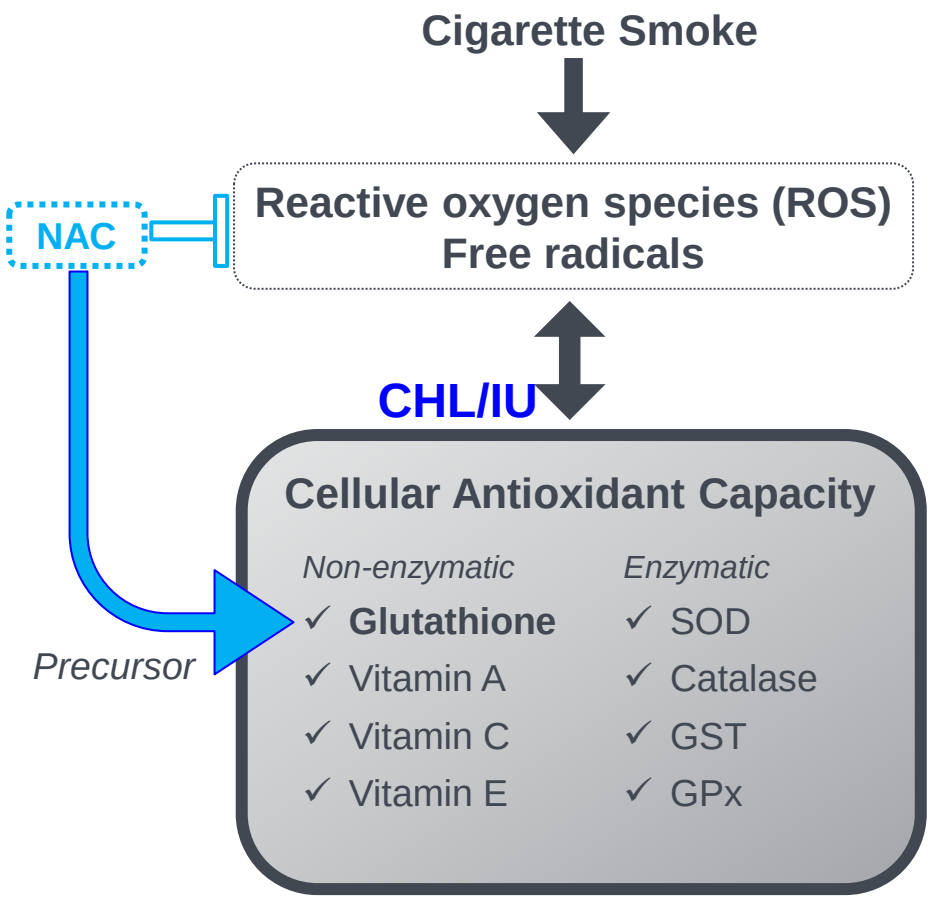


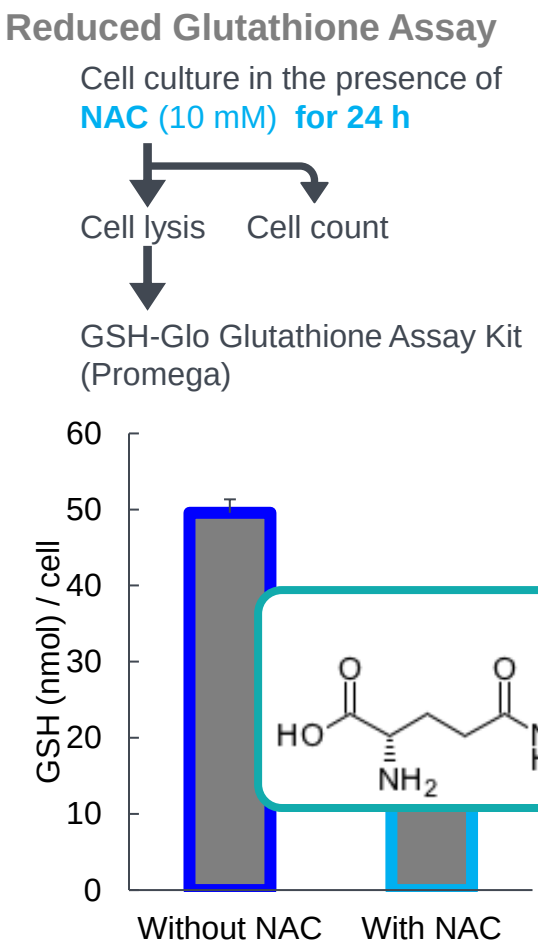


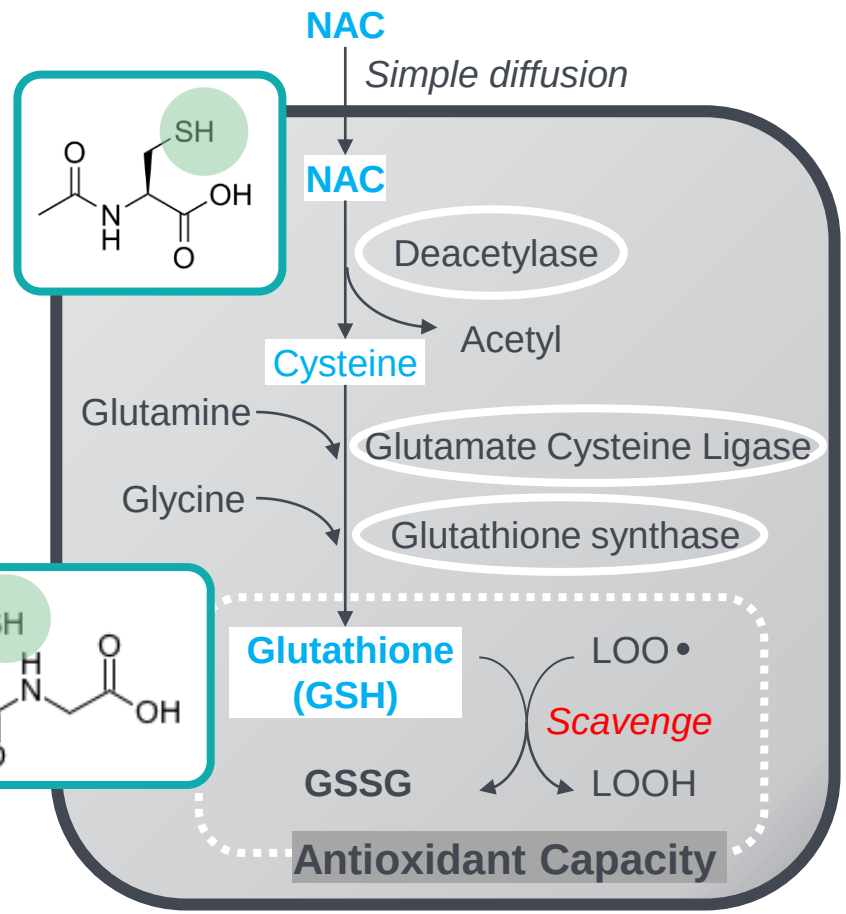
Unexpectedly, we observed reproducible results indicating that NAC pretreatment reduced cellular GSH regardless of the cell line.

** Significantly different from the without NAC (P < 0.05, paired t-test).*
Error bars indicate the standard error (N=3).

