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THE ROLE OF GLUTATHIONE AS A CLEARANCE SYSTEM IN SKIN

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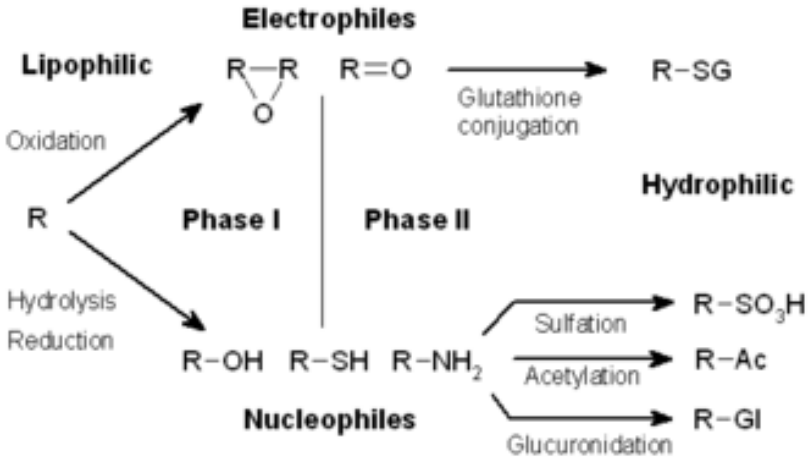
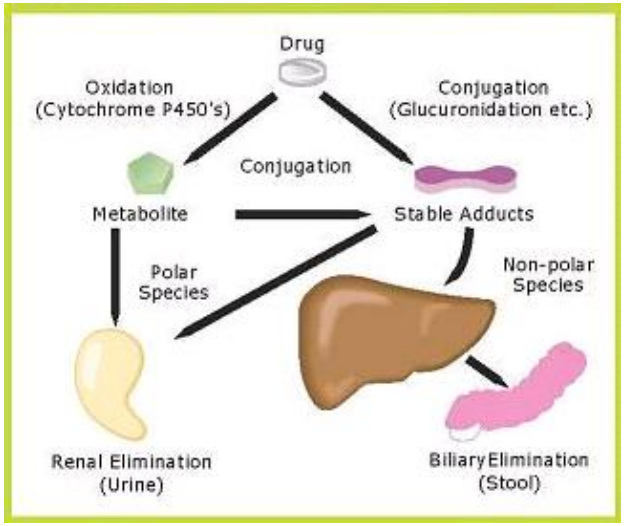
BACKGROUND - METABOLISM

Detoxification is the physiological or medicinal removal of toxic substances from a living organism.

Phase II metabolic activity in skin: Glutathione S-transferase / N-acetyltransferase (protein and mRNA level), Sulfotransferase/ catechol-O-methyltransferase (mRNA level)

