Selenium sources differentially impact protection of pig intestinal cells following Cadmium-induced oxidative DNA damage.

Cadmium is genotoxic to pig intestinal cells
-some selenium sources can protect the cells in these circumstances

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6th Bioanalytical School, Londrina City, Brazil.

November 29th, 2018

Cadmium – an environmental toxin

- **Cadmium**
  - Toxic metal - ranks 7th on the US Agency for Toxic Substances & Disease Registry's priority list of hazardous substances
  - Affects DNA replication/repair, cell proliferation, cell cycle progression etc.

- Sources
  - Natural – volcanic, forest fires
  - Human activity - mining and agriculture

- Cadmium exposure
  - Humans: < 2.5 μg/kg body weight per week
  - Agri-Food industry –animal feed contamination (pig feed in particular)

Cadmium – an important contaminant in animal feed

RASFF
Rapid Alert System for Food and Feed

Date	Case no.	EU Country	Contaminated source and level
16/01/2014	2014.0062	Belgium	Cd (**160 ppm**) Poultry feed additive from Belgium
15/01/2014	2014.0057	Italy	Cd (**2.81; 3.90 ppm**) Fish meal from Spain
04/07/2014	2014.0924	Sweden	Cd (**1.08 ppm**) Compound feeds from the UK
03/08/2015	2015.1008	Germany	Cd (**6.66 ppm**) Dog feed from Poland
23/09/2013	2013.1293	Germany	Cd (**1.43 ppm**) Cattle feed materials from Germany
04/06/2013	2013.0777	Belgium	Cd (**52 ppm**) ZnO in feed additives from Turkey, via Denmark

efsa
European Food Safety Authority

Cadmium in animal feeds

- 60.6% of samples tested contained toxic levels of Cd (*EFSA Report 2012*)

DCU

Selenium -an essential trace mineral

- A vital dietary component - 50 – 100 μg/day
  - Necessary for growth and reproduction
  - Healthy brain, immune system, thyroid, reproductive, and lung function
  - Selenoproteins play major roles in antioxidant (anti-ROS) functions, DNA repair

- Agri-Food industry: low selenium status causes problems
  - white muscle disease, poor reproductive performance and failure to thrive

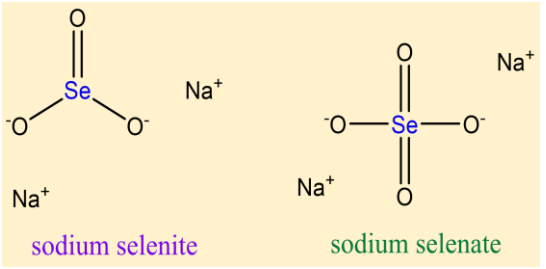
- Se supplementation/animal feed industry
  - Market size of ~$200 M
  - Selenium sources can be inorganic or organic selenium

DCU

Selenium -an animal feed additive

Inorganic Se sources

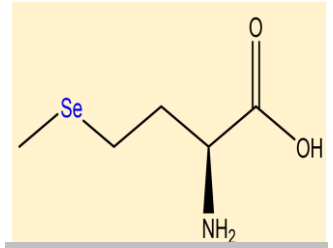
- sodium selenate, sodium selenite
- cheap but problematic
  - Evidence of DNA damage & mutation, compromised gut integrity
  - Reduced Feed conversion (FCR) ratio & performance



Sodium selenite Sodium selenate

Organic Se sources

- Seleno-methionine (Se-M)
- Selenised yeast products (Se-Y)
- Improved *bioavailability*
  - Increased assimilation by cells



Seleno-methionine

Selenised yeast (Se-Y)

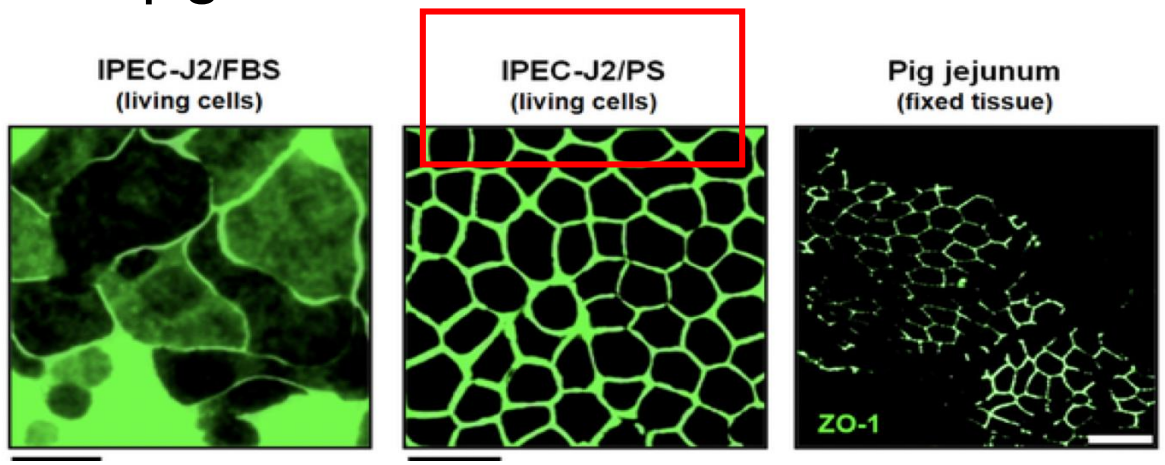
levedura selenizada!

Objective

To investigate the effects of Se supplementation on porcine (pig) intestinal cells following insult with Cadmium

A porcine (pig) intestinal cell culture model

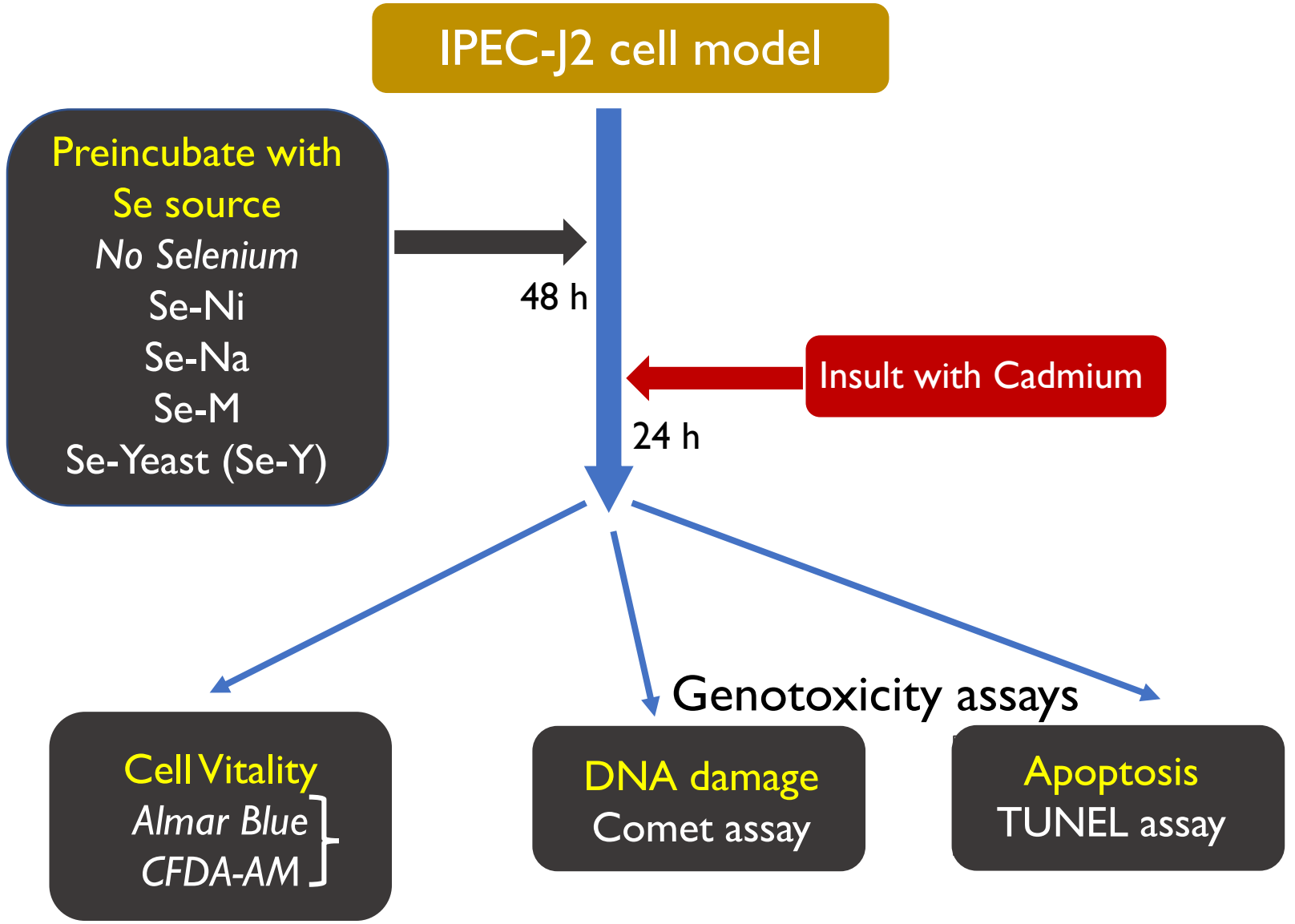
- **IPEC-J2 cells**
  - primary porcine intestinal enterocytes
  - isolated from the jejunum of a neonatal pig