Effect of NAC on Cellular GSH Level per Cell







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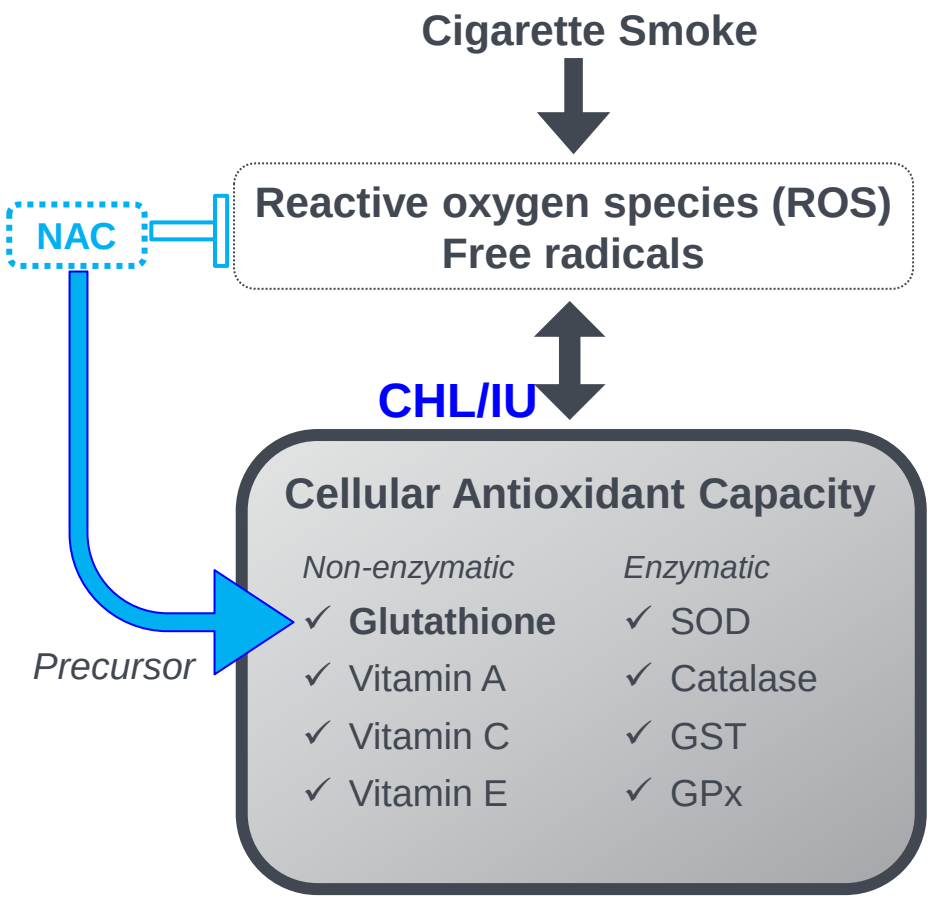