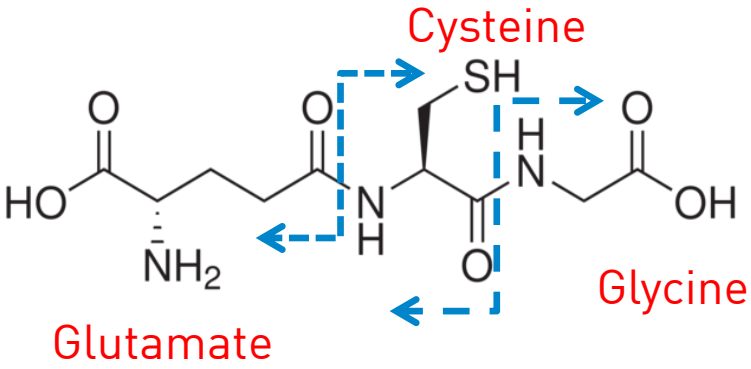


http://en.wikipedia.org/wiki/Drug_metabolism



http://www.thebody.com/content/art875.html

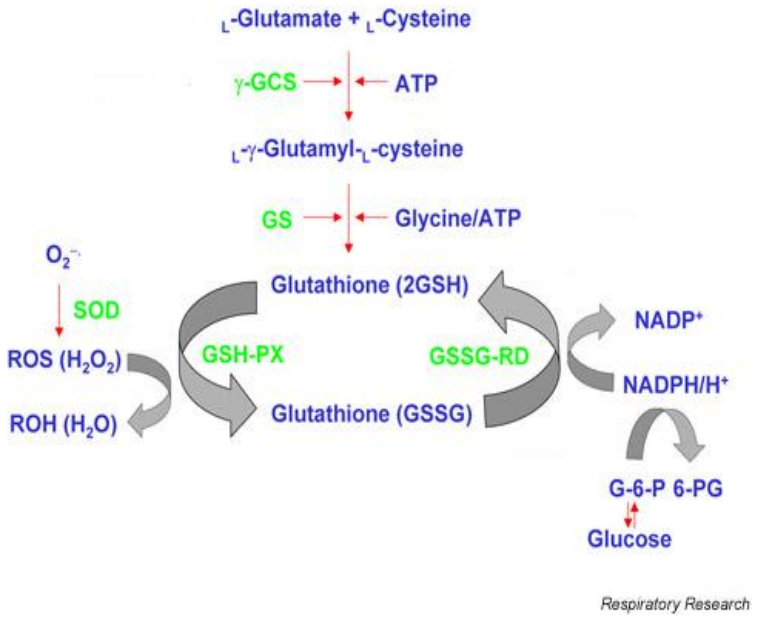
BACKGROUND - GLUTATHIONE



GSH is used as a protective mechanism. It is not only synthesized by the cells but can be regenerated after oxidative stress.

Glutathione is present at mM concentrations in cells.

Characterisation of GSH cycle and enzymes involved done on liver tissue (Meister et al)

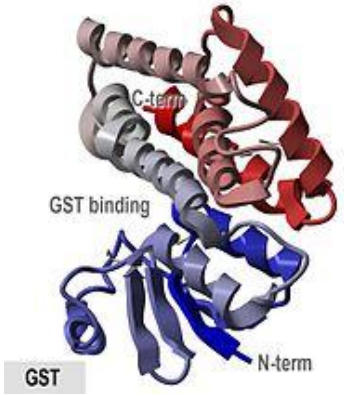


GSH cycle described by Meister et al.

BACKGROUND – HUMAN SKIN MEASUREMENTS

Total GSH (nmol/g)	Technique used	Number of donors	Reference
480 (epidermis) 85 (dermis)	Ellman's reagent-GR recycling assay	6 (healthy)	Shindo *et al* (1994)
250-450 (epidermis) 280-530 (dermis)	Ellman's reagent-GR recycling assay	7 (mean 21y.old) 9 (mean 71y.old)	Rhie *et al* (2001)
195 (healthy whole skin) 442 (lesional skin)	Ellman's reagent-GR recycling assay	5 (healthy skin) 6 (ICD or ACD on hands)	Kaur *et al* (2001)

BACKGROUND - GLUTATHIONE S TRANSFERASES



GSTs : superfamily of enzymes for detoxification, containing two reactive domains N-term (Location for GSH) and C-term (location for electrophile).