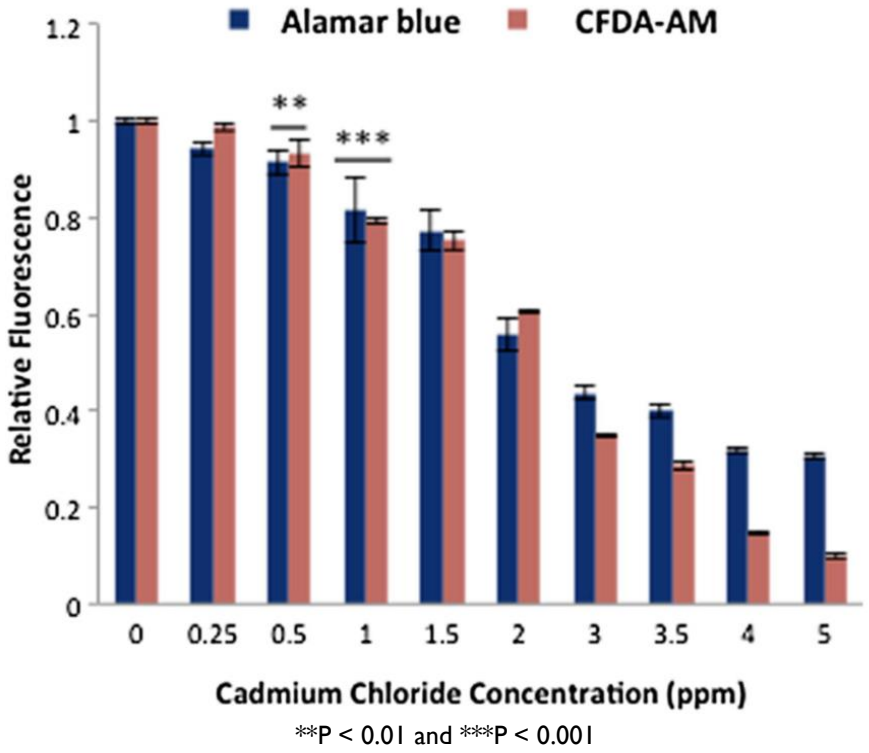
- Cultured with ***Porcine Serum (PS)***
  - Architecture
  - Cell morphology
  - Junction barrier properties / transport functions
  - Trans-epithelial resistance (TER) values
  - ***An improved tool for pig small intestine, toxicology and digestive studies***

Zakrzewski SS, et al. (2013). PLOS ONE 8(11): e79643.

Experimental strategy – to compare Se sources



$CdCl_2$ is toxic to IPEC-J2 Cells



Alamar blue assay – metabolic activity

CFDA-AM assay – membrane integrity

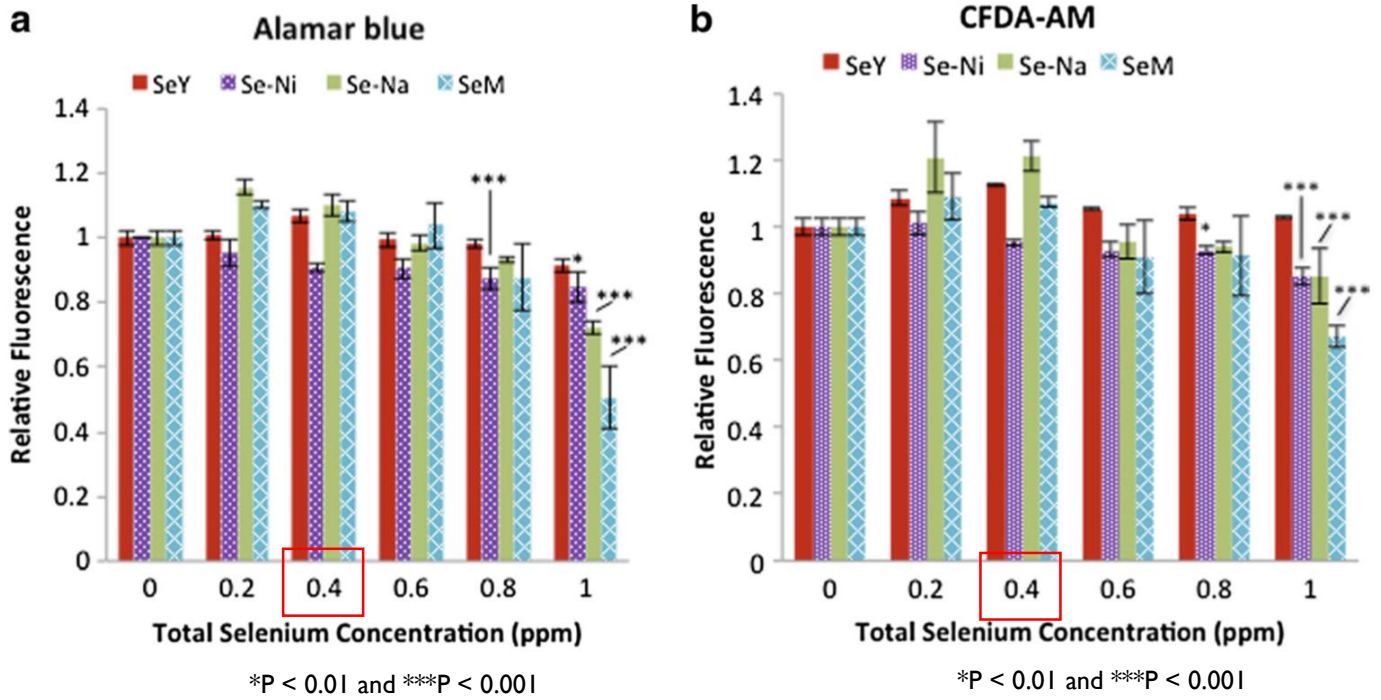
Response to $CdCl_2$ (no added Selenium)

- Dose-dependent decrease in cell vitality
- Significant at 0.5 ppm Cd

Lynch et al, 2016. *Biological Trace Element Research,* 176 (2) 311-320

Effect of Se sources on IPEC-J2 vitality

Toxicity



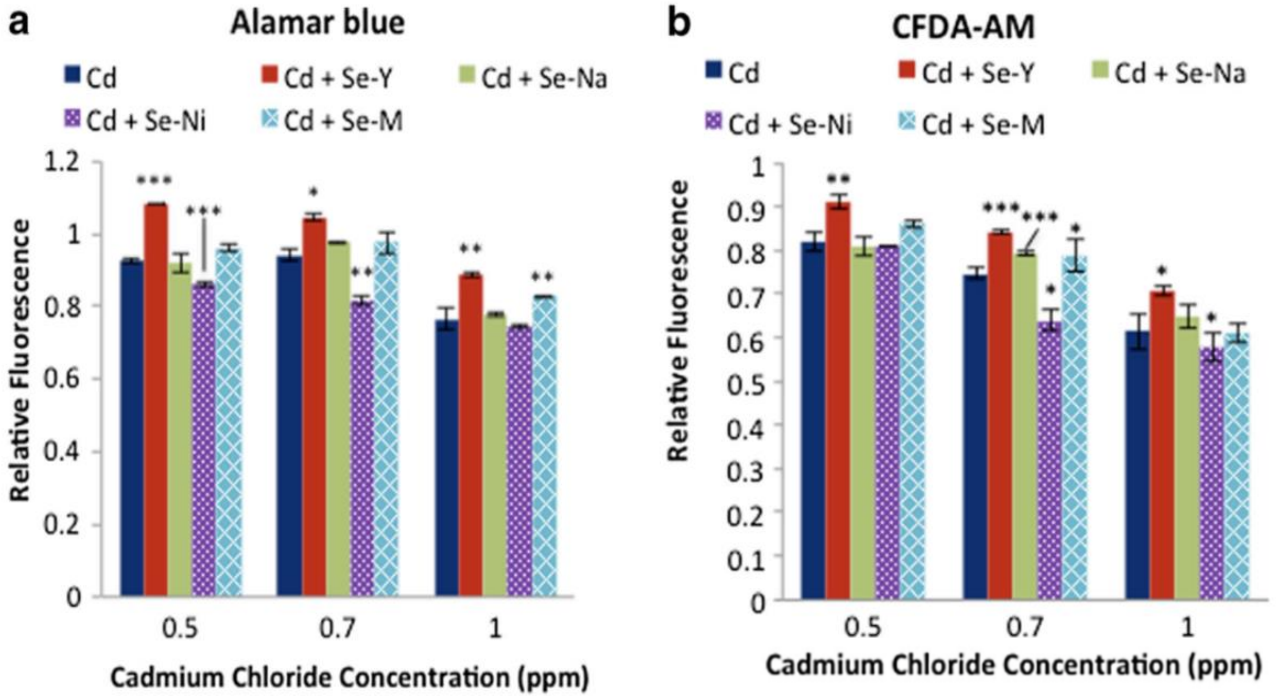
EFSA recommended Se level for supplementation – 0.4 ppm

- Se sources alone – none are cytotoxic at 0.4 ppm Se
- Se-Ni - toxic above 0.8 ppm Se – both assays
- Se-Y - no toxicity up to 1 ppm Se

