THE ROLE OF GLUTATHIONE IN ALLERGIC CONTACT DERMATITIS

SANDRINE SPRIGGS- 28TH SEPTEMBER 2017

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BACKGROUND- ALLERGIC CONTACT DERMATITIS

Allergic Contact Dermatitis (ACD) is a delayed-type hypersensitivity response to external compounds characterised by a visible redness or rash on the skin after successive exposures.

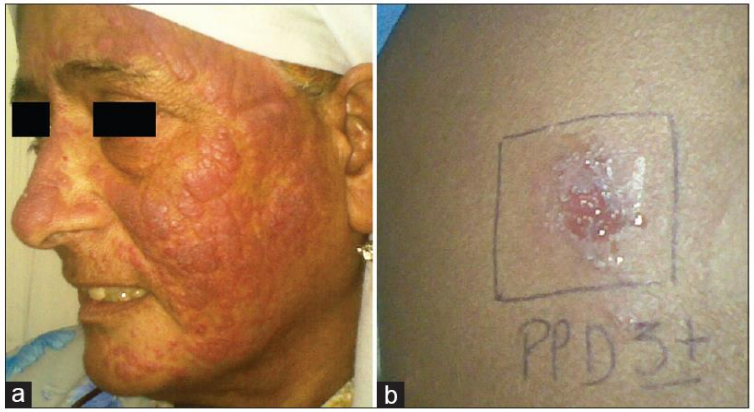
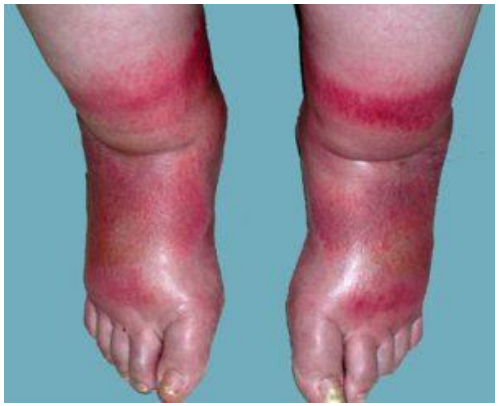


Figure 4: (a) Chronic actinic dermatitis (actinic reticuloid) due to PPD. (b) Patch test shows 3+ reaction to PPD. Gupta et al., Indian Dermatology Online Journal, 2015.

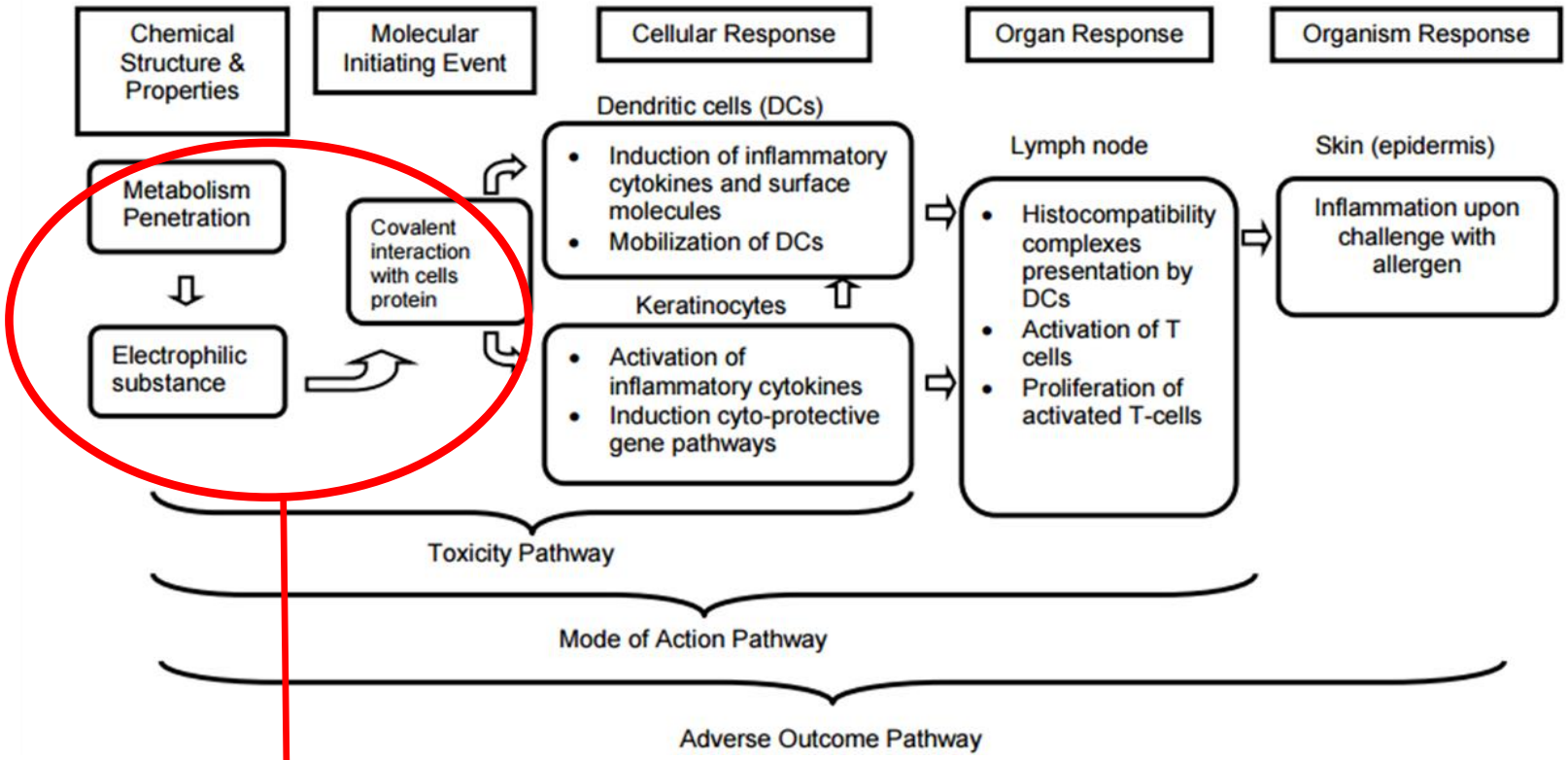
Based on data collected between 1966 and 2007, an average of 20% of the Western European and North American population suffers from contact dermatitis to at least one allergen.



Contact dermatitis can occur from antiseptic agents (povidone-iodine solution, Betadine®). http://iacdworld.org/contact-dermatitis-happ/

In the UK, there are currently 17000 people (estimated) with a skin condition caused or made worse by work. http://www.hse.gov.uk/statistics/causdis/dermatitis/

BACKGROUND- SKIN ALLERGY ADVERSE OUTCOME PATHWAY

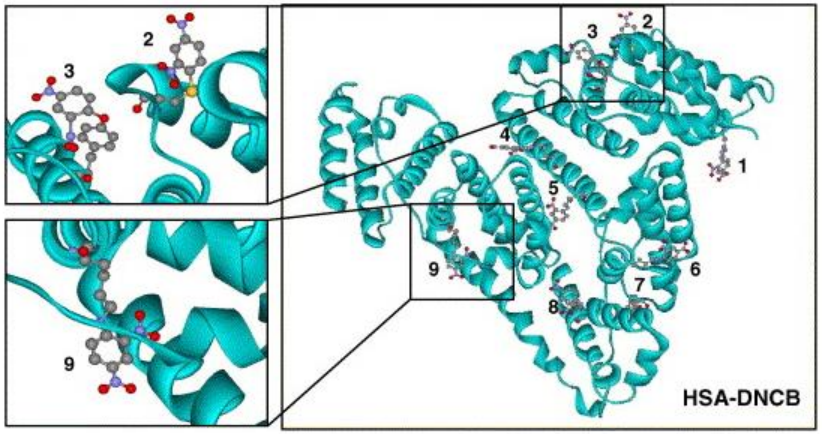


The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins. Part 1: Scientific Evidence.Series on Testing and Assessment.No.168. ENV/JM/MONO(2012)10/PART1

GSH metabolism (conjugates formation) occurs simultaneously to the molecular initiating event of skin allergy (protein haptenation)

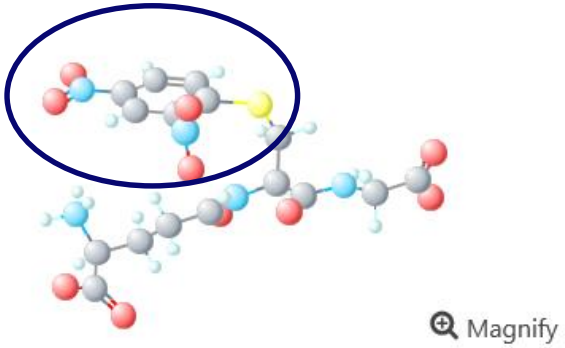
PROTEIN HAPTENATION

Hapten: chemical able to permanently modify the nucleophilic sites available on proteins within the skin.
The protein-hapten adducts resulting from these reactions can be recognised as an antigen by the human body.