http://en.wikipedia.org/wiki/Glutathione_S-transferase

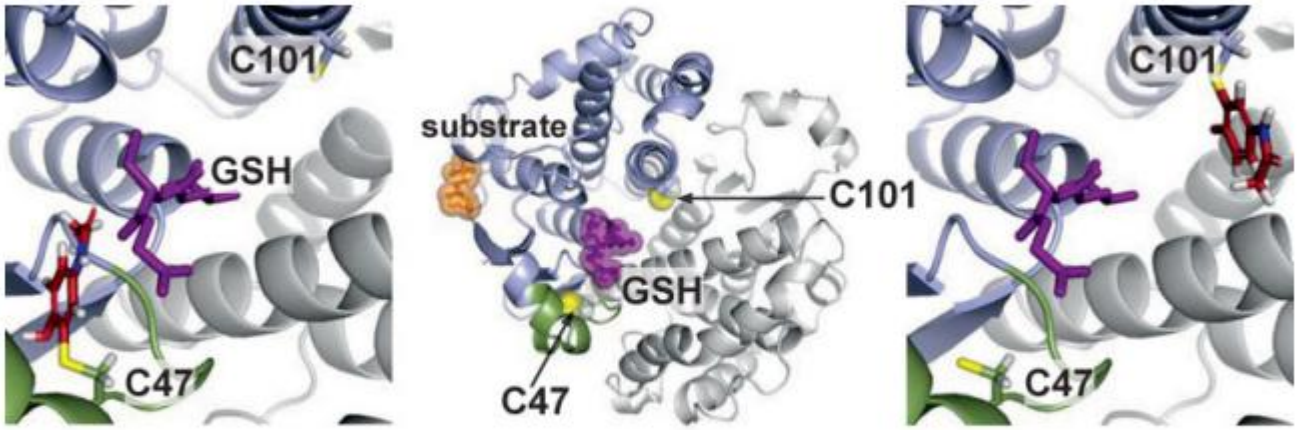


Figure 2. Molecular model of human GSTP. The central panel shows the full structure of the GSTP dimer, indicating the GSH (magenta) and substrate (orange) binding sites and the position of Cys47 and Cys101, highlighted in yellow. The left-hand side panel is an adapted model indicating modification of Cys47 with a single molecule of APAP. Similarly, the right-hand side panel shows modification at Cys101. In each case helix 2 is represented in green indicating the proximity of this helix to APAP modified Cys47. Each figure is based on structure 6GSS contained in the Protein Data Bank [30] and modified using the program PyMol [42].

BACKGROUND – HUMAN SKIN MEASUREMENTS

3 main isozymes of GST found in skin all belonged to either the alpha or pi family of GSTs (Raza, 1991) (Singhal, 1993).

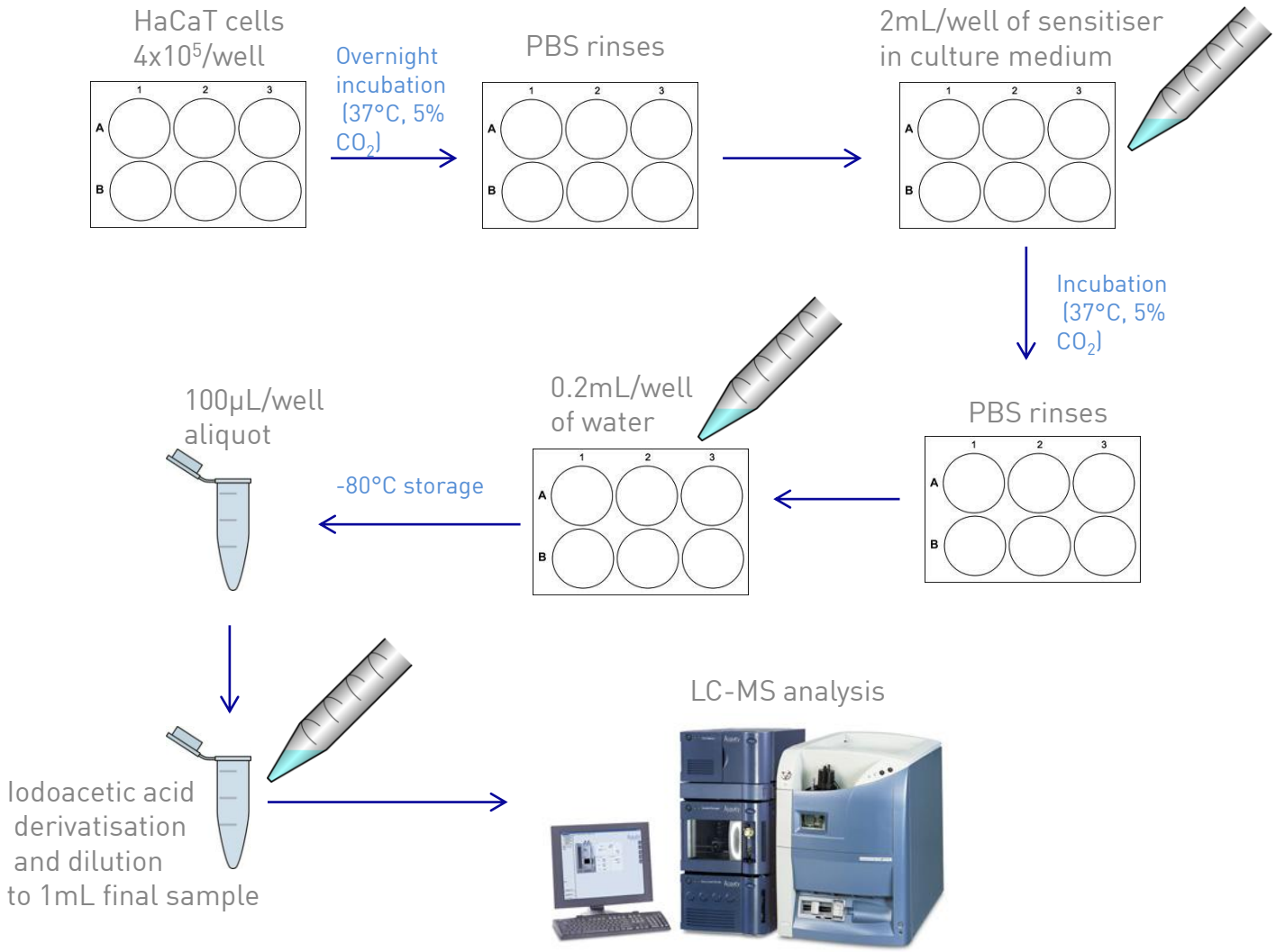
GST activity, expressed as mU per g of wet tissue, has been reported to be 1320±180 in male skin samples (n=8) and 760 ±120 for women donors (n=6) (Singhal, 1993).

