Electrophilic and oxidative stress: molecular basis for interindividual variability?

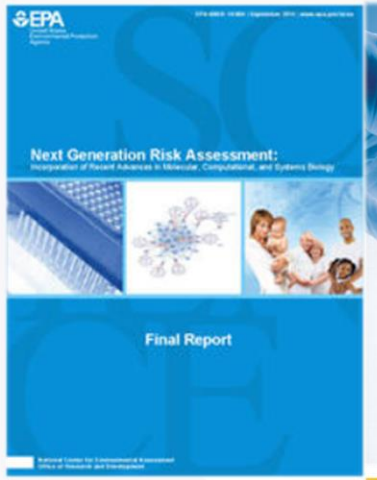
Maja Aleksic

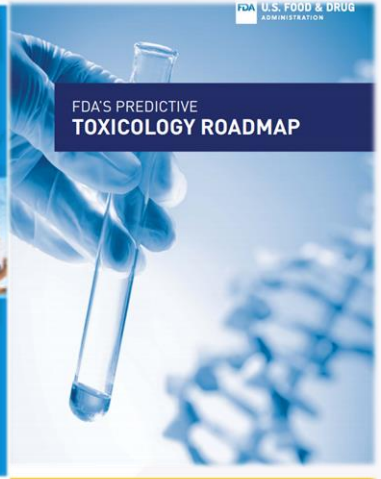
ESCD Amsterdam 8-10th June 2022

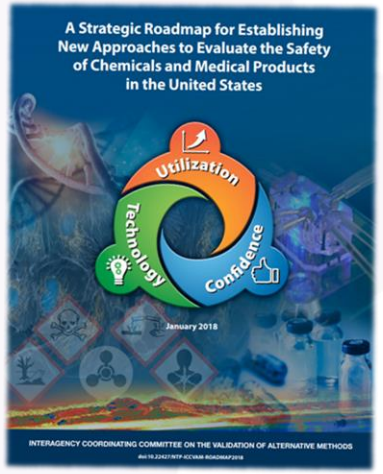
Unilever

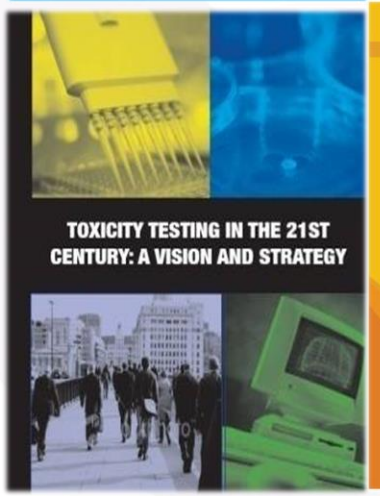
Assessing ingredient & product safety without animal testing

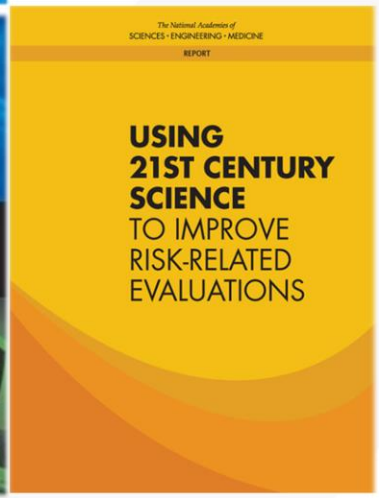
Next Generation Risk Assessment (NGRA)