DNCB covalently bound to 1)His, 2)Cys, 3)Tyr, 4-9)Lys residues of Human Serum Albumin proteins.
Aleksic et al., Toxicology in vitro, 21, issue 4, 723-733, 2007

Defence mechanisms can operate in the same way. Products formed (i.e metabolites) are small and excreted from the body (here DNP-SG) .



DNCB covalently bound to GSH

BACKGROUND- IN CHEMICO/ IN VITRO TEST FOR SKIN SENSITISATION

In chemico test assessing skin sensitisation

OECD guideline: Test 442C
Direct Peptide Reactivity Assay

Assess cysteine reactivity

Ac-RFAACAA-COOH +100mM Chemical
1:10 ratio, incubation at 25°C for 24h
Measure peptide depletion by HPLC.
Positive result: over 14% depletion

In vitro test assessing skin sensitisation

OECD guideline: Test 442D
KeratinoSens™

ARE-Nrf2 luciferase test method

Modified keratinocytes (containing a luciferase gene)
Cultured with test chemical for 24h.
Cell viability should be 70% or over.
Positive result if 50% increase in luciferase activity.

-> Indirect measure of cysteine reactivity

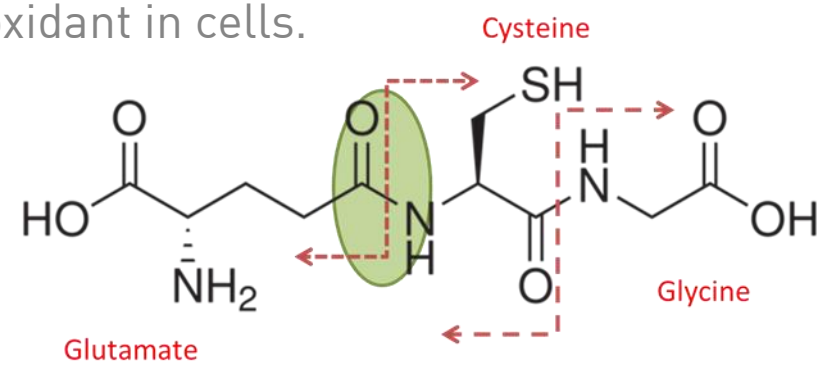
GLUTATHIONE

What is glutathione?

- GSH is an endogenous tripeptide (Glu-Cys-Lys) present intracellularly, mostly in the cytosol.

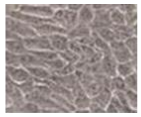
- It has a free thiol that can react in the same way as cysteine residues on proteins. Small GSH conjugates are subsequently excreted and are mostly considered non-allergenic.

- GSH also exhibits a protective effect against ROS or any allergen with oxidative properties as it is oxidised into GSSG (disulfide). It's the most prominent antioxidant in cells.



Glutathione with characteristic γ-glutamyl bond (green)

RESEARCH OUTLINE



Cell lines (here HaCaT)

3D skin models (here Reconstructed Human Epidermis, Episkin, France)

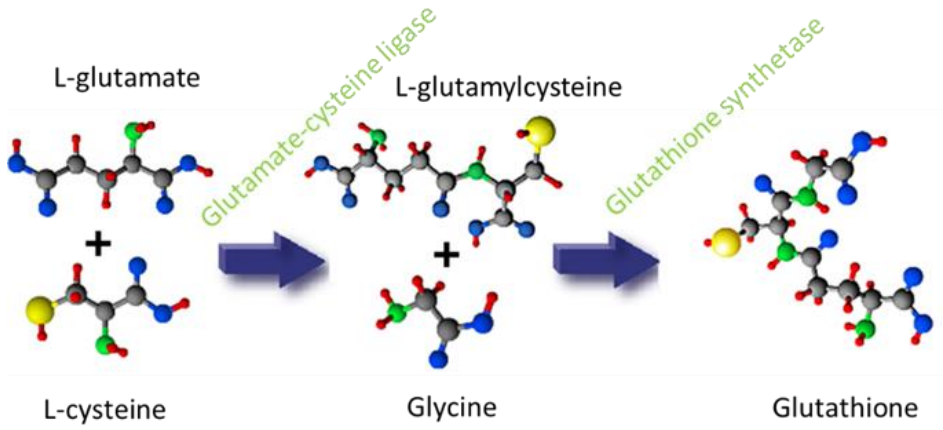
Fresh ex vivo human skin in culture
(Difficult to source, ethical issues)

Characterise the GSH cycle in skin using in vitro models (cells, 3D reconstructed epidermis).

Investigate detoxification of model sensitisers by the GSH pathway *in vitro.*

Study the metabolic capacity of Reconstructed Epidermis after multiple exposures to a model skin sensitiser.

GSH METABOLISM IN SKIN



Intracellular GSH synthesis

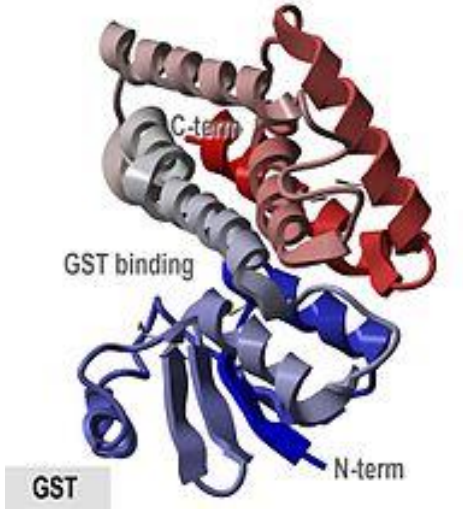
GSH is present in the low to mid hundreds of nmol/g tissue (Shindo *et al* ,1994, Rhie *et al*, 2001 and Kaur *et al,* 2001)

<u>GST activity in human skin:</u>

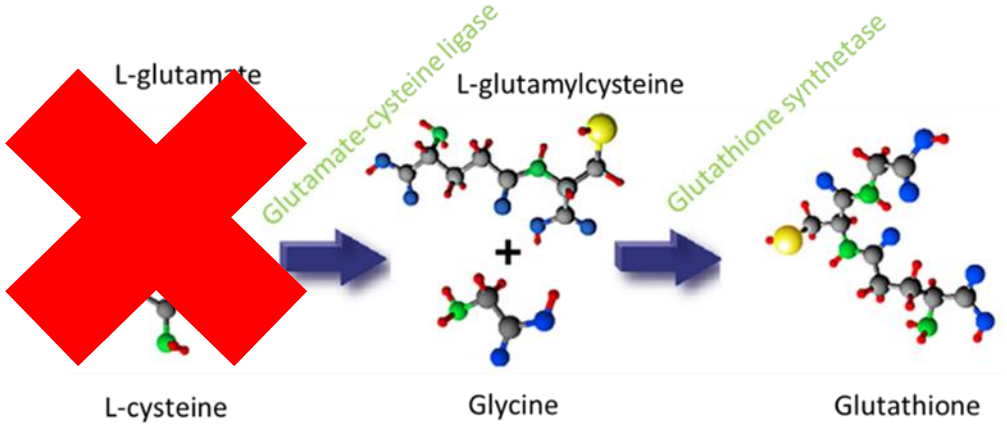
30 nmol/min/µg cytosolic protein (epidermis)(Harris et al, 2002)
90 nmol/min/mg of whole skin (van Eijl et al, 2012)

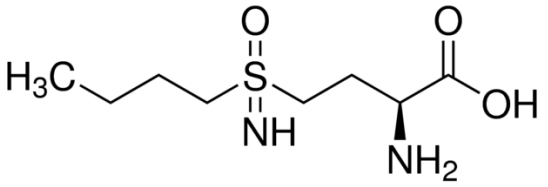
<u>GST activity in human liver:</u>

119nmol/min/mg (Baars et al, 1981)