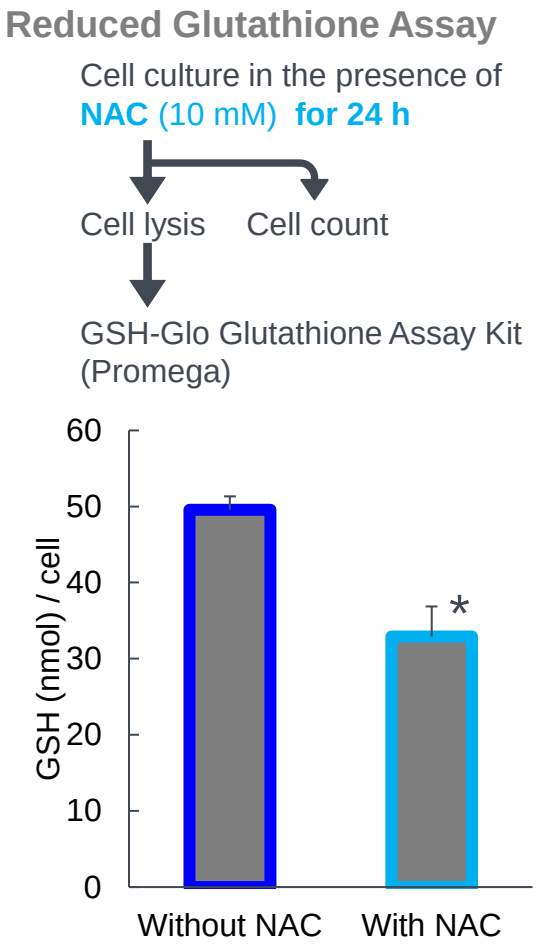

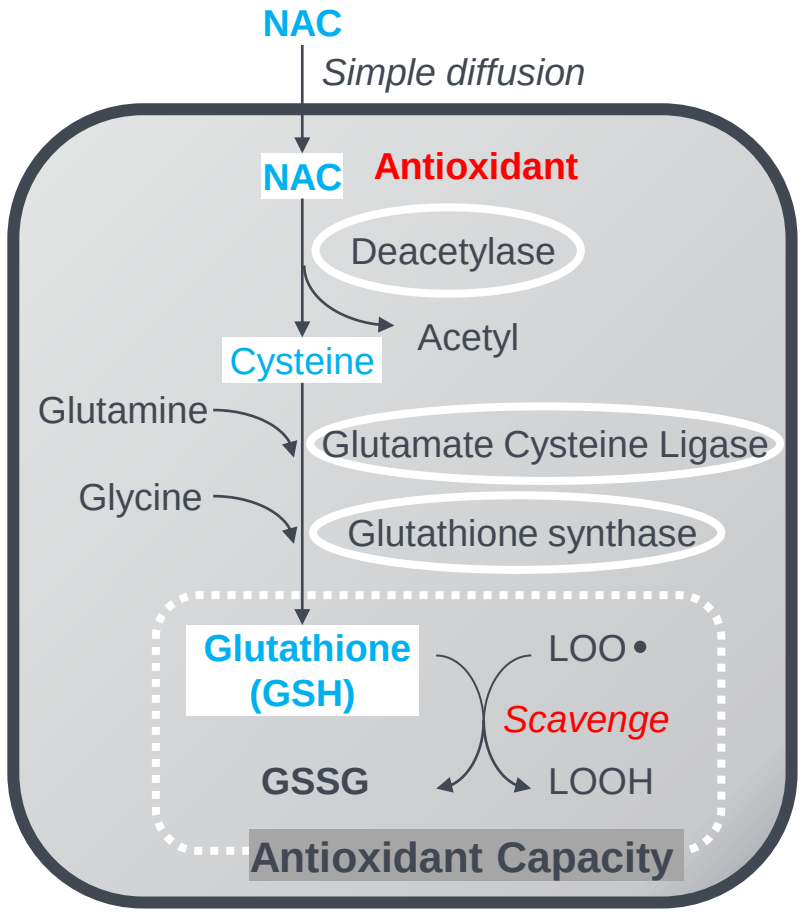

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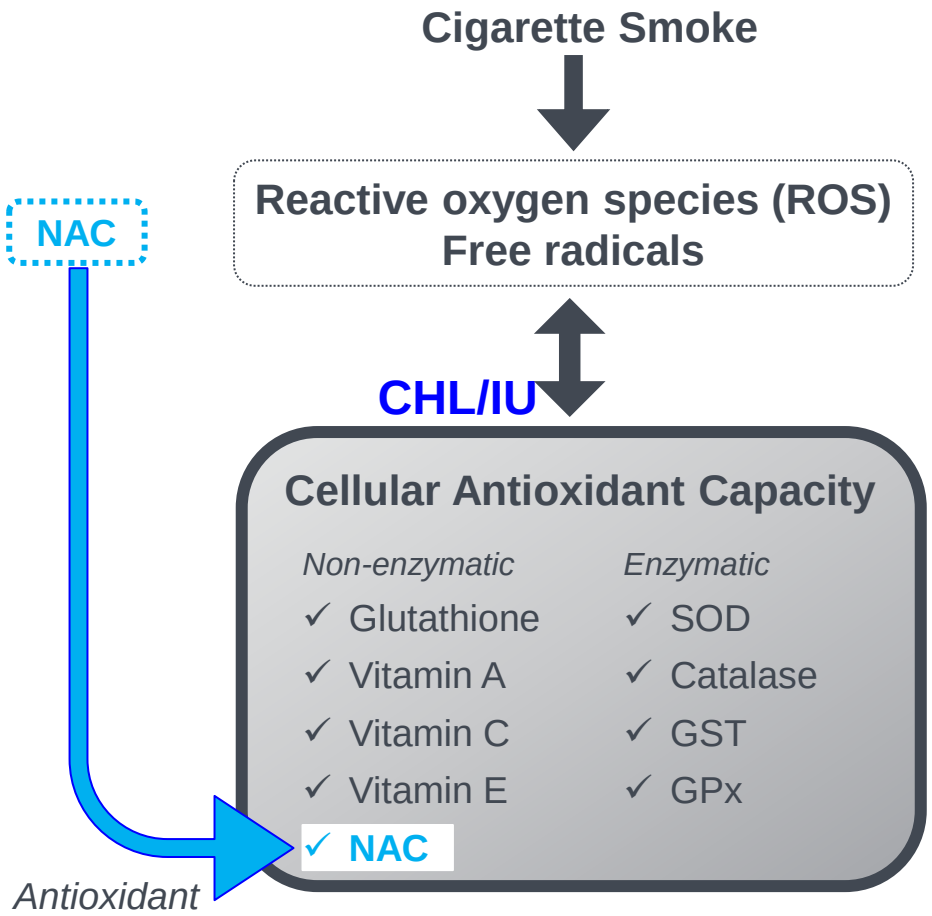
Effect of NAC on Cellular GSH and Thiol Level per Cell