Lynch et al, 2016. *Biological Trace Element Research*, 176 (2) 311-320

Cd-induced Cell toxicity is modulated by Se sources

Toxicity



All Se at 0.4 ppm

*P < 0.05, **P < 0.01 and ***P < 0.001

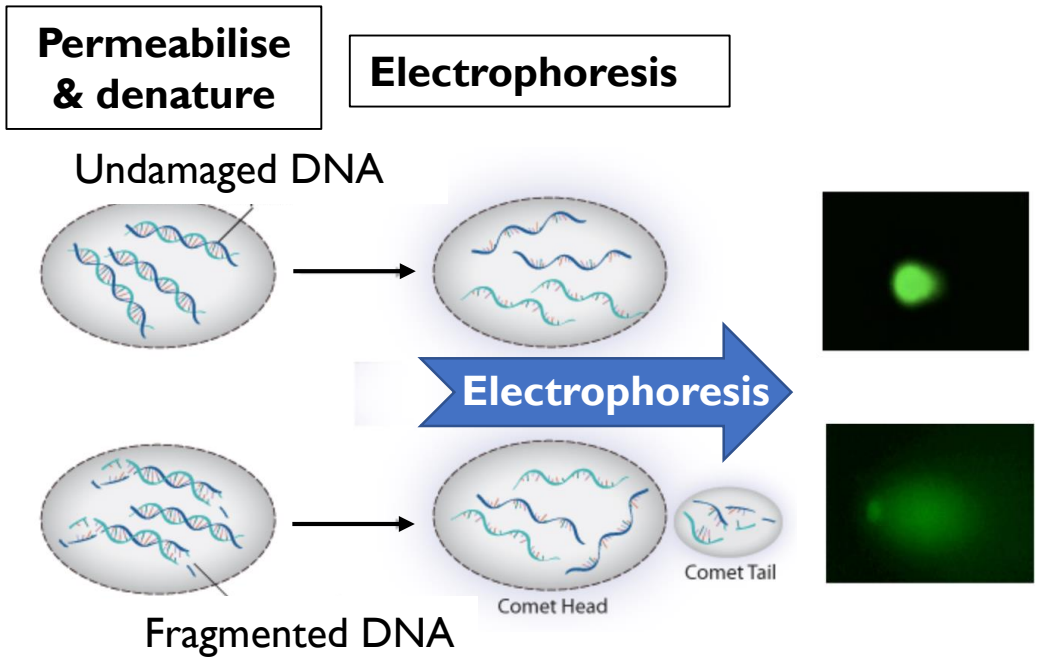
- Se-Y (Selenised Yeast) showed a protective effect at all Cd concentrations used
- <u>Protective effects: Organic Se = best; Inorganic Se = worst</u>
- Cd plus Se-Ni – enhanced cell toxicity!

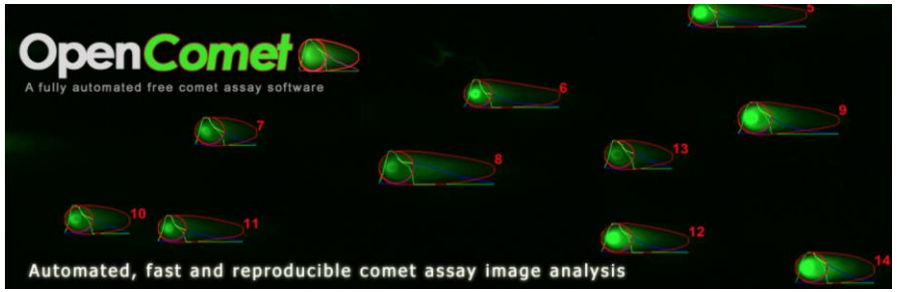
Lynch et al, 2016. *Biological Trace Element Research,* 176 (2) 311-320

Genotoxicity testing – by Comet assay

(Single Cell Gel Electrophoresis Assay)

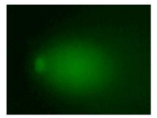
- A sensitive assay to detect DNA damage
- Used in genotoxicity testing –industrial/agro chemicals, biocides, pharmaceuticals
- Measures DNA damage/apoptosis, DNA repair



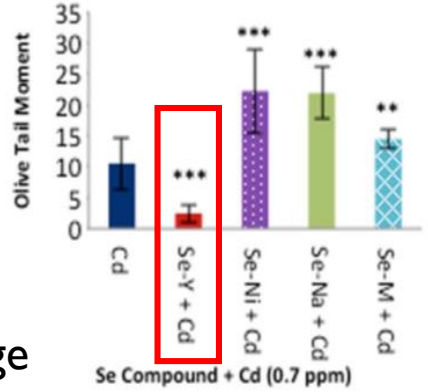


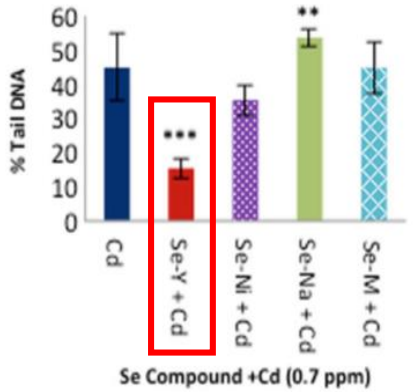
Denatured cleaved DNA fragments migrate out of immobilized cells following permeabilisation and electrophoresis

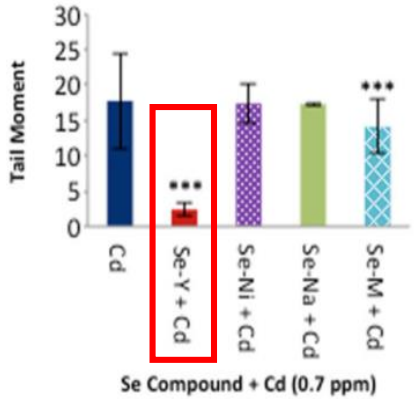
Cd-induced genotoxicity is modulated by Se sources

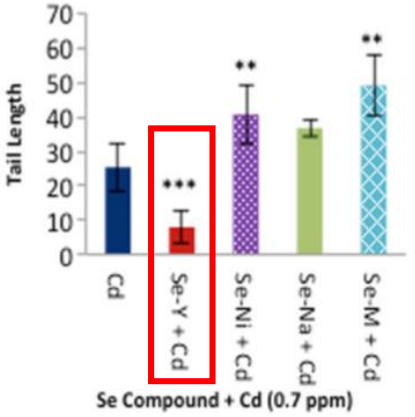


DNA damage









*P < 0.01 and ***P < 0.001

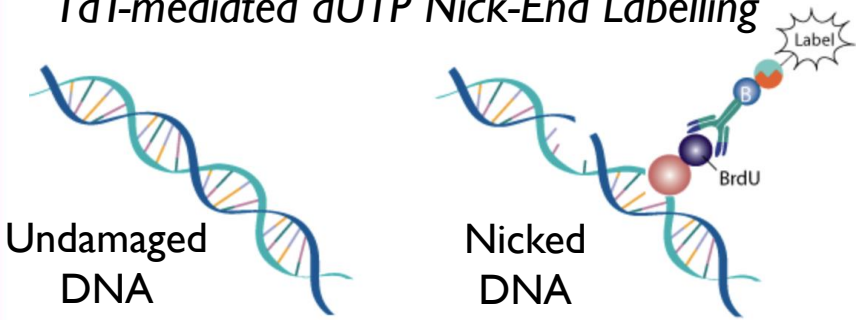
- Cd induces DNA damage in IPEC-J2
- Se-Y most effective at protecting against Cd-induced DNA damage
- Inorganic Se –cells were most sensitive to Cd-induced DNA damage (also 1 ppm Cd, not shown)

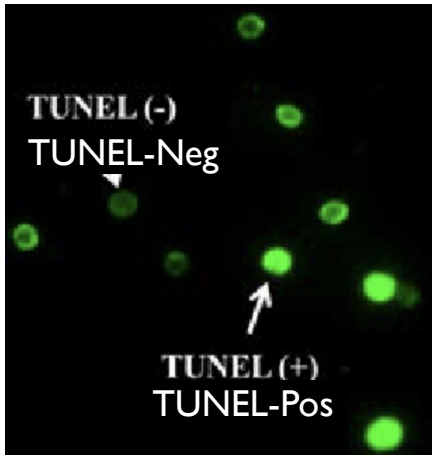
Lynch et al, 2016. *Biological Trace Element Research,* 176 (2) 311-320