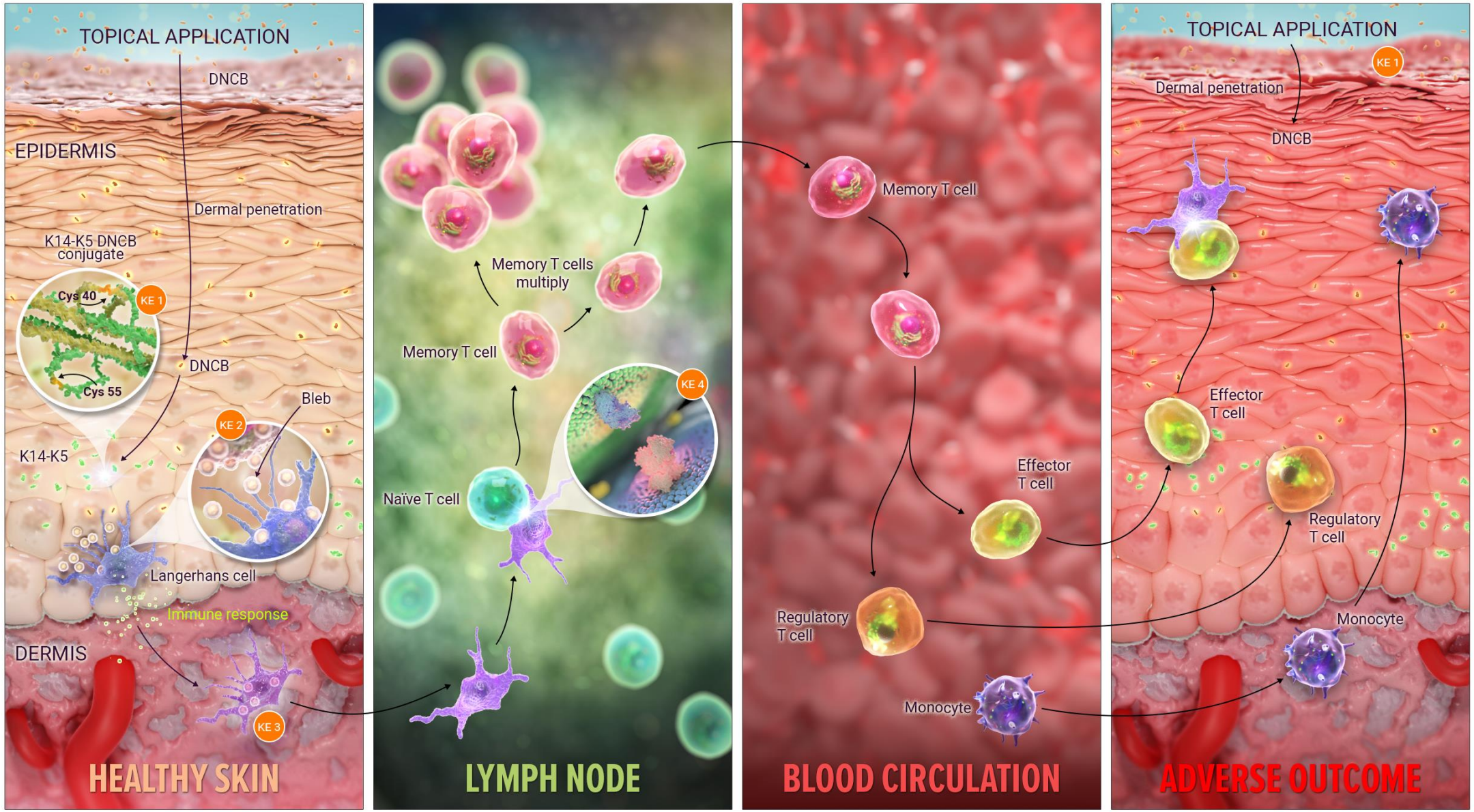




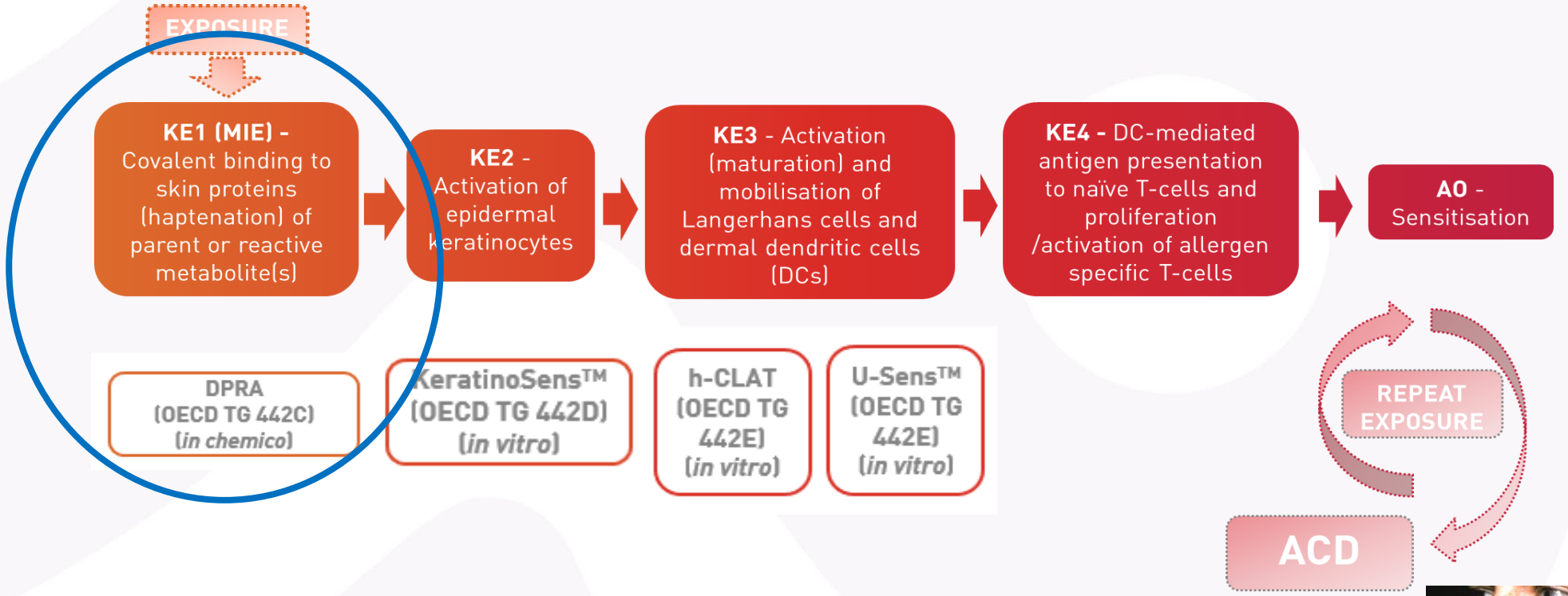
Is it safe to include x% of chemical y in product z?