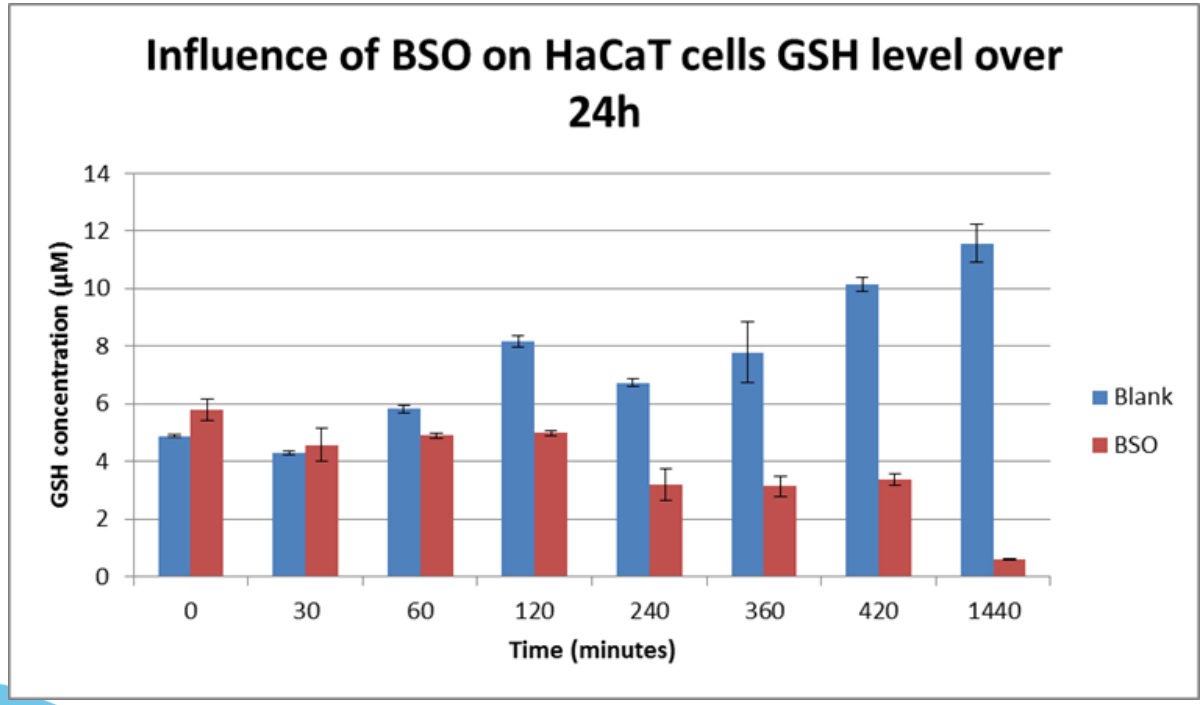


GSH SYNTHESIS IN KERATINOCYTES



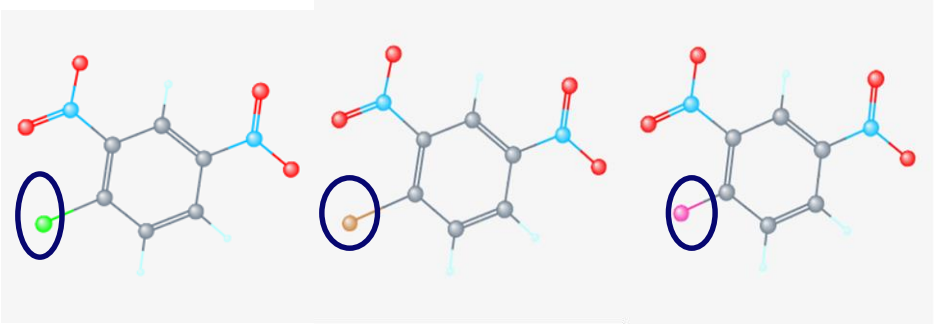


L-buthionine-sulfoximine
Used as a GCL inhibitor

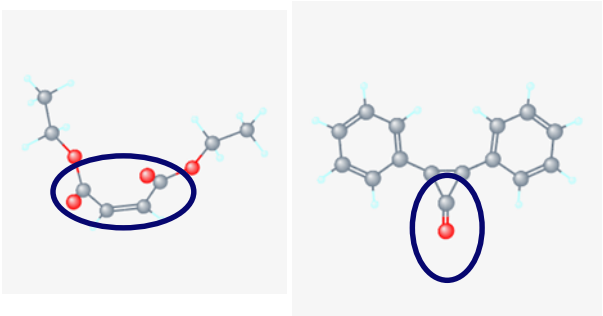


CHOICE OF MODEL SENSITISERS

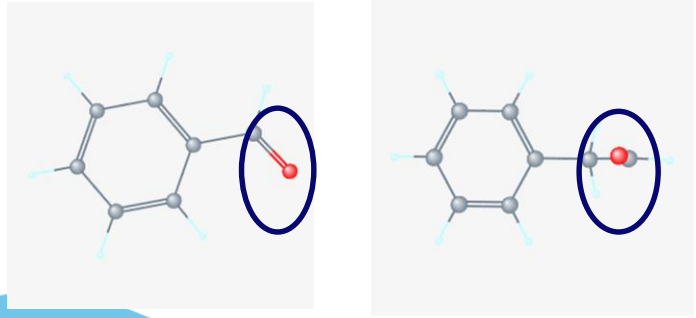
3 related dinitrohalobenzenes: DNCB, DNFB and DNBB