More recent values, of approximately 30 nmol/min/µg cytosolic protein for epidermis samples (Harris, 2002) and 90 **nmol/min/mg** of whole skin (van Eijl, 2012) have been reported.

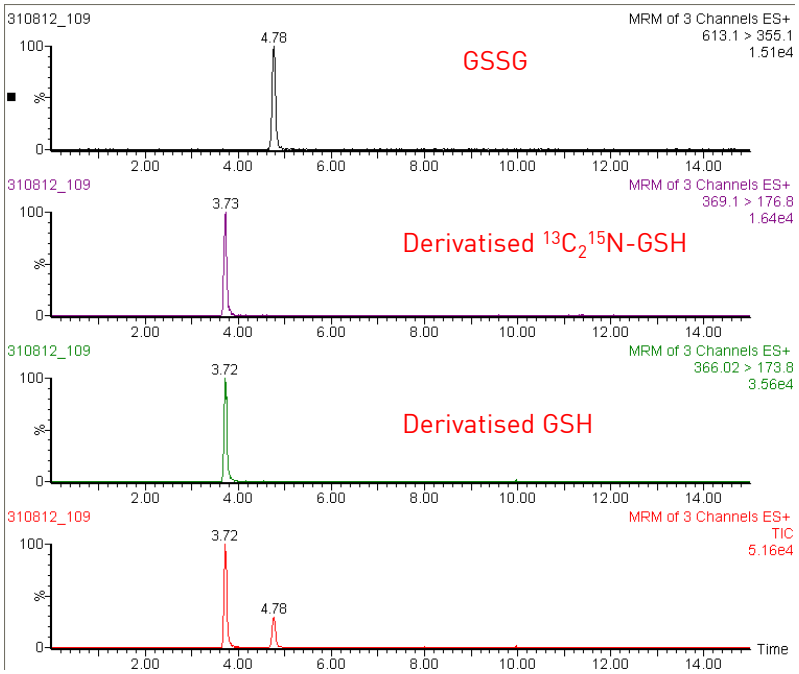
Cell cultures have also been used to try and understand the involvement of GST-P1 1(pi family)in **signalling pathways linked to detoxification**, even though the total GST activity measured in this study seemed to be significantly different depending on the type of cell used (Zang, 2002).

GSH EVOLUTION IN HACAT CELLS

Experimental design

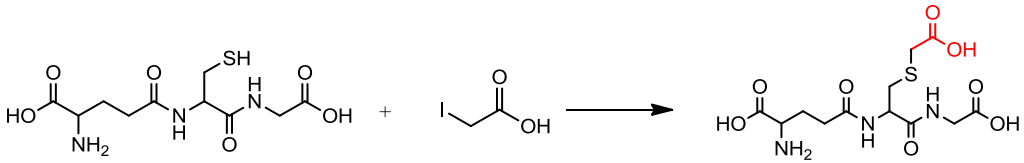


GSH EVOLUTION IN HACAT CELLS



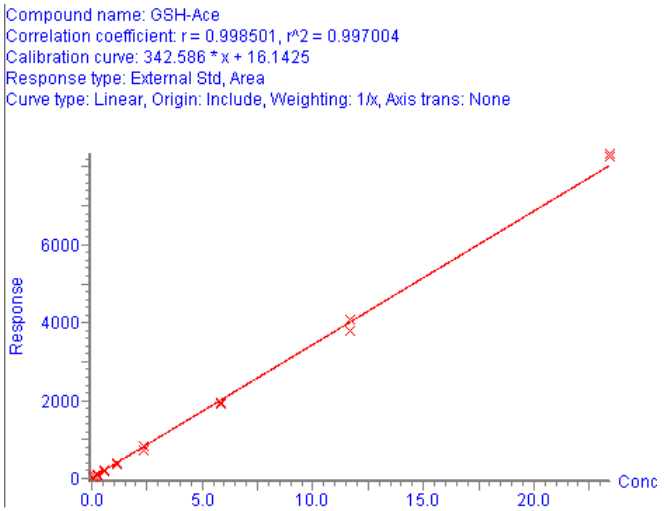
Analysis by LC-MS:

GSSG is directly measured.
GSH is derivatised (with iodoacetic acid) and the resulting product is detected.