Unilever

SKIN SENSITISATION OVERVIEW



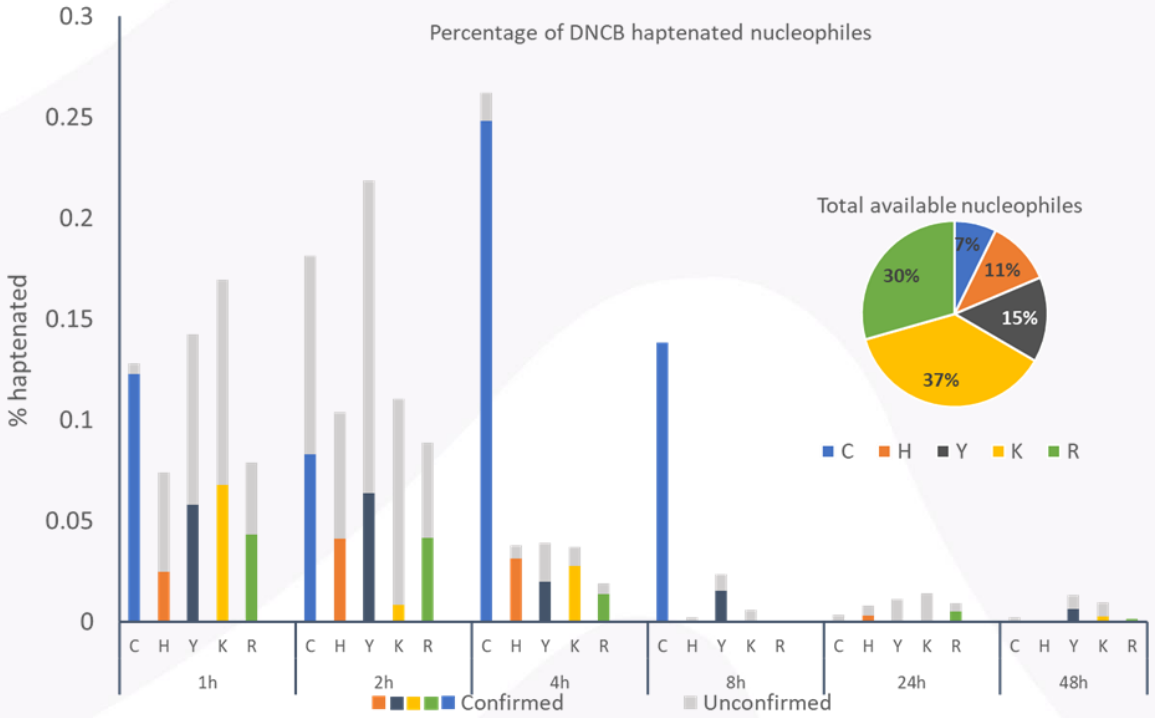
Adverse Outcome Pathway for Skin Sensitisation