2 alpha-beta unsaturated ketones (prone to Michael addition): DEM and DPCP

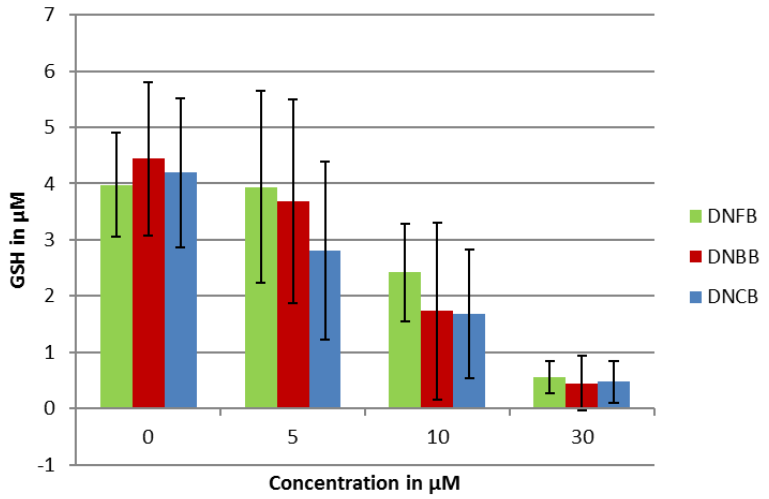


2 aldehydes with potential for cysteine oxidation: BA and PA



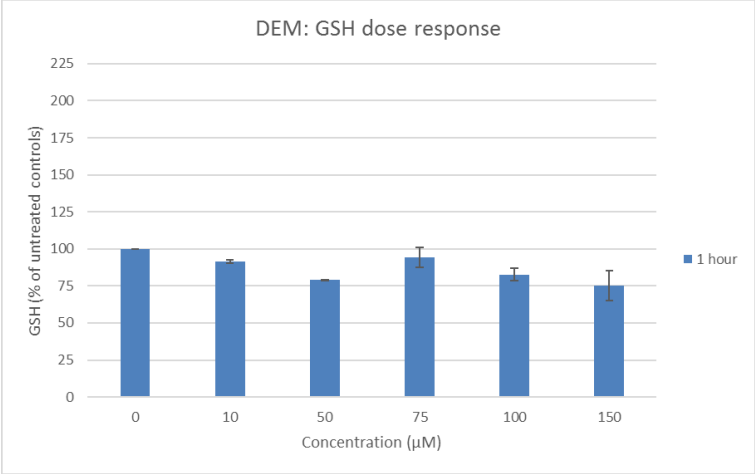
GSH DEPLETION BY ELECTROPHILES

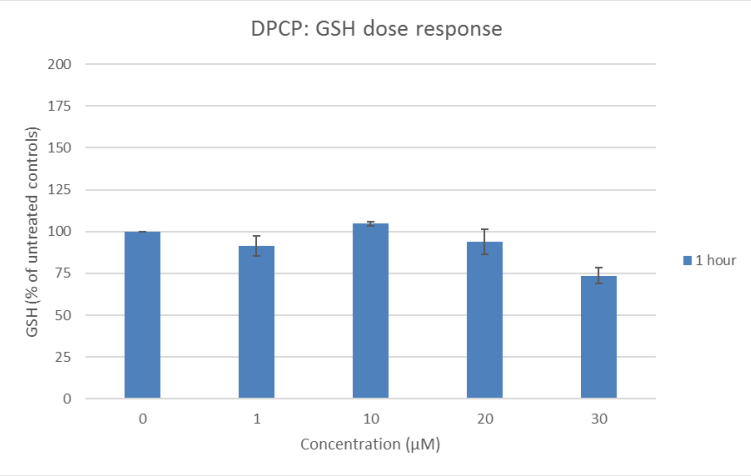
GSH level measured after 1 hour



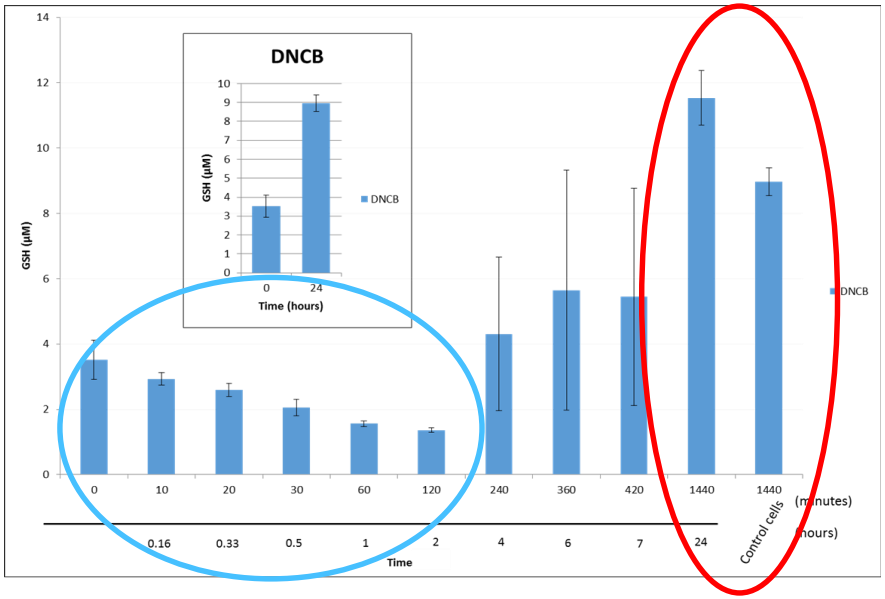
✓ GSH depletion is significant for all dinitrohalobenzenes

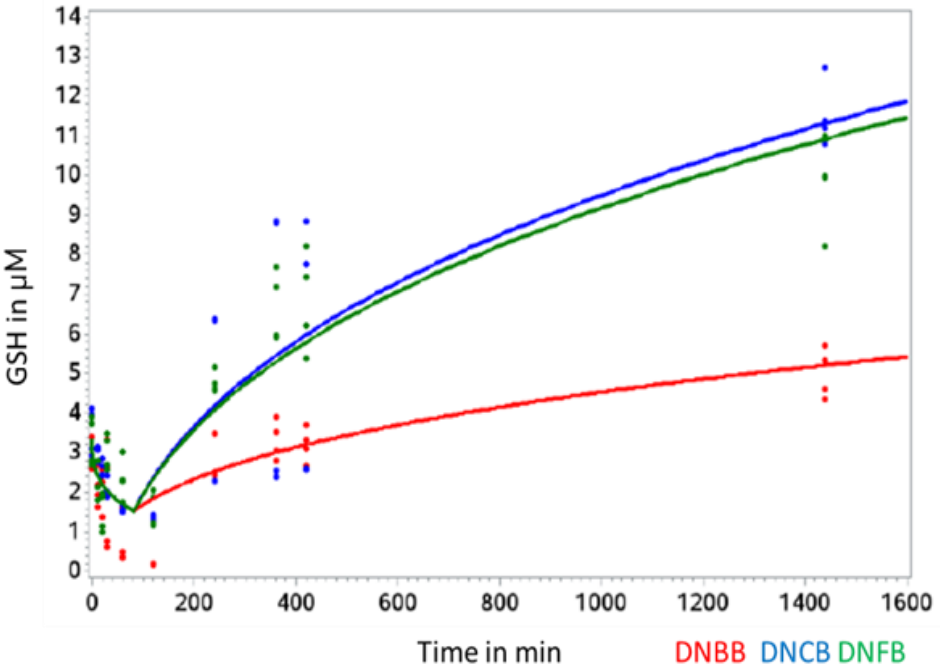
X GSH depletion is difficult to see for DEM and DPCP without increasing doses above toxic levels (cell death over 20% after 24h exposure)





GSH UPREGULATION: DINITROHALOBENZENES

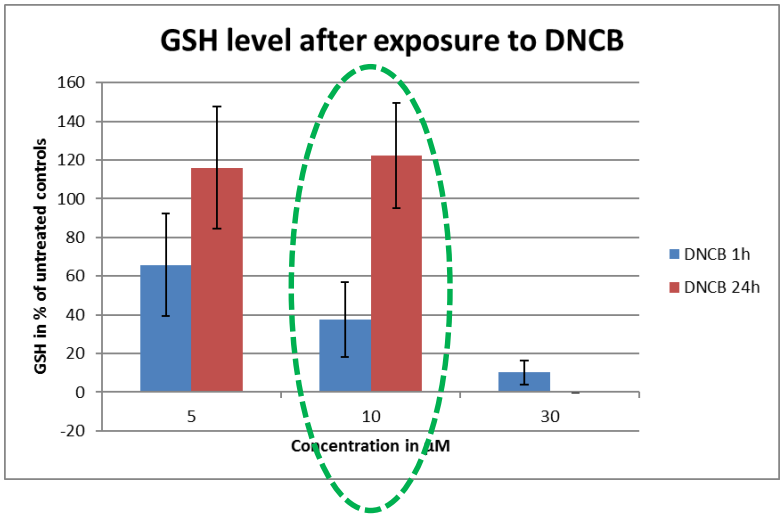




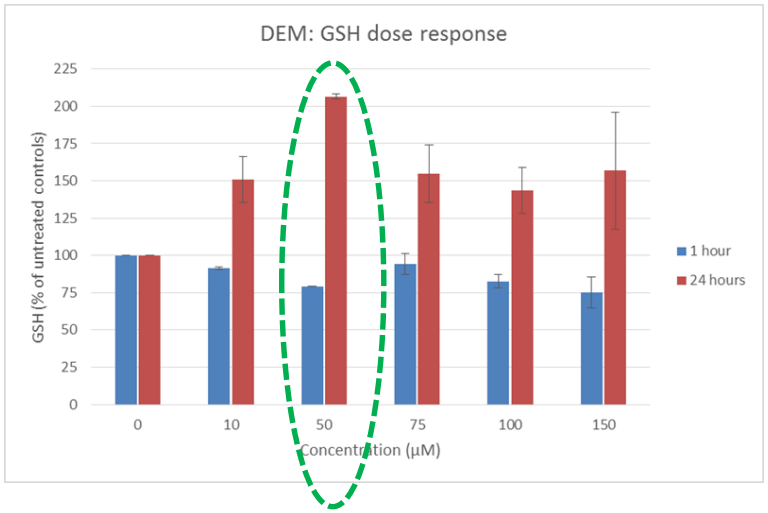
- ✓ 10µMDNCB dose depletes GSH stock within 2h
- ✓ Stock is replenished after 24h
- ✓ GSH upregulation compared to untreated cells
- ✓ Upregulation level is a differentiating factor for related chemical reacting via the same mechanism

GSH REPLETION AFTER DOSING

✓ GSH upregulation level cannot be anticipated from the level of depletion



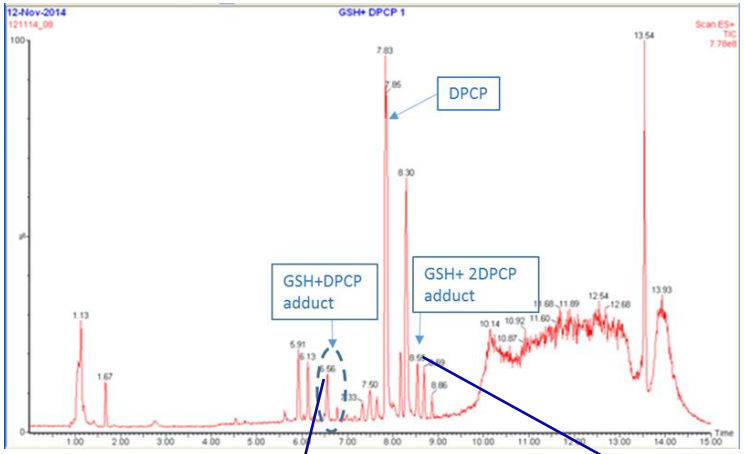
GSH upregulation of 20% compared to untreated cells observed after a 60% depletion measured at 1h