Genotoxicity testing – by TUNEL assay

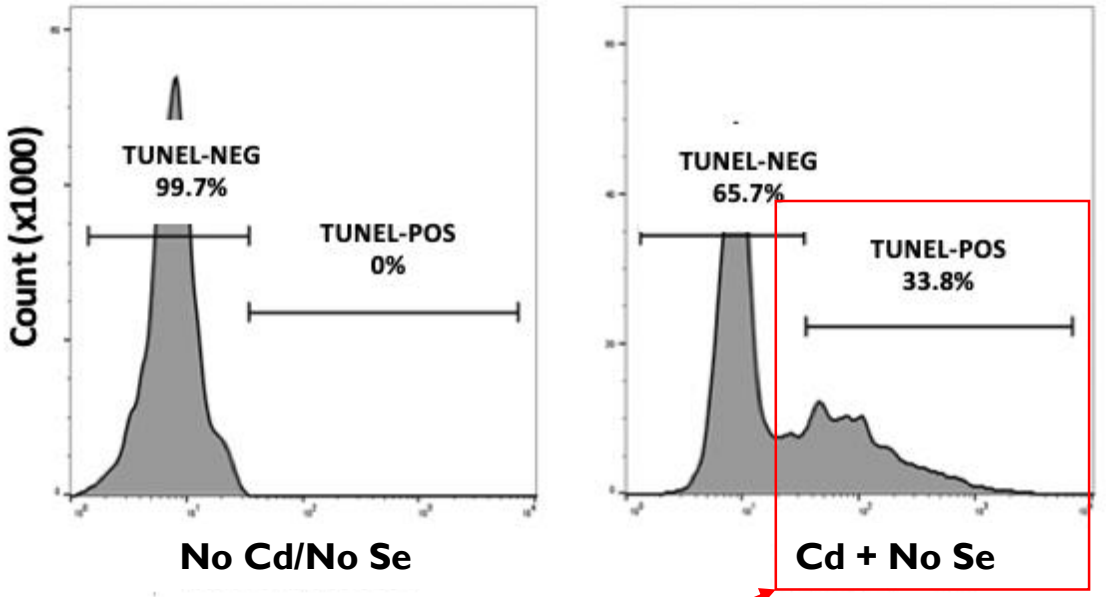
TUNEL Assay

TdT-mediated dUTP Nick-End Labelling





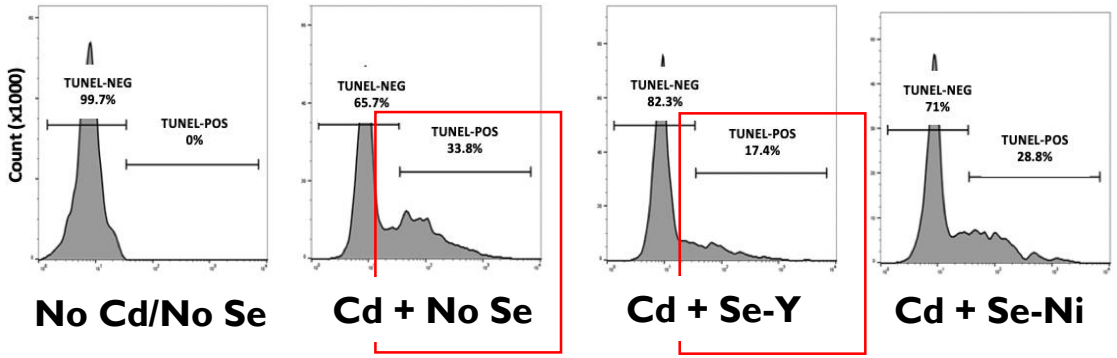
Flow Cytometry



- TUNEL-labelled cells contain damaged (nicked) DNA
- Cd induces apoptosis-associated DNA fragmentation

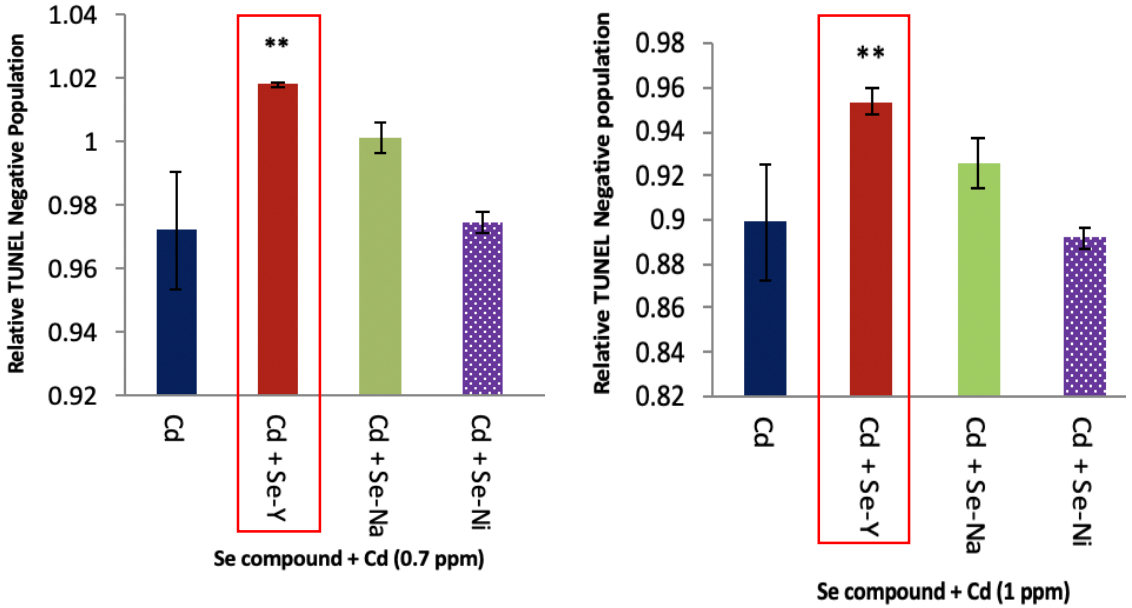
Lynch et al, 2016. *Biological Trace Element Research*, 176 (2) 311-320

Se-Y protects IPEC-J2 against Cd-induced apoptosis



TUNEL assay

DNA damage

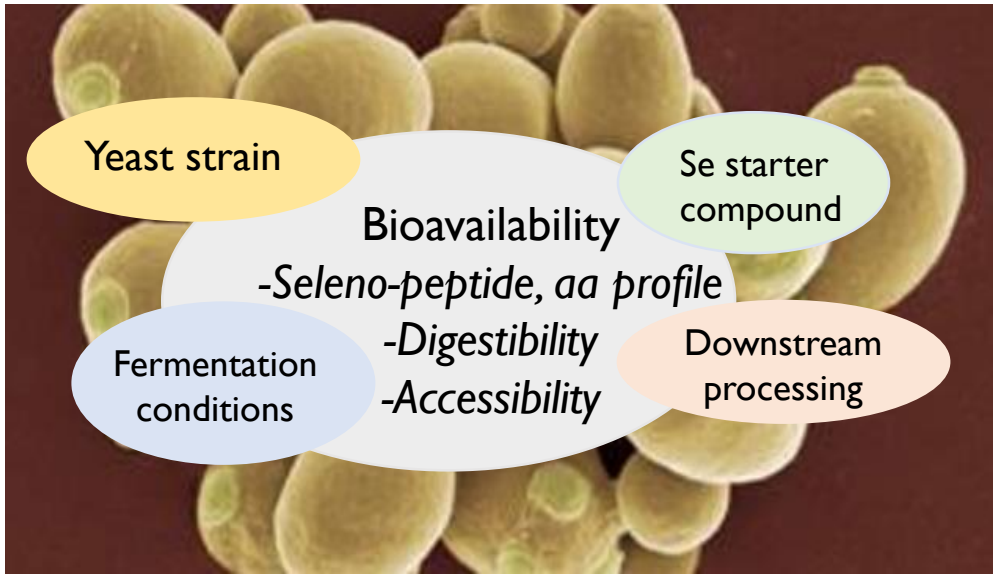


- Se-Y protects against Cd-induced apoptosis
- Se-Ni does not

Lynch et al, 2016. *Biological Trace Element Research*, 176 (2) 311-320

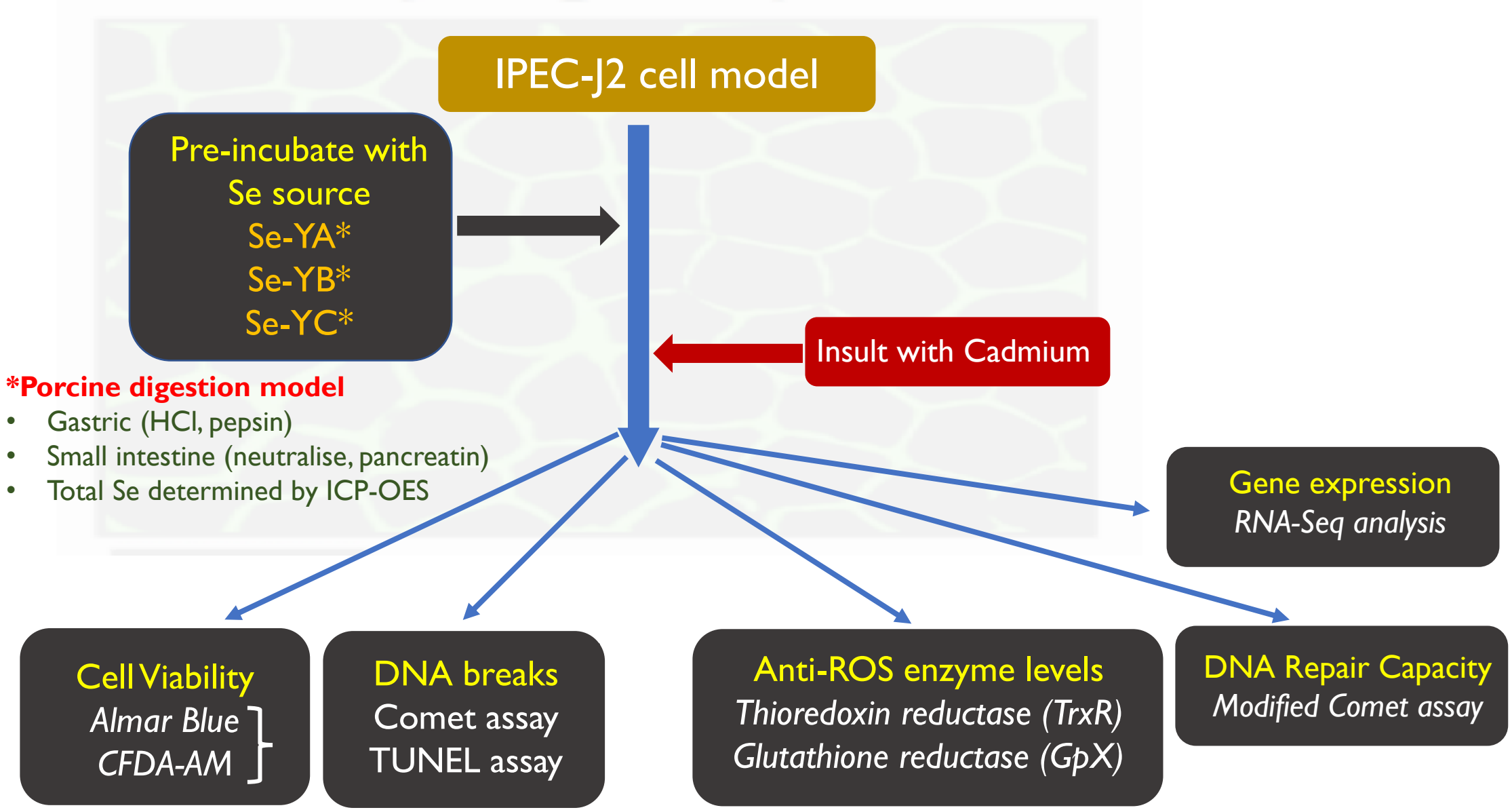
Se-Y sources –are there differences in bio-efficiency?