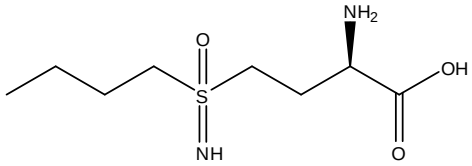
GSH is quantified over the range 0.05 to 20µM

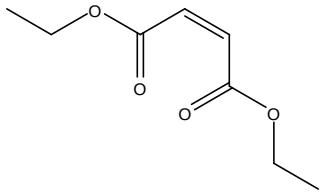
GSSG is quantified over the range 0.1 to 10µM

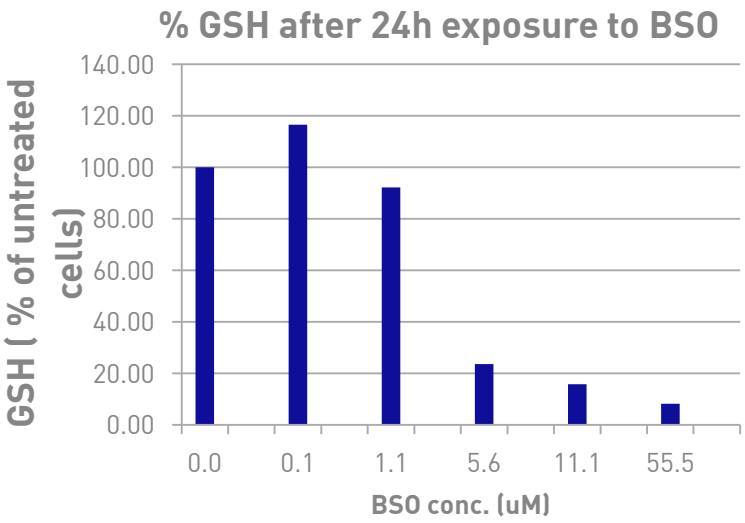


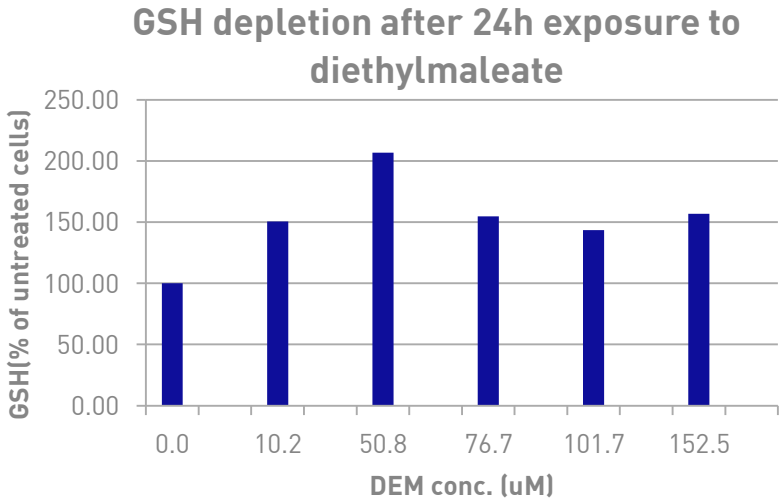
GSH EVOLUTION IN HACAT CELLS

Controls: L-Buthionine sulfoximine and Diethylmaleate



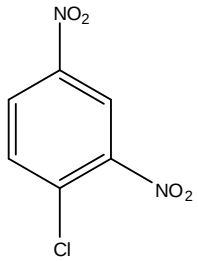


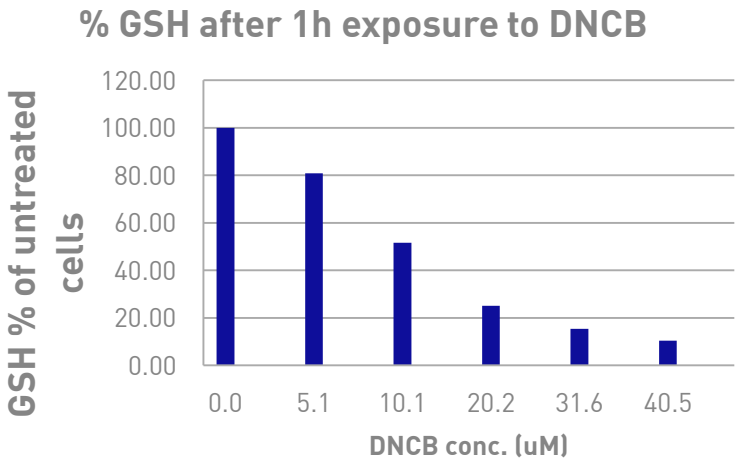


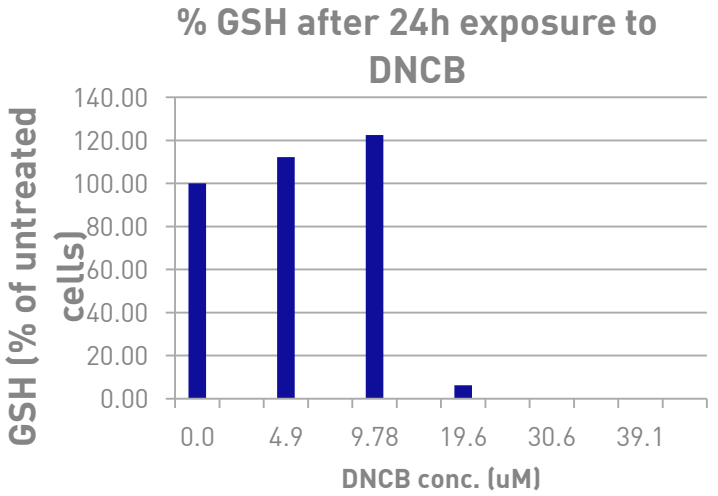


GSH EVOLUTION IN HACAT CELLS

Dose response to DNCB:

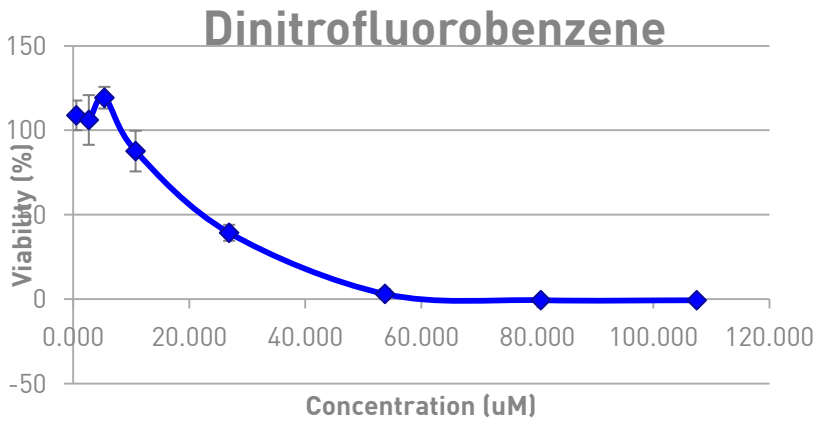


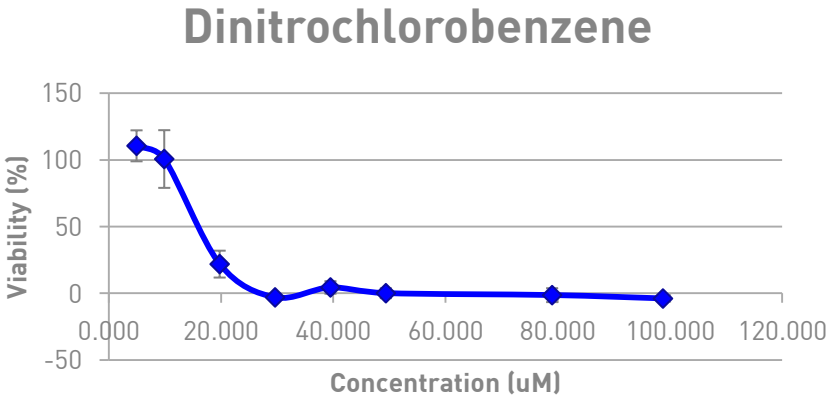


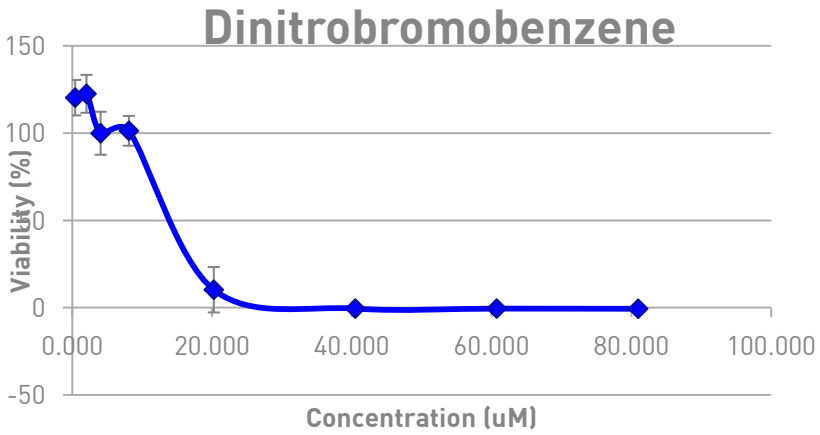


GSH EVOLUTION IN HACAT CELLS

Cytotoxicity of 1-halo-2,4-dinitrobenzenes





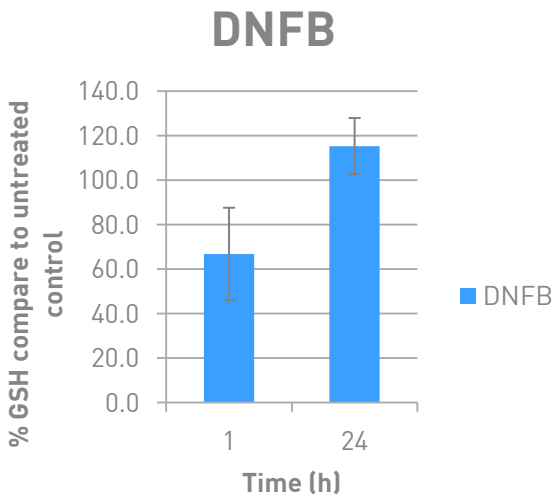


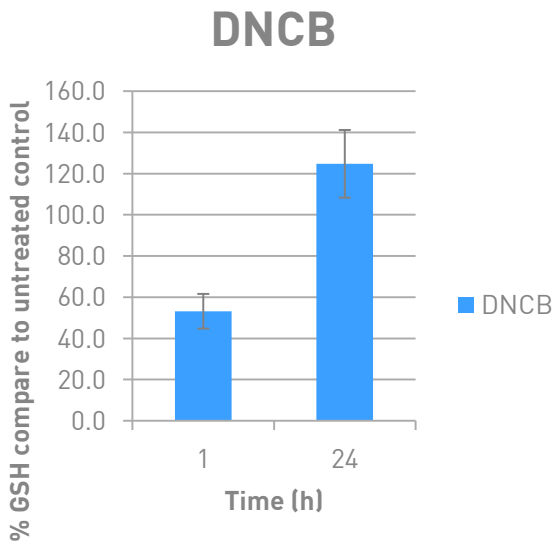
The evolution of toxicity followed the trend DNFB < DNCB < DNBB. For example, for a solution at 20µM in contact for 24hours, approximately 70% of cell viability would be observed for DNFB compared to approximately 60% for DNCB and approximately 35% for DNBB

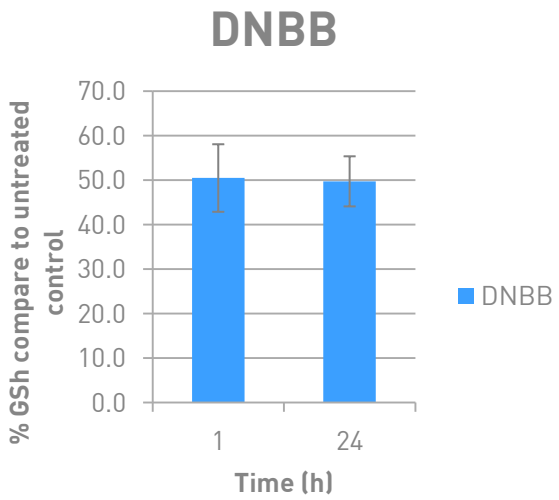
GSH EVOLUTION IN HACAT CELLS

GSH is quickly depleted over the first two hours of exposure for all compounds.