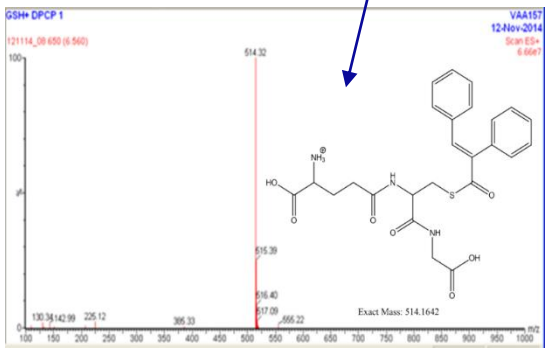


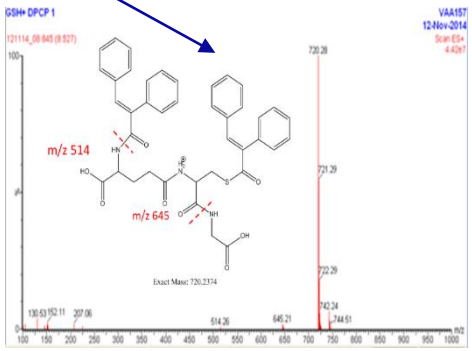
GSH upregulation of 100% compared to untreated cells does not correlate with the 25% depletion observed at 1h

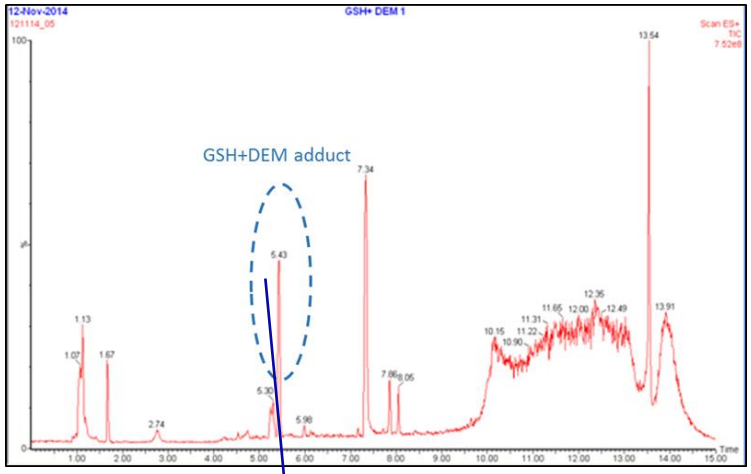
GSH REACTIVITY: IN CHEMICO VS IN VITRO

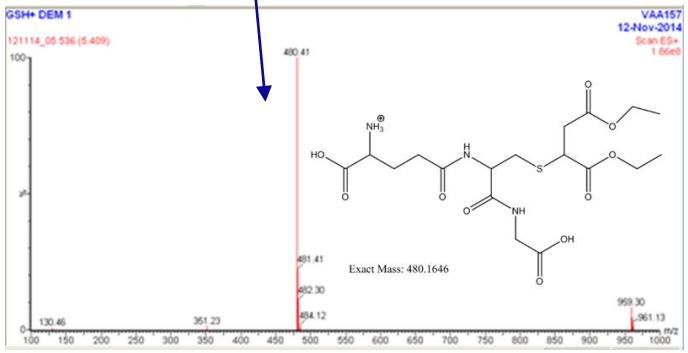
Bioavailability is a key factor to consider: GSH reacts with both DEM and DPCP *in chemico*





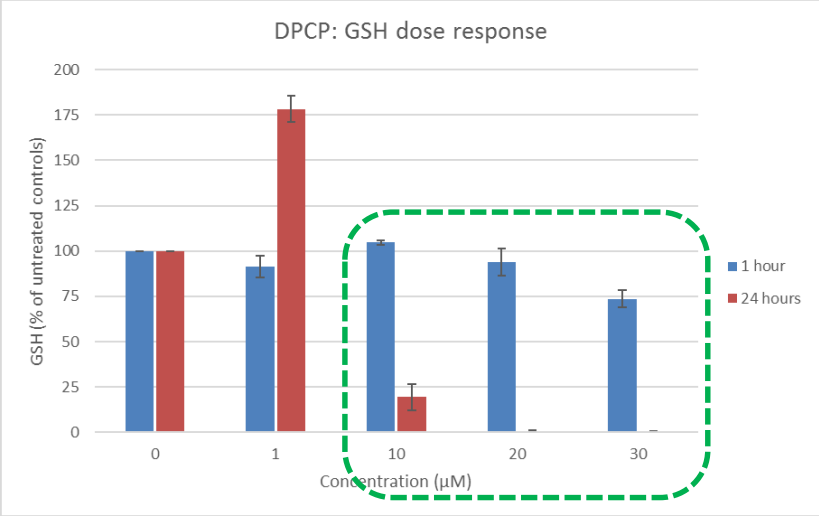


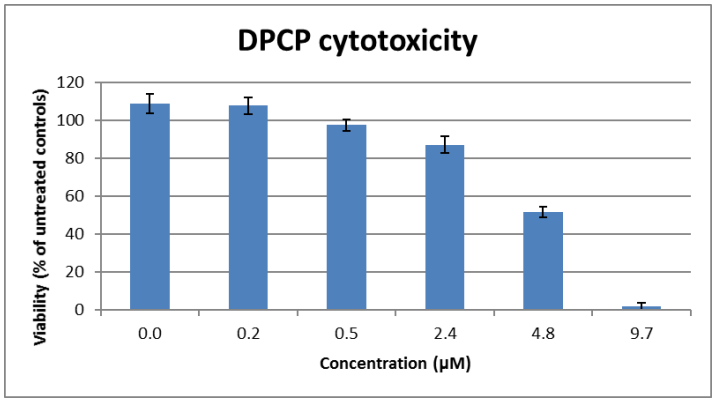




GSH REPLETION AFTER DOSING

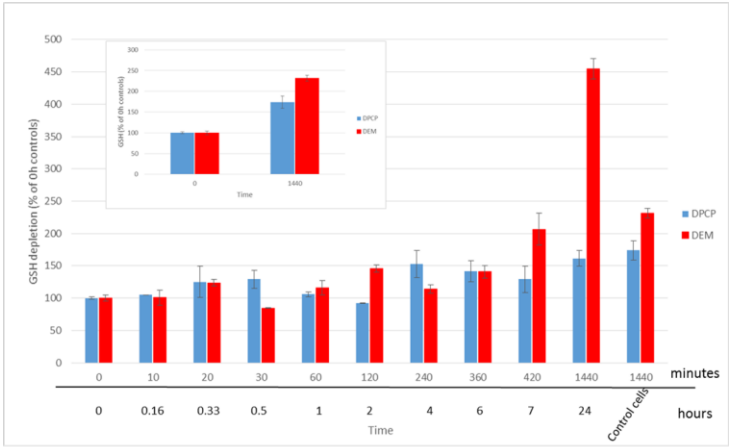
But DPCP reactivity with GSH *in vitro was minimal. Toxicity was observed at low concentrations…*





>10µM inducing 100% cell death at 24h

…while DEM depleted GSH at a rate similar to *de novo* GSH synthesis before synthesis rate overtook clearance as the dose to clear diminished.

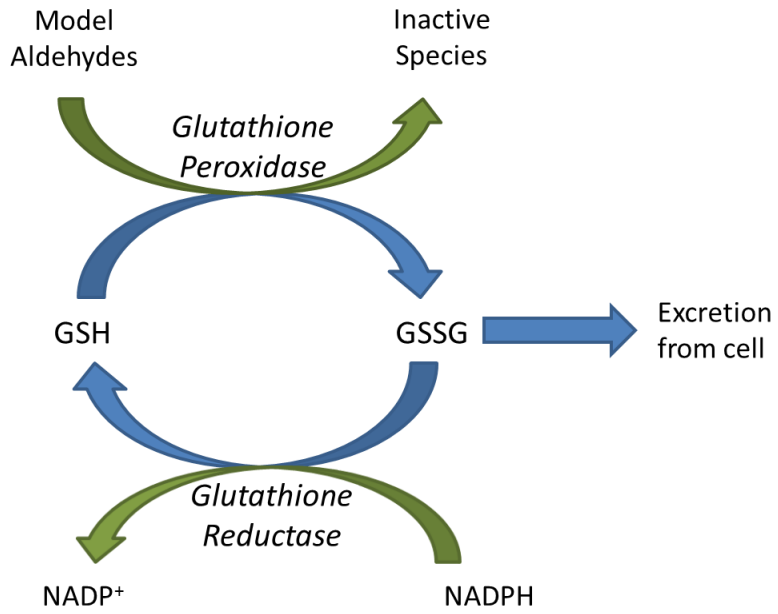


GSH IN OXIDO-REDUCTIVE MECHANISM FOR CLEARANCE: BA AND PA

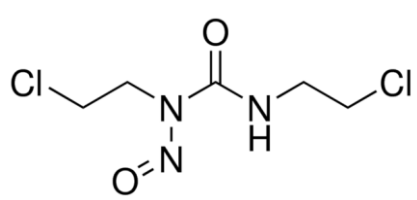
Expected reactivity with thiol: *in chemico* both BA and PA have shown to deplete Cys residues by oxidising the small peptide studied into a dimer.