- **Selenised Yeast (Se-Y)**
  - Animal welfare, economic & environmental advantages
  - ***Do commercial Se-Y preparations differ?***
    - Protein-rich yeast matrices
    - Physiological, nutritional and bioprocess factors
    - Differ in bioavailability of Se forms
      - *Composition, digestibility and accessibility*

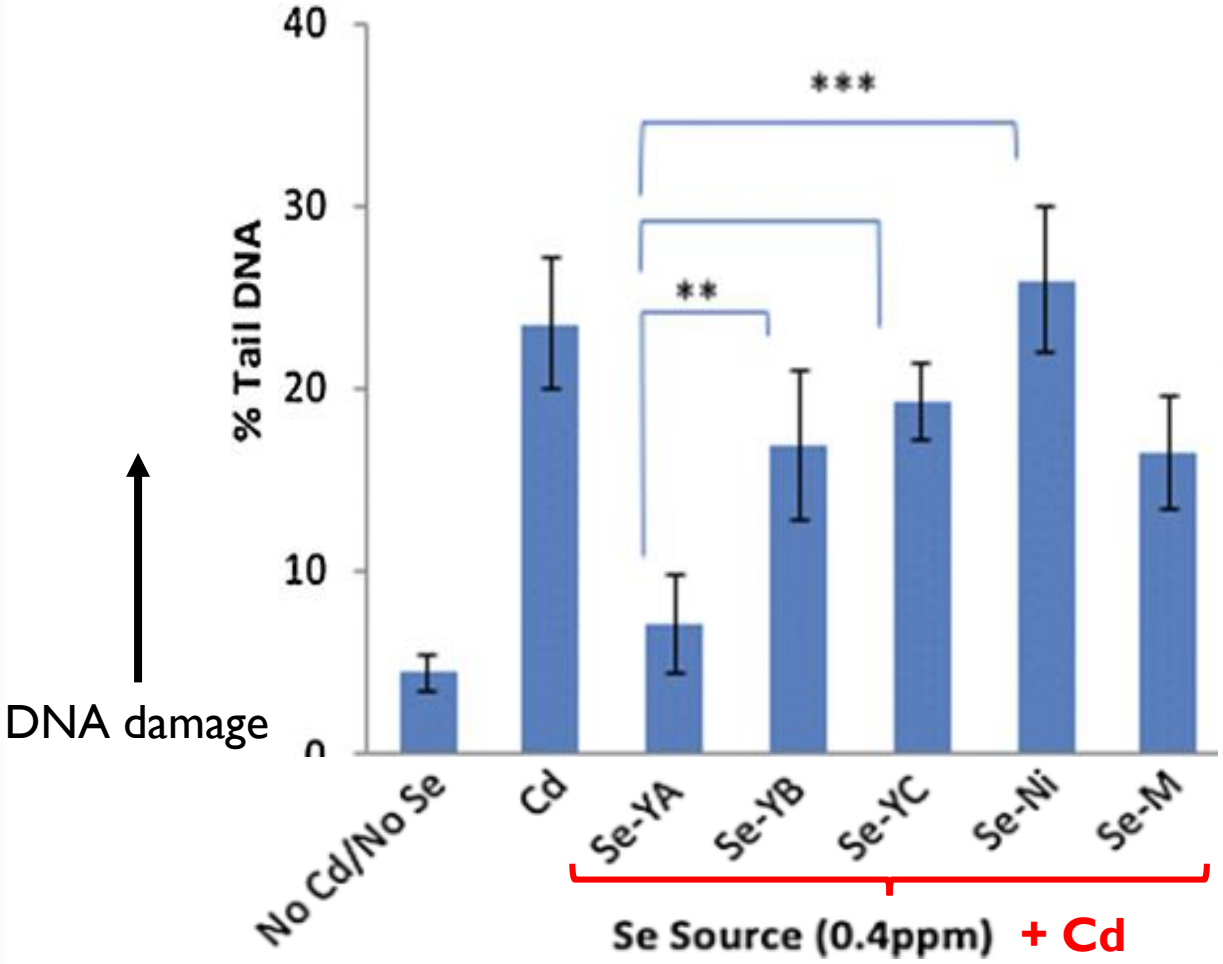


Fagan et al, 2015. *Biological Trace Element Research,* 166 (1) 245-259

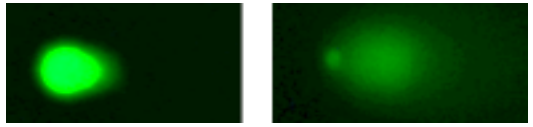
Comparing Se-Y sources - experimental strategy



Se-Y sources differ -protecting from Cd-induced DNA damage



Alkaline Comet assay



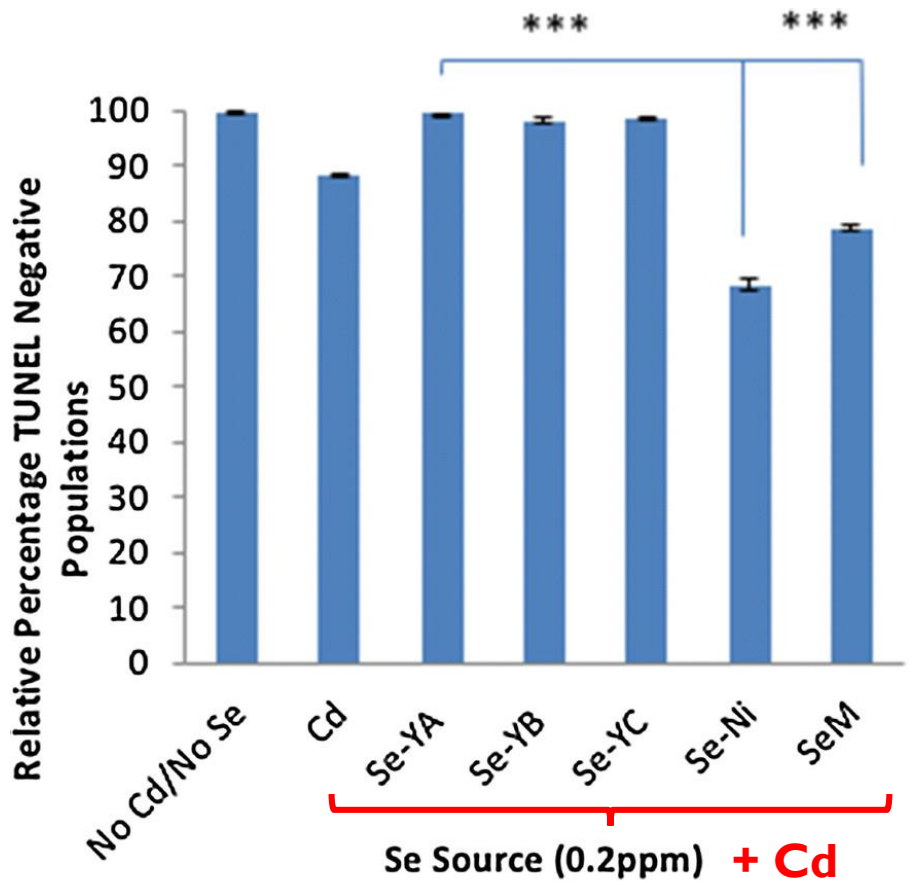
Cd-induced DNA damage

- Inhibited by Se-YA
- Not by Se-YB/YC, Se-M and Se-Ni

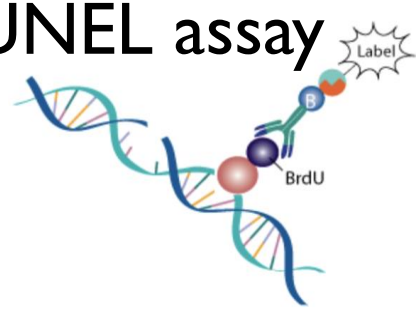
Lynch et al, 2018. *Biometals* (2018) 31:845–858