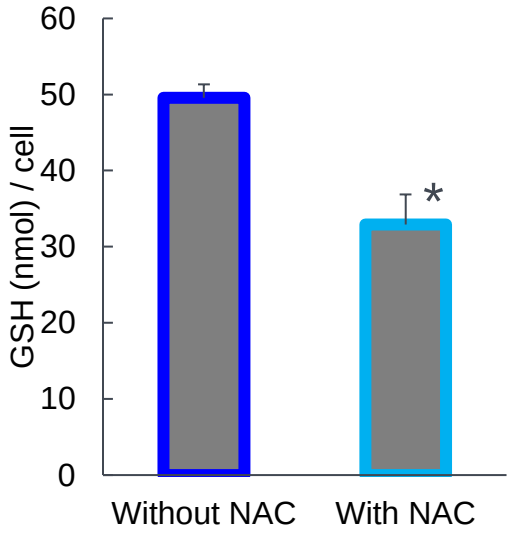


Reduced Glutathione Assay

Cell culture in the presence of **NAC** (10 mM) **for 24 h**

Cell lysis → GSH-Glo Glutathione Assay Kit (Promega)

Cell count

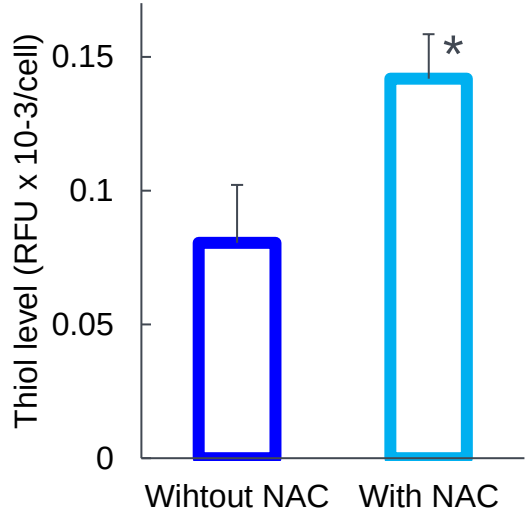


Free Thiol Assay

Cell culture in the presence of **NAC** (10 mM) **for 24 h**

Cell lysis → SensoLyte 520 Thiol Quantitation Kit (AnaSpec)