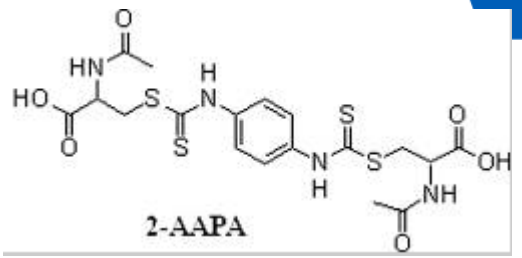
Chemical	Potency category (%EC3)[a]	Cys (pH 7.4) % dep	Cys (pH 7.4) SD	Lys (pH 10) % dep	Lys (pH 10) SD
Oxazolone	Extreme (0.003)	82.3	0.38	99.2	0.17
Benzoquinone	Extreme (0.0099)	100	0.00	100	0.00
DNCB	Extreme (0.06)	97.4	0.31	97.7	0.90
4-Nitrobenzyl bromide	Extreme (0.05)	95.0	0.48	100	0.00
Glutaraldehyde	Strong (0.1)	74.4	1.15	100	0.00
Hydroquinone	Strong (0.11)	100	0.00	98.2	0.26
PPD	Strong (0.16)	99.0	0.82	25.0	5.61
Benzyl bromide	Strong (0.2)	100	0.00	77.3	1.61
n-Dodecyl gallate	Strong (0.3)	72.8	3.77	85.3	2.53
Formaldehyde	Strong (0.61)	31.5	2.16	26.2	6.26
Isoeugenol	Moderate (1.7)	90.4	0.14	96.1	0.71
MPT	Moderate (1.4)	100	0.00	61.5	10.5
Glyoxal	Moderate (1.4)	−7.23	1.50	42.7	1.41
2-Hydroxyethyl acrylate	Moderate (1.4)	100	0.00	100	0.00
Trans-2-decenal	Moderate (2.5)	91.7	0.38	57.9	3.77
Cinnamaldehyde	Moderate (3.0)	97.3	0.00	58.8	1.64
Phenylacetaldehyde	Moderate (3.0)	100	0.00	100	0.00
2,4-Heptadienal	Moderate (4.0)	90.8	0.39	90.7	2.02
3,4-Dihydrocoumarin	Moderate (5.6)	−5.13	2.84	38.1	3.20
12-Bromo-1-dodecanol	Moderate (6.9)	51.1	11.6	13.0	8.63
Hexylcinnamaldehyde	Weak (11)	7.59	1.90	6.27	3.21
1-Bromododecane	Weak (18)	74.4	11.3	10.2	1.90
Phenyl benzoate	Weak (20)	76.2	3.16	20.3	5.60
Cinnamyl alcohol	Weak (21)	22.7	1.13	8.38	3.17
Cyclamen aldehyde	Weak (22)	33.5	2.79	7.19	5.48
Ethyl acrylate	Weak (28)	92.4	0.14	94.0	2.75
Hydroxycitronelal	Weak (33)	72.7	4.18	10.9	6.48
Glycerol	Nonsensitizer	−1.47[b]	2.07	1.04	2.23
1-Bromobutane	Nonsensitizer	11.0	4.68	0.04	1.46
2-Acetylcyclohexanone	Nonsensitizer	74.8	1.80	49.6	1.03
6-Methyl coumarin	Nonsensitizer	−7.59	0.78	12.7	3.48
Salicylic acid	Nonsensitizer	−8.59	2.87	−9.06	5.93
Lactic acid	Nonsensitizer	−12.7	0.38	1.16	0.26
Benzaldehyde	Nonsensitizer	100	0.00	26.7	0.14
2-Hydroxypropyl methacrylate	Nonsensitizer	100	0.00	33.1	1.65

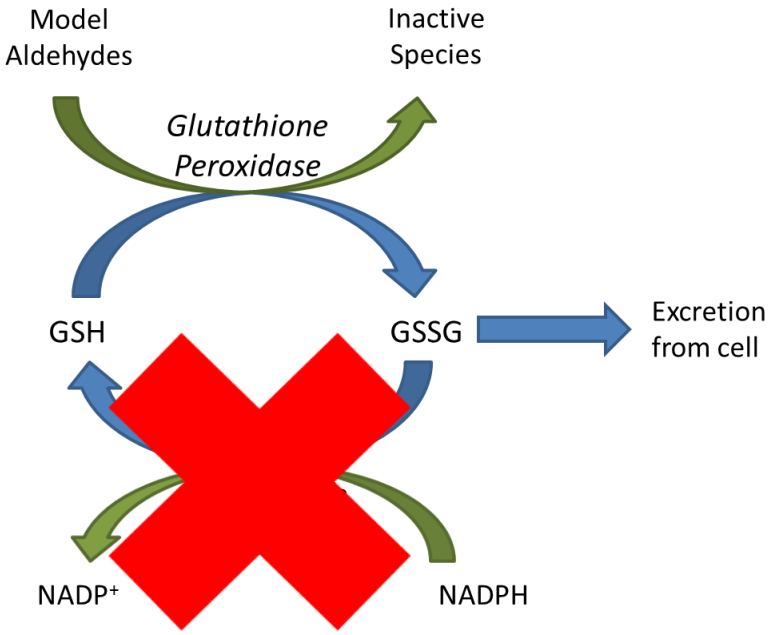
Hence, GSH is expected to be oxidised into GSSG *in vitro*



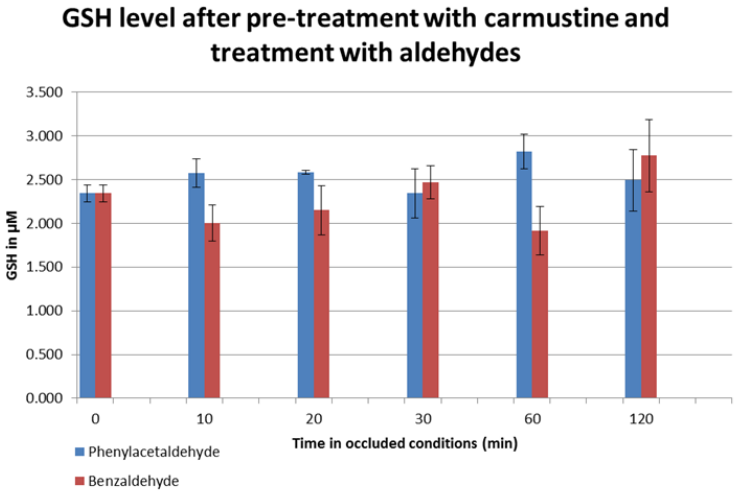
GSH IN OXIDO-REDUCTIVE MECHANISM FOR CLEARANCE: BA AND PA