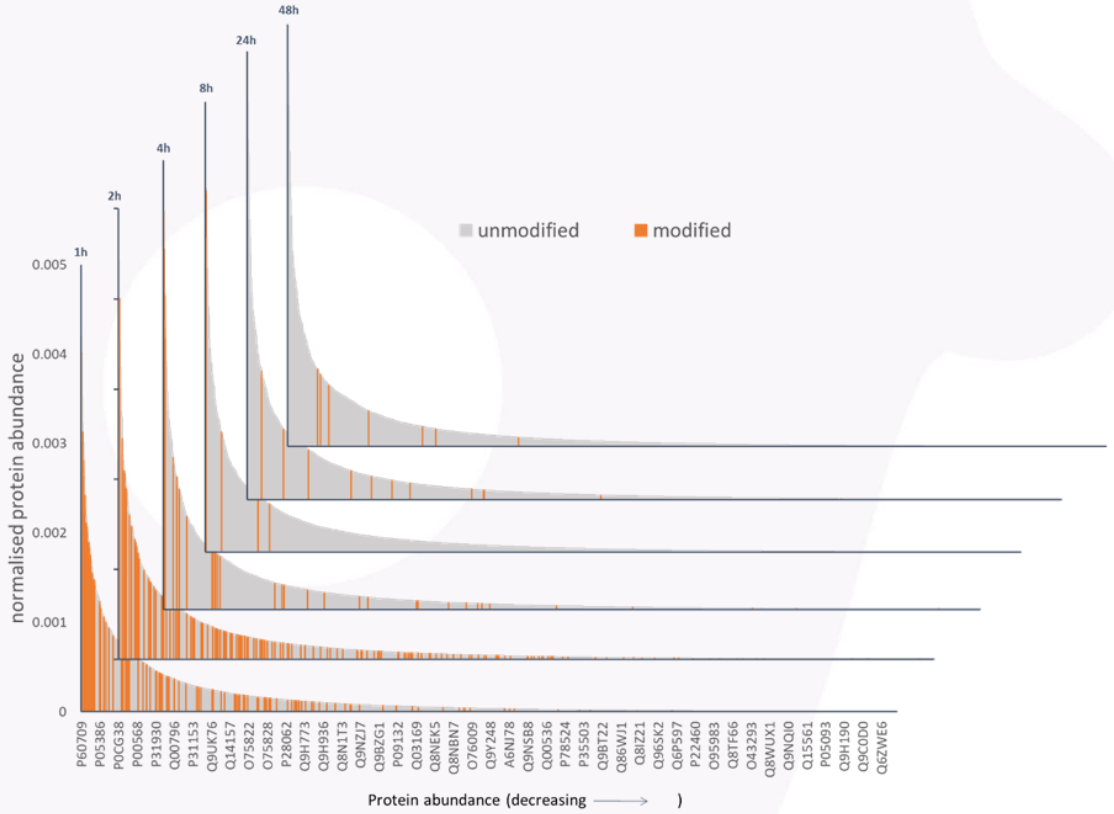


OECD (2014), *The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins*, OECD Series on Testing and Assessment, No. 168, OECD Publishing, Paris, https://doi.org/10.1787/9789264221444-en.