Se sources differ -protecting from Cd-induced apoptosis

DNA damage



TUNEL assay



Cd-induced apoptosis

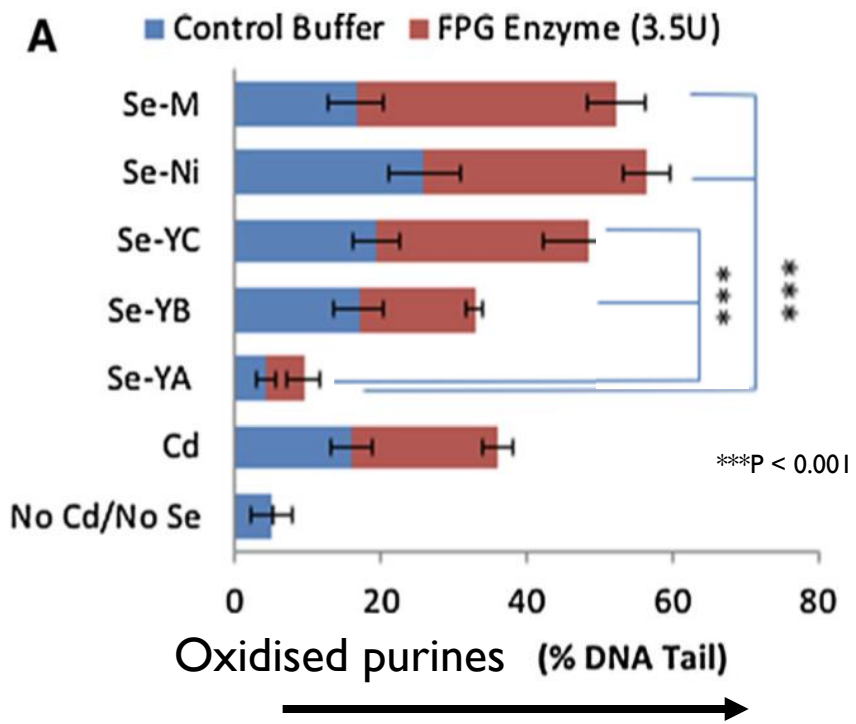
- Inhibited by Se-YA/YB/YC
- Enhanced by Se-Ni and Se-M

Lynch et al, 2018. *Biometals* (2018) 31:845–858

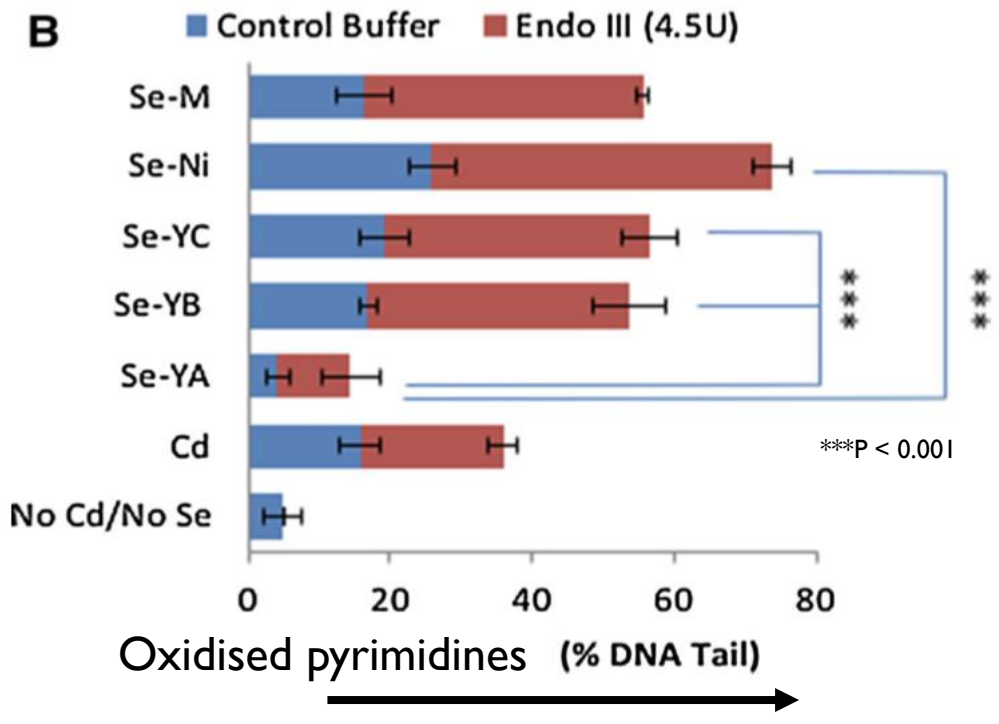
Se sources differ - moderating Cd-induced oxidative base damage

Modified Comet assay

FPG-specific lesions
(oxidised purines, eg. 8-oxoG)



Endo III-specific lesions
(oxidised pyrimidines)