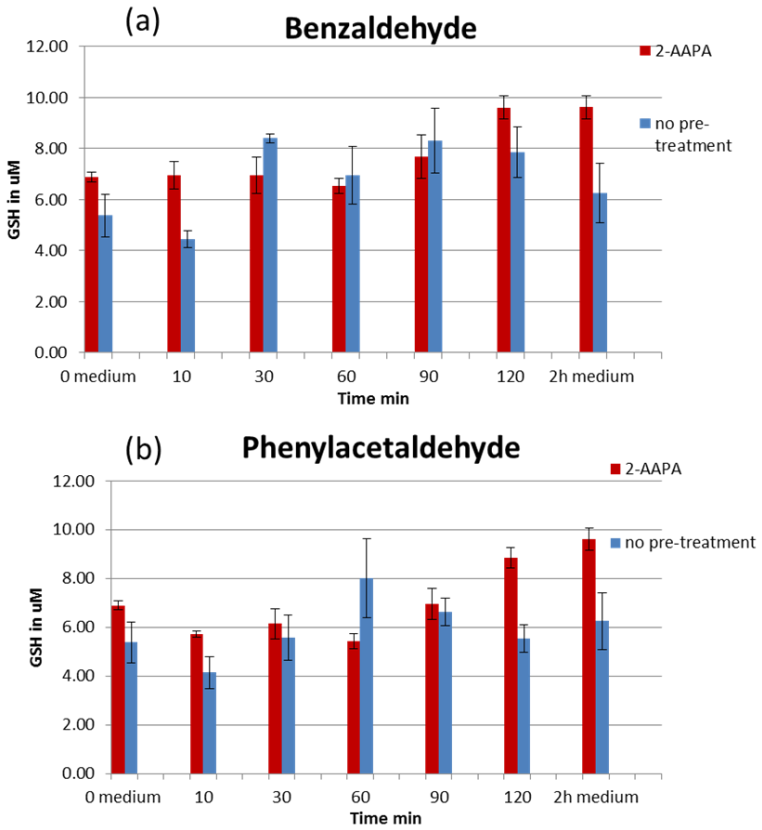


Carmustine and 2-AAPA are two GR inhibitors

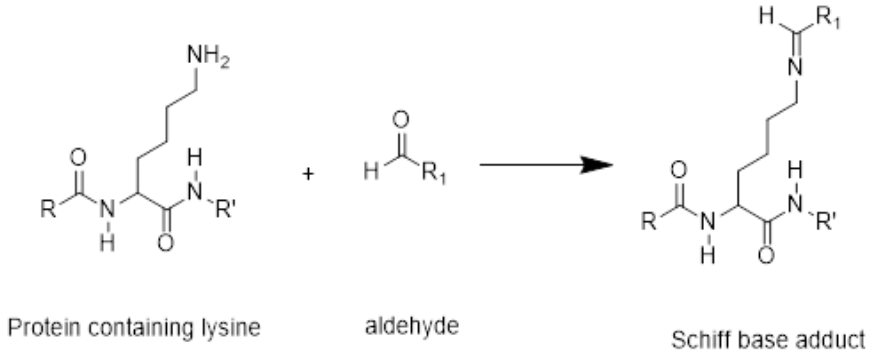


GSH IN OXIDO-REDUCTIVE MECHANISM FOR CLEARANCE: BA AND PA

GSH 2h time course after pre-treatment with 2-AAPA



- Conclusion: GR inhibition did not facilitate GSH-> GSSG by aldehydes
- In vitro aldehydes might be more likely to react with amines via Schiff base



CONCLUSION ON CELL LINE EXPERIMENTS

- The GSH pathway is an important clearance pathway for electrophiles such as dinitrohalobenzenes that generate conjugates with GSH.

- Antioxidant properties can be more difficult to modulate (GSH depletion due to oxidation into GSSG is concomitant with cell death)

- GSH clearance in vitro cannot be extrapolated from *in chemico* data alone

  -> need to consider availability of other nucleophilic sites (ex: amines for aldehydes)

  -> need to consider general bioavailability of the compound (Can it penetrate into the cells? Is it volatile?)

- HaCaT cells exposure to sensitisers is limited by time as cell division will occur within the well. A model stable for several days is required to mimic *in vivo* exposure more realistically (i.e multiple exposures scenario).

RECONSTRUCTED EPIDERMIS MODEL

4cm² RHE model (in insert)

Models are grown from keratinocytes:

Sold after 17days of culture
Size: 0.5cm² up to 4cm²
Stable for several days in growth medium

Specificities of this model:

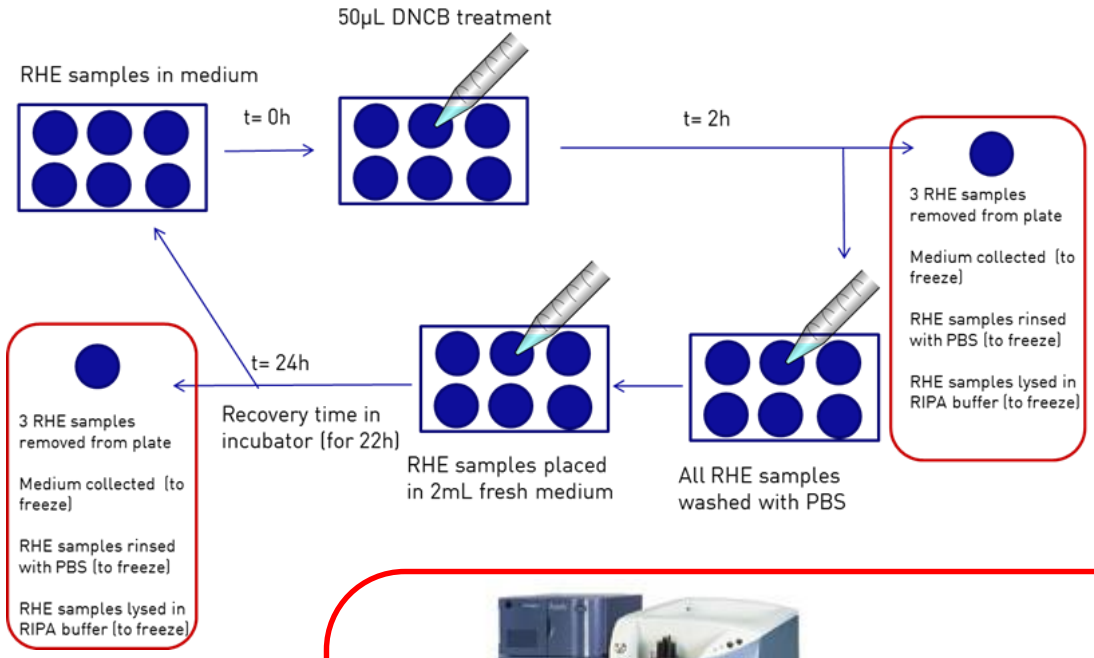
- Different levels of specialisation of the keratinocytes (closer to ex vivo skin than HaCaT cell line)
- Readily available for purchase

-> Could be used to investigate successive exposures to sensitisers thus mimicking product use, drug treatment, repeated exposure on place of work…

Time point in h (day)	GSH (in µg/mg soluble protein)
0 (1)	5.63
24 (2)	5.40
48 (3)	5.04
72 (4)	6.39
96 (5)	7.68
144 (7)	6.15

GSH level in RHE models cultured for 7 days in RHE growth medium is consistently in the µg/mg soluble protein range

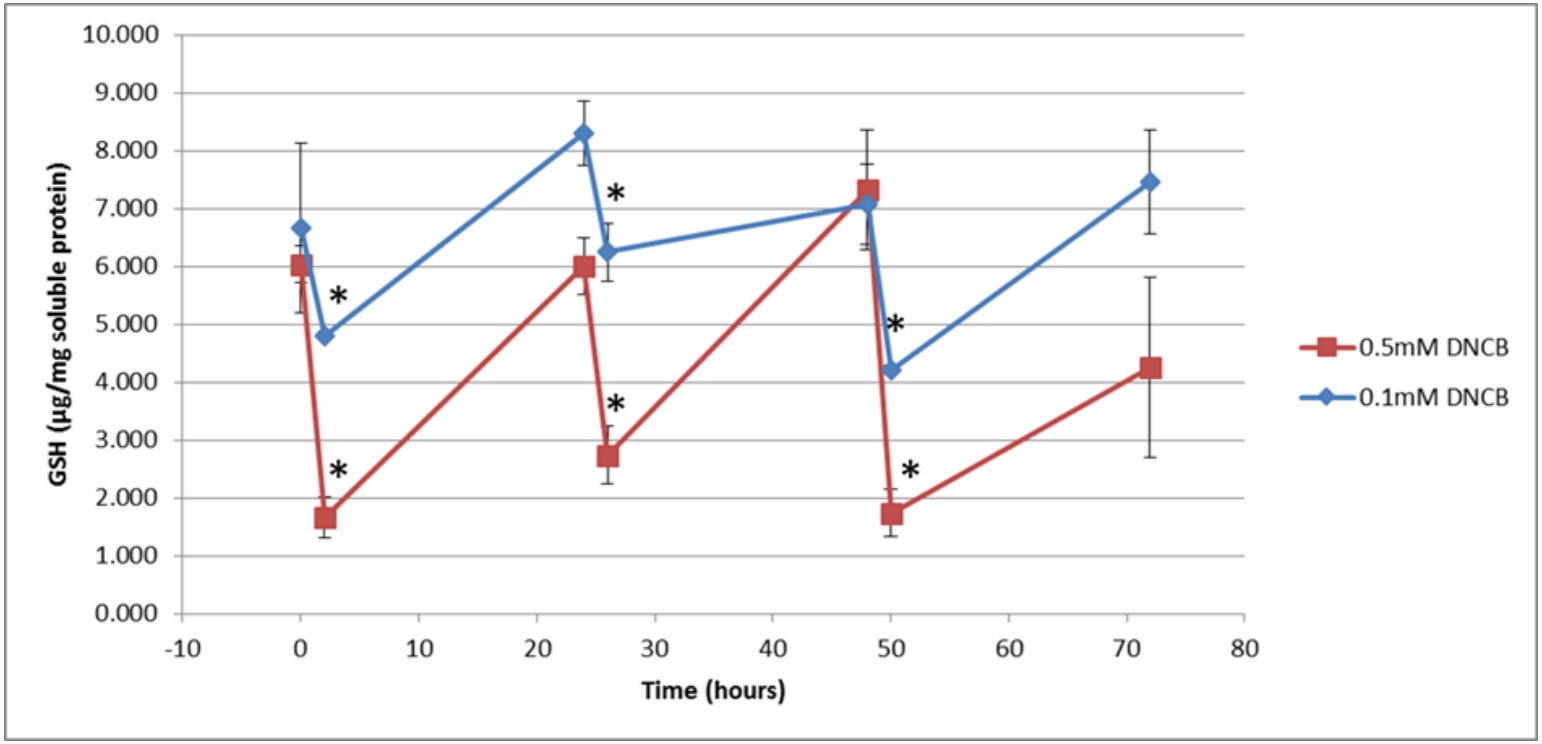
"REPEATED DOSE": EXPERIMENTAL SET UP



LC-MS analysis :