The Dynamics of Haptenation by DNCB in living HaCaT cells

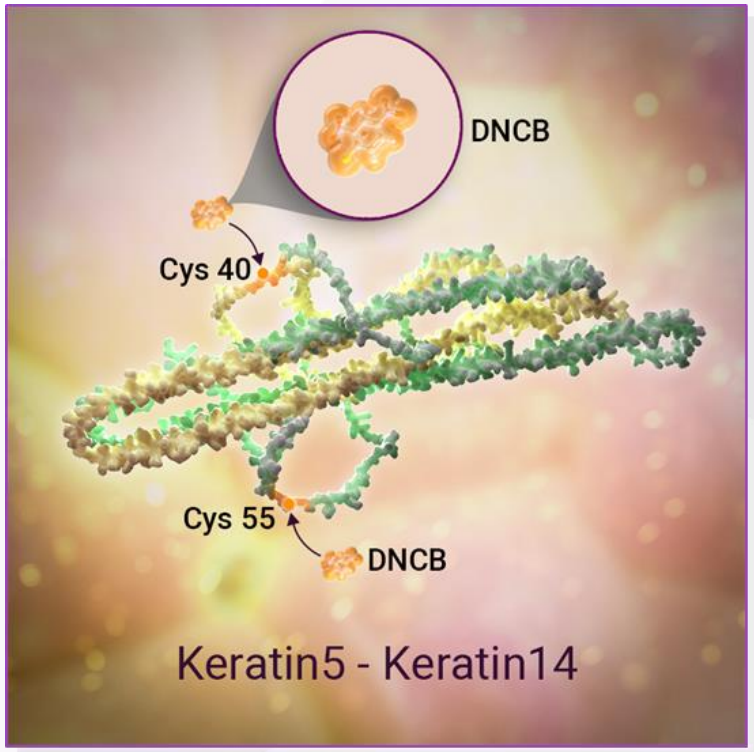
No change in differential protein expression throughout 48h of experiment

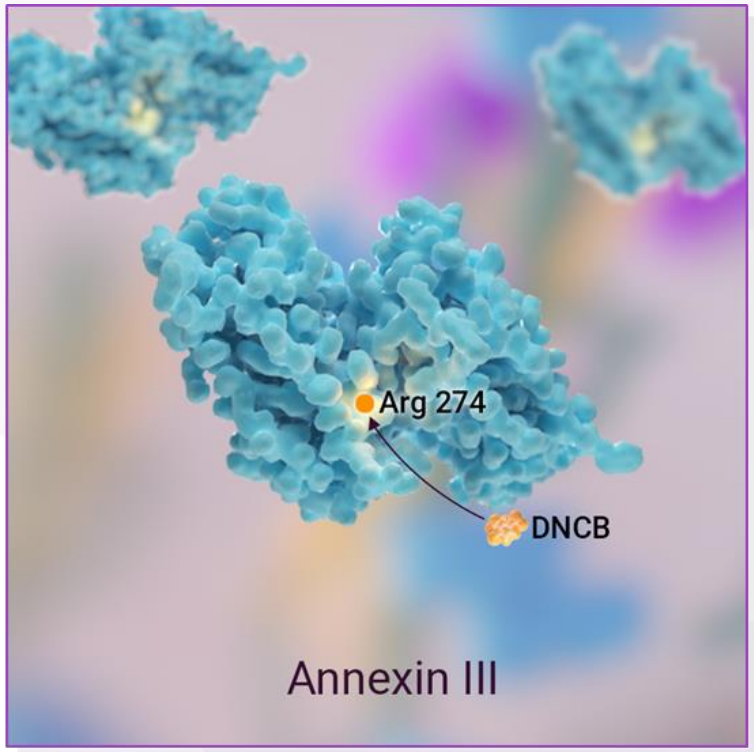