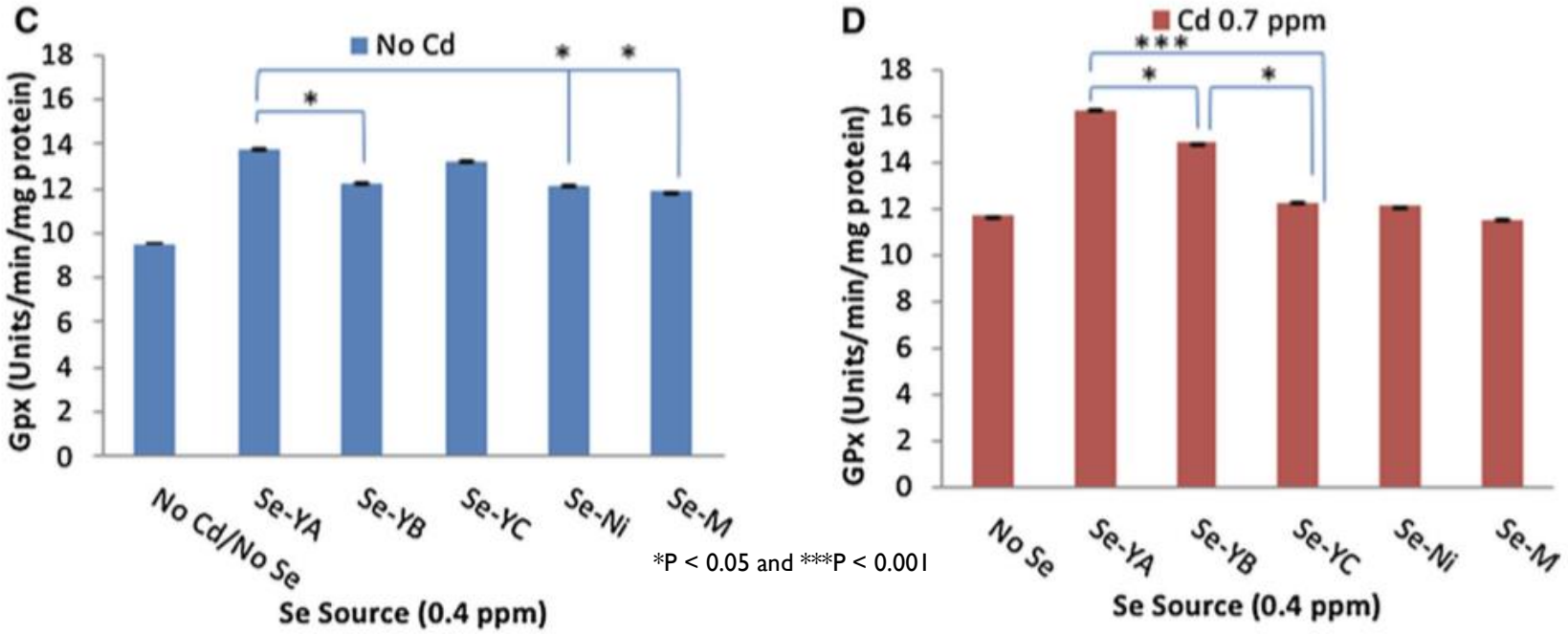


- Se-YA – least Cd-induced DNA base oxidation

Lynch et al, 2018. *Biometals* (2018) 31:845–858

Modulation of anti-ROS enzyme activity by Se sources

Glutathione peroxidase (GpX) activity

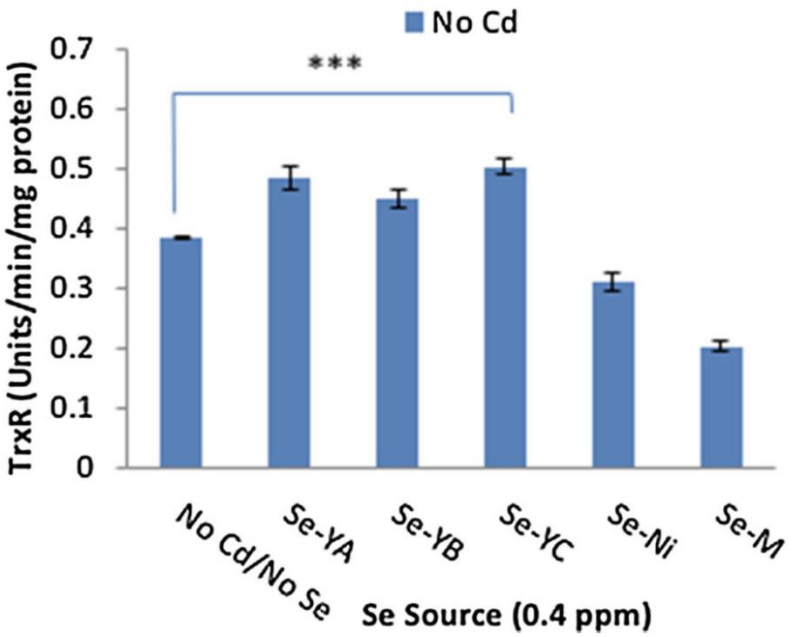


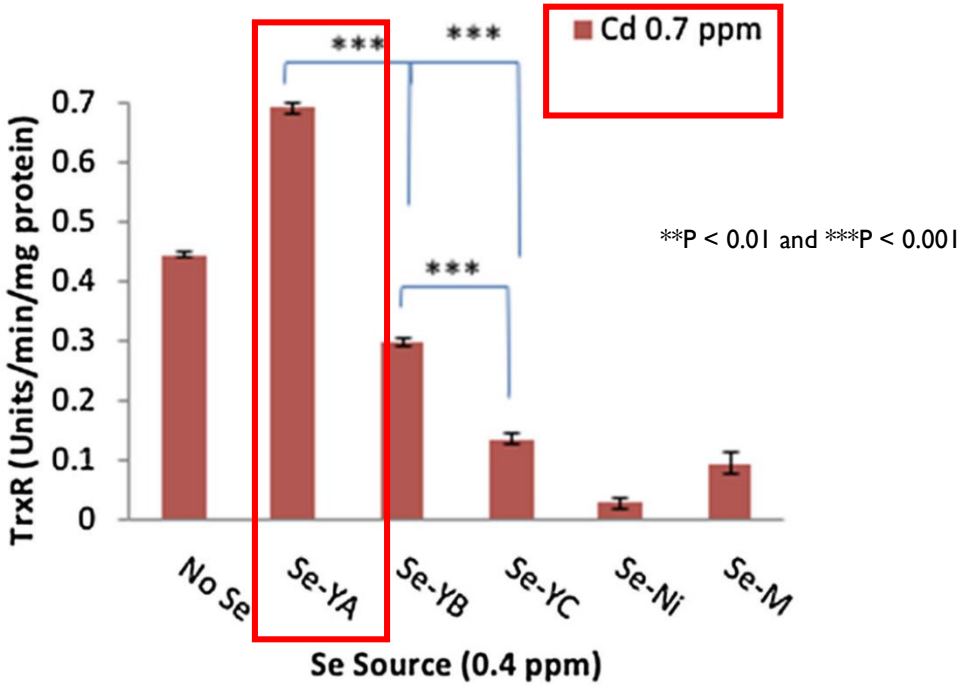
- Se sources all maintained GpX activity following Cd insult
- Se-YA most effective

Lynch et al, 2018. *Biometals* (2018) 31:845–858

Modulation of anti-ROS enzyme activity by Se sources

Thioredoxin reductase (TrxR) activity

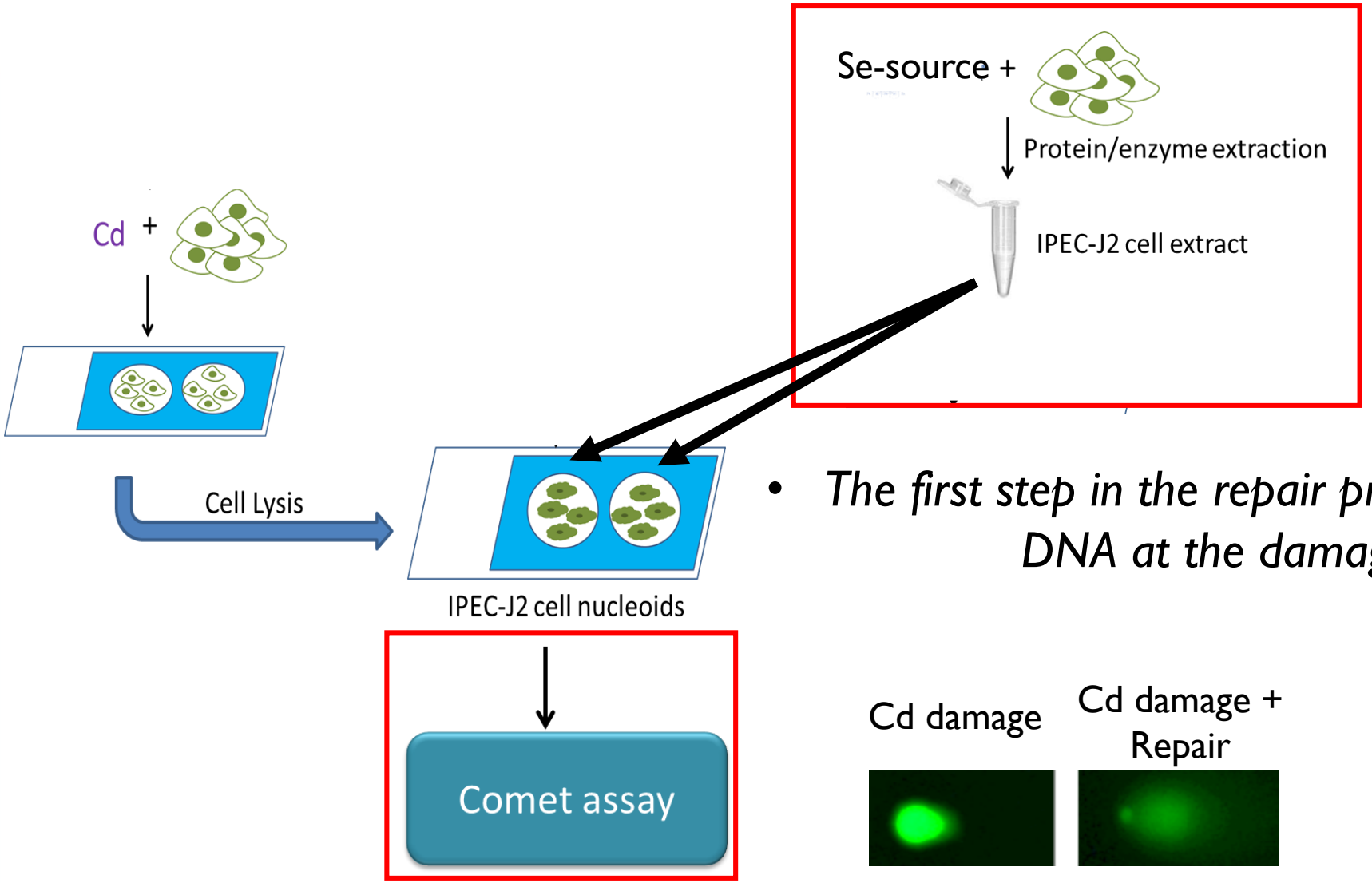




- Only Se-Y sources maintained TrxR activity in absence of Cd
- Only Se-YA maintained TrxR levels in presence of Cd

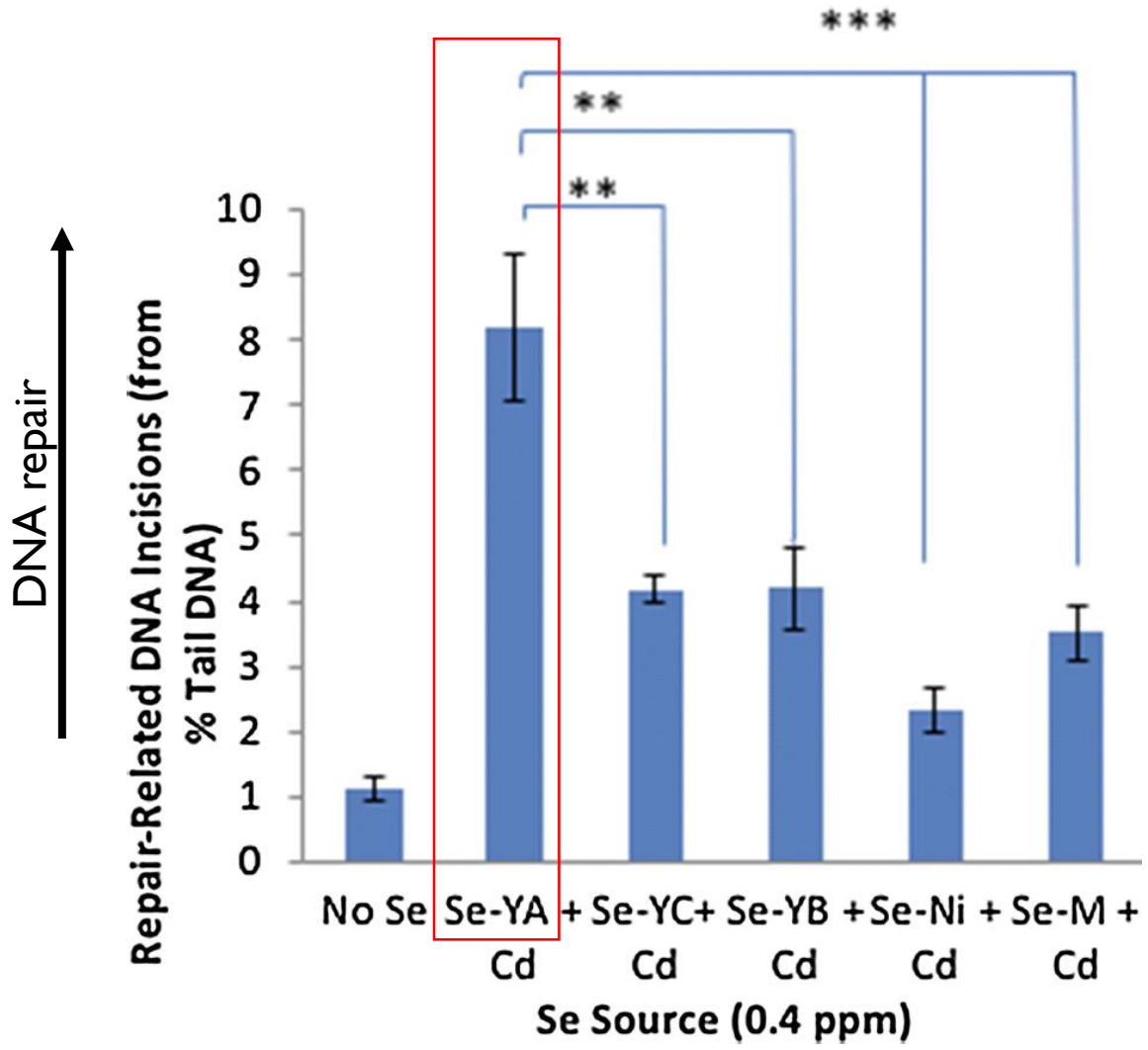
Lynch et al, 2018. *Biometals* (2018) 31:845–858