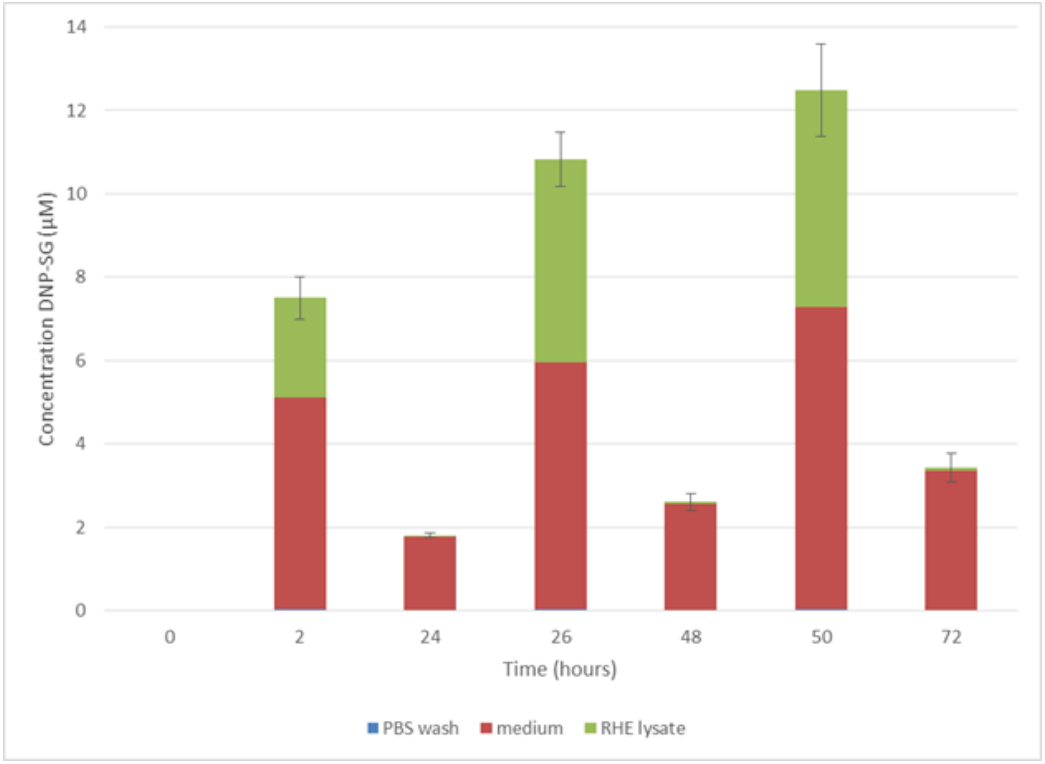
GSH depletion/repletion cycle (in RHE)
DNP-SG formation (in RHE and medium)

DNCB DOSING: "REPEATED DOSE"



GSH level decreases within the two hours of exposure to DNCB
GSH is replenished top basal level within the 22h recovery period

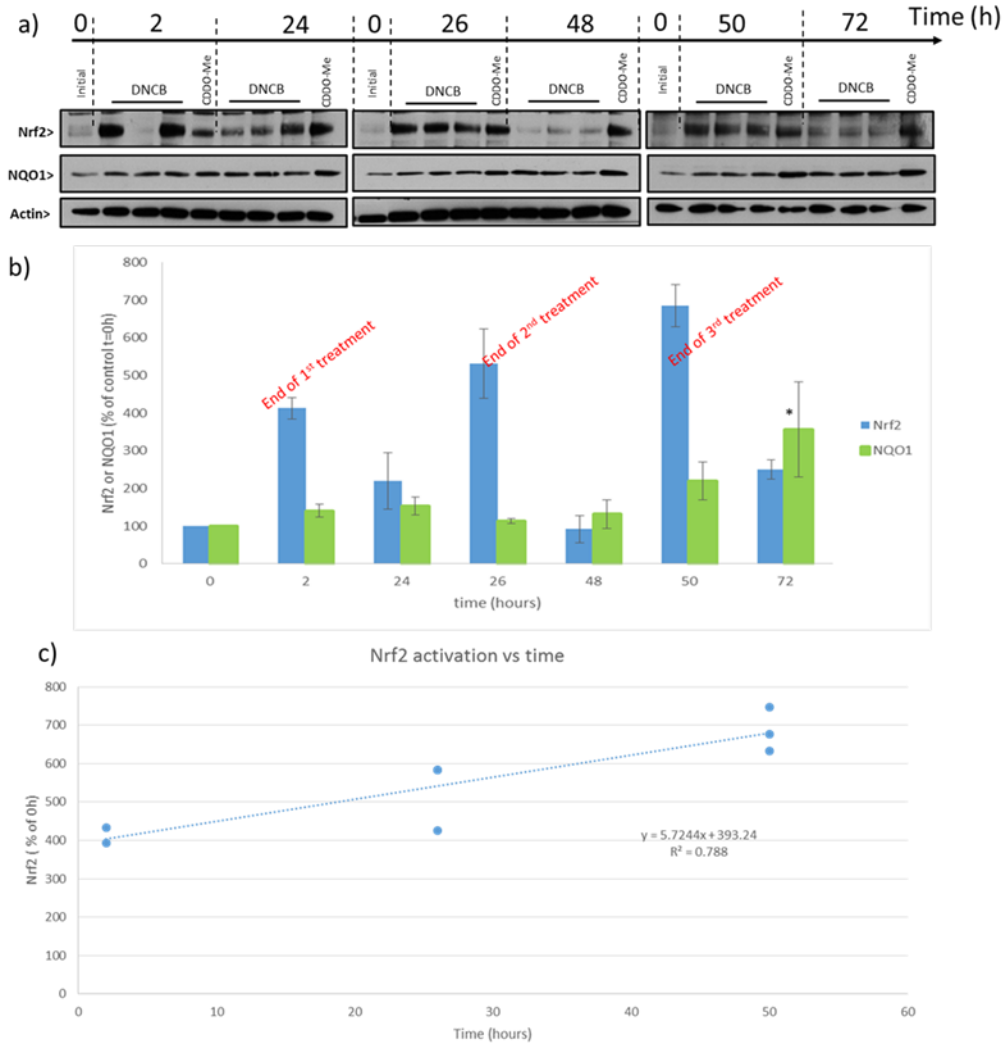
DNCB DOSING "REPEATED DOSE"



DNP-SG formed during the two hours of exposure (in medium and model) but also during the recovery period (despite the model being rinsed with PBS and placed in fresh medium).

GSH upregulation results in more DNP-SG formed after each exposure cycle.

"REPEATED DOSE": NRF2 ACTIVATION



- Nrf2 acts like a rapid "ON/OFF switch" that induces the production of the Phase II enzymes of the GSH pathway
- The amount of Nrf2 released in the cytosol increases after each exposure to DNCB

CONCLUSION

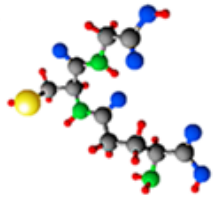
GSH synthesis in skin models is sufficient to detoxify small amounts of model sensitisers and does not rely on "GSH import" from the pool generated elsewhere in the body (liver).

Dual role of GSH (antioxidant and conjugating agent) fully functional in skin.

GSH pathway can be induced to enhance skin defence systems *in vitro* (Nrf2 activation is linked to GCL upregulation for increased GSH synthesis and GST upregulation for increased GSH conjugation and GR upregulation for GSSG recycling).

In vitro to *in vivo* extrapolations should include the potential for induction after several exposures. Skin biology needs to be integrated into mathematical models that rely on reactivity data to assess the potential for skin sensitisation.

Skin metabolism as a defence system include other pathways that should be investigated (Phase II enzymes).



Glutathione

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THANK YOU