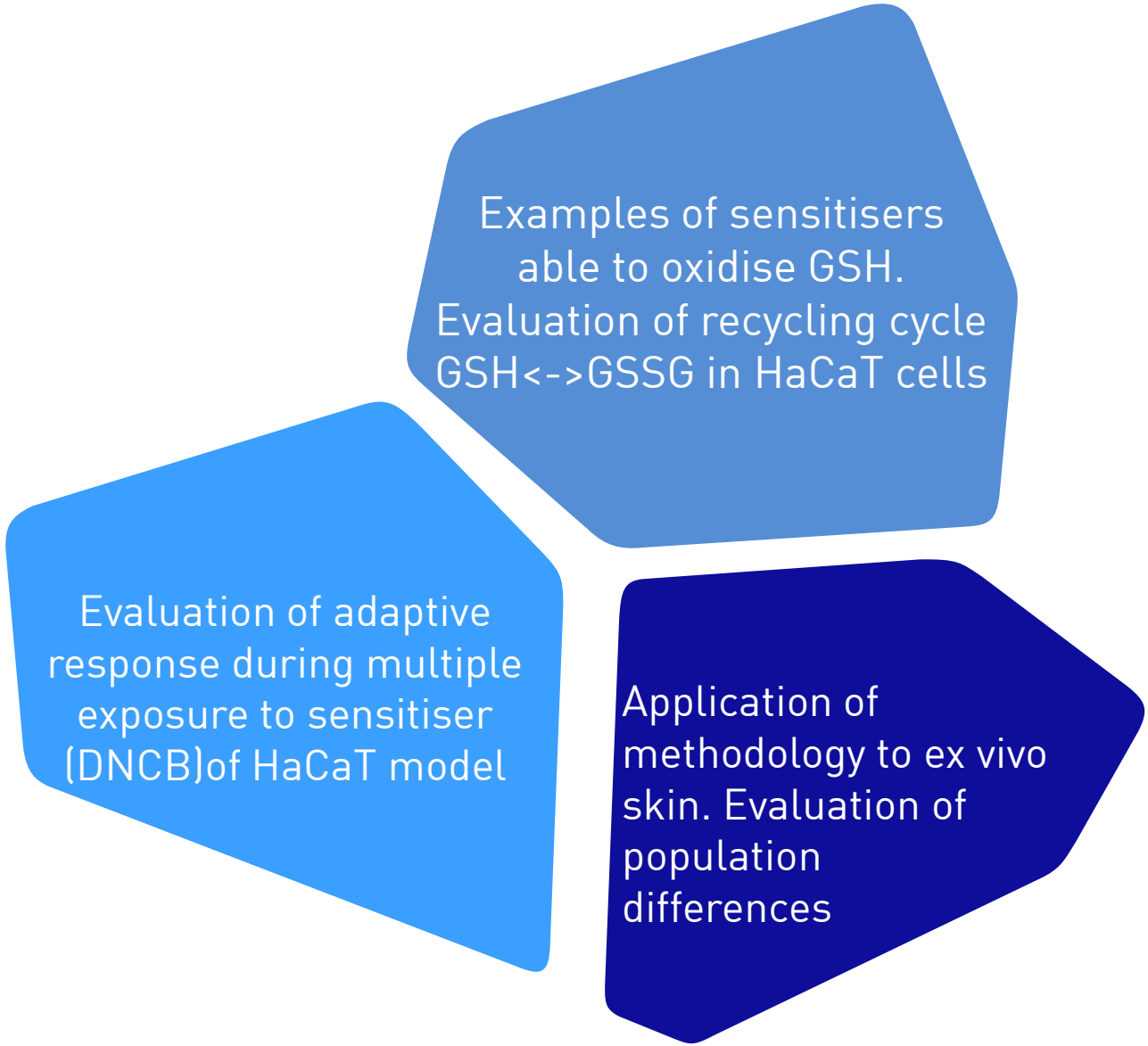
Upregulation at 24h time point for 2 compounds out of 3







FURTHER INVESTIGATION OF GSH MODEL



CONCLUSION

The HaCaT cell line is a suitable model for demonstrating the metabolic activity of keratinocytes for the depletion/repletion of GSH stock after treatment with sensitisers.

Future work includes the use of frozen ex vivo skin to determine the basal levels of glutathione in the epidermis and dermis of healthy and poly-sensitised donors, the hypothesis being that people that are prone to contact allergies have a natural capacity of detoxification that is lower than that of the general population.

THANK YOU

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