Measuring DNA Repair activity: Base Excision Repair (BER)



- *The first step in the repair process is cleaving DNA at the damage site*

Enhanced DNA repair activity in Se-Y IPEC-J2

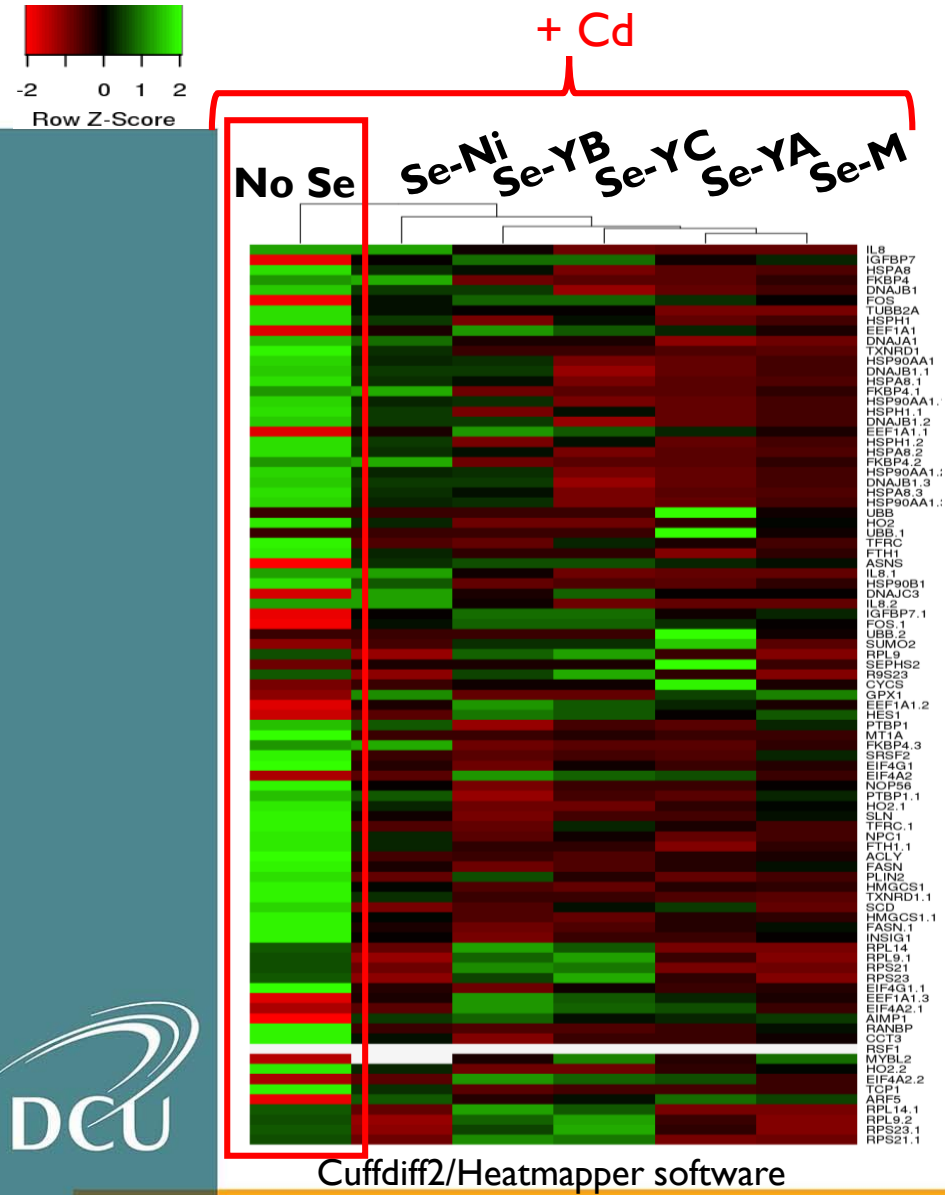


Measuring Base Excision Repair (BER)
-Modified Comet assay

- Most significant enhancement of BER capability with Se-YA

Lynch et al, 2018. *Biometals* (2018) 31:845–858

Effect of Se sources on gene expression in Cd-treated IPEC-J2



Cuffdiff2/Heatmapper software

- Impact of Cd (no Se)
  - Cell response to stress/external stimuli
  - Attenuation phase
  - Heat shock response
  - Protein folding/unfolding response
- Hierarchical clustering
  - Se-Ni response differed from organic Se sources
  - Se-YA - clustered most closely with Se-M
  - Se-YA DNA repair genes

Lynch et al, unpublished

Conclusions

Does nutritional supplementation with Se protect porcine gut cells from damage due to Cd?

- *Levels of DNA breakage*
- *Induction of apoptosis*
- *Extent of DNA base oxidation*
- *Levels of anti-oxidant enzyme activity*
- *Levels of DNA repair activity*
- *Differential Gene expression*

- *Se sources do not all perform the same (when used at EFSA guideline levels)*
- Inorganic Se often enhanced the negative effects of Cd
- Organic Se forms performed significantly better at ameliorating Cd-induced damage
- However - there are important differences in the bio-efficiency of Se-Yeast sources

DCU

Acknowledgments

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Prof. Dermot Walls

School of Chemical Sciences

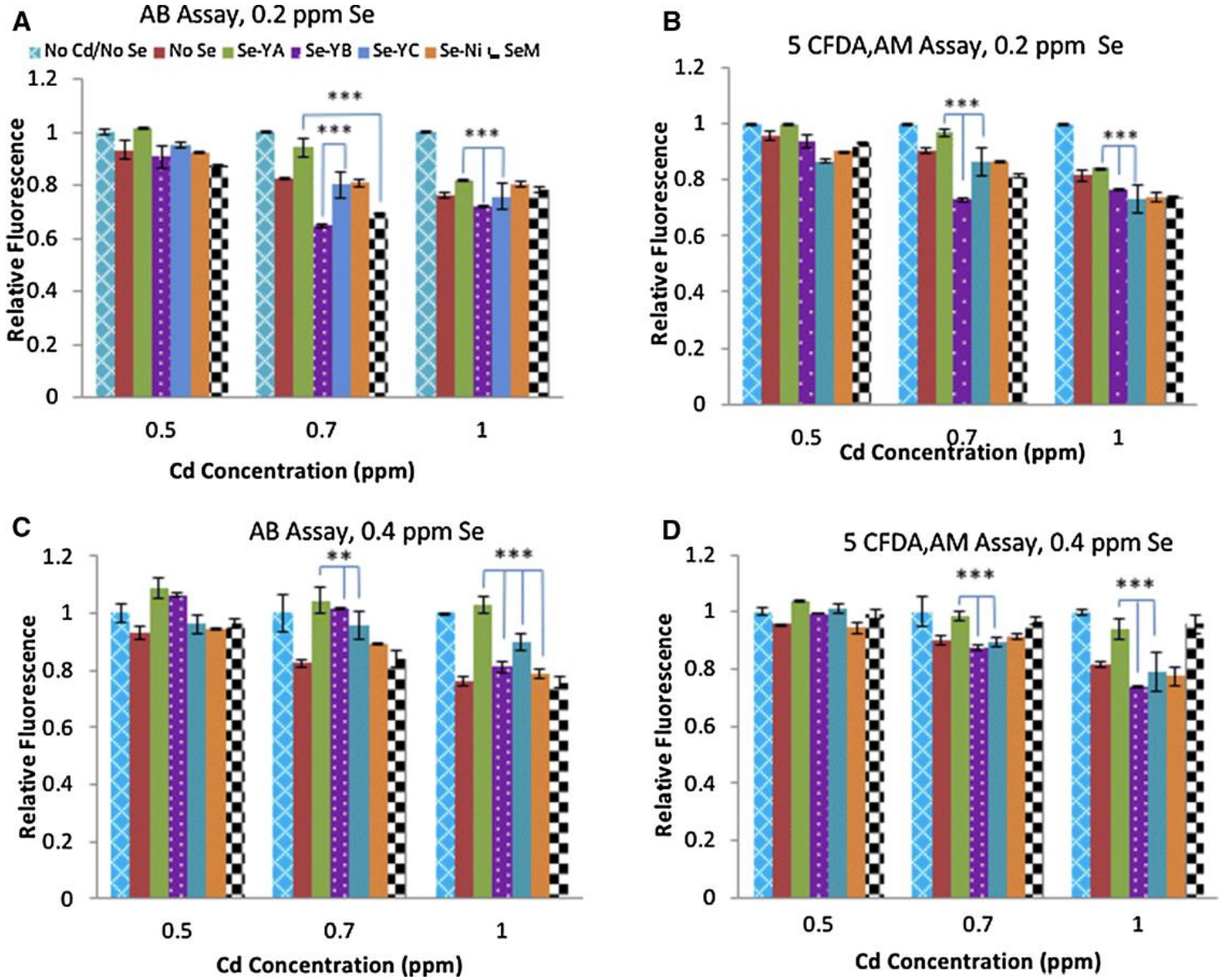
Asmita Thapa
Prof. Blánaid White

Alltech Biotechnology Centre
Dunboyne, Ireland

Dr. Karina Horgan

EU COST Action CA15132 - The comet assay as a human biomonitoring tool (hCOMET)

Obrigado!



Maybe skip this one…?

Lynch et al, 2018. *Biometals* (2018) 31:845–858