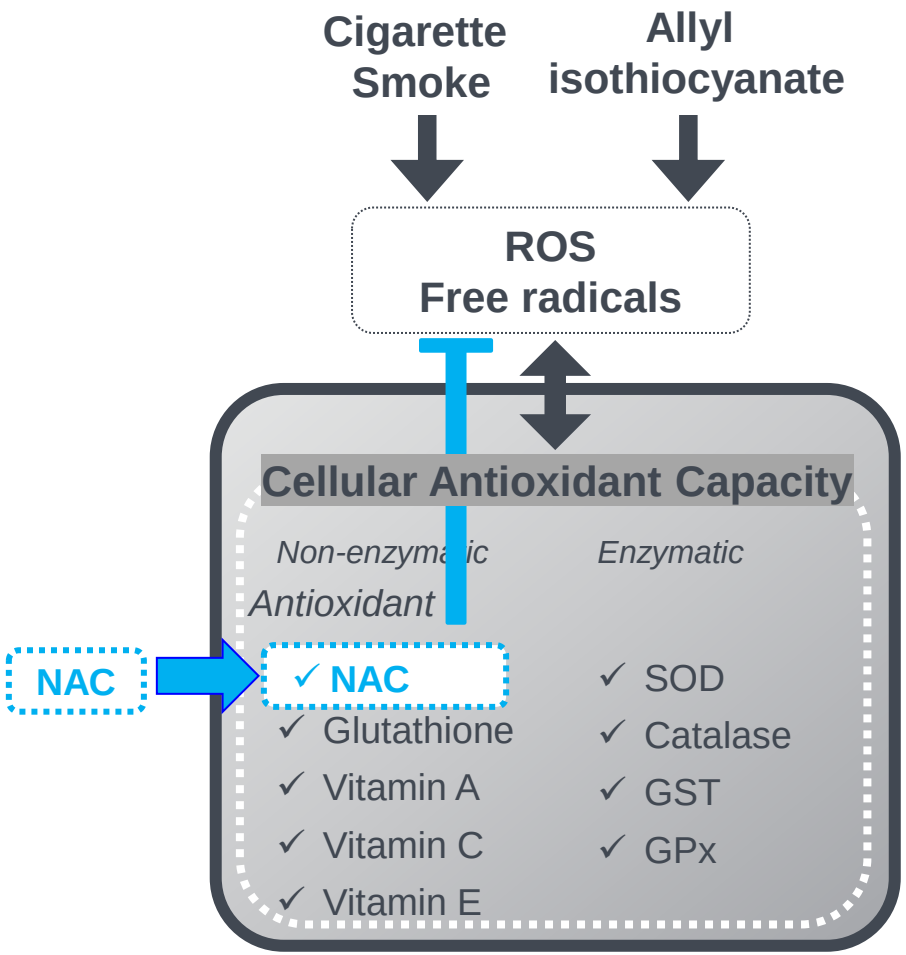
Cell count



NAC was sufficiently incorporated into the cell, which suggested that thiol exerted a protective effect against oxidative stress.

** Significantly different from the without NAC (P < 0.05, paired t-test).*
Error bars indicate the standard error (N=3).

Comparison of Thiol Level Between Each Sample Treatment



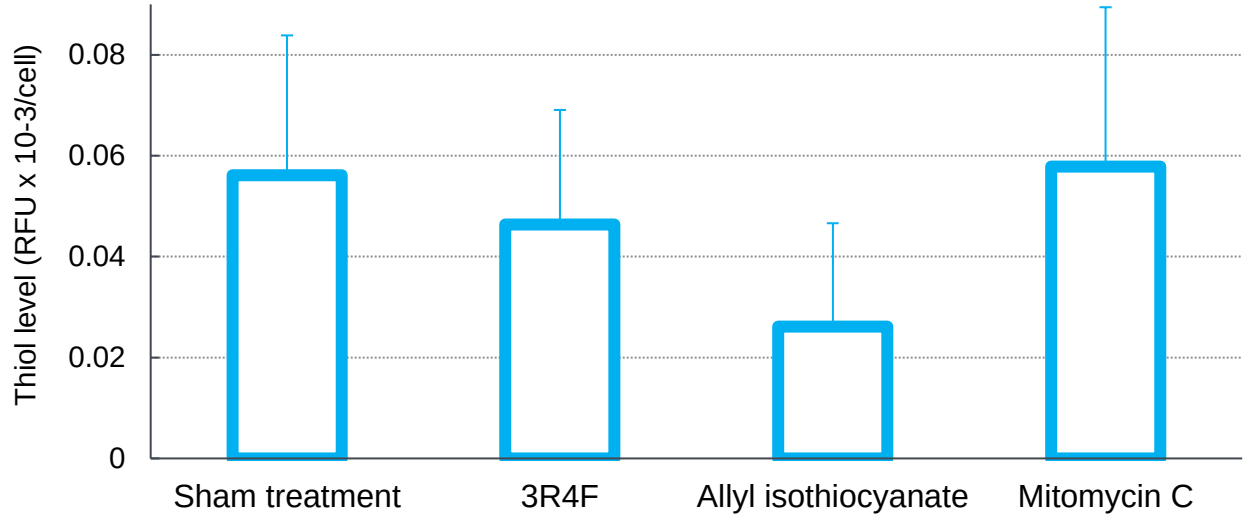
Free Thiol Assay

Cell culture in the presence of **NAC** (10 mM) **for 24 h**

3-h Treatment with 3R4F, allyl isothiocyanate, mitomycin C.

Cell lysis Cell count

SensoLyte 520 Thiol Quantitation Kit (AnaSpec)



Thiol in CHL/IU cells protects against oxidative stress caused by 3R4F TPM as well as allyl isothiocyanate.

Error bars indicate the standard error (N=3).

Summary

- CHL/IU is more resistant than L5178Y or TK6 to genotoxicity of 3R4F cigarettes.
- Antioxidant capacity and glutathione in CHL/IU are higher than those in L5178Y.
- NAC or BSO pretreatment of CHL/IU attenuates or enhances 3R4F genotoxicity.
- Cellular thiol increased by NAC pretreatment acts as an antioxidant.
- Antioxidant capacity in each cell line might be related to sensitivity to 3R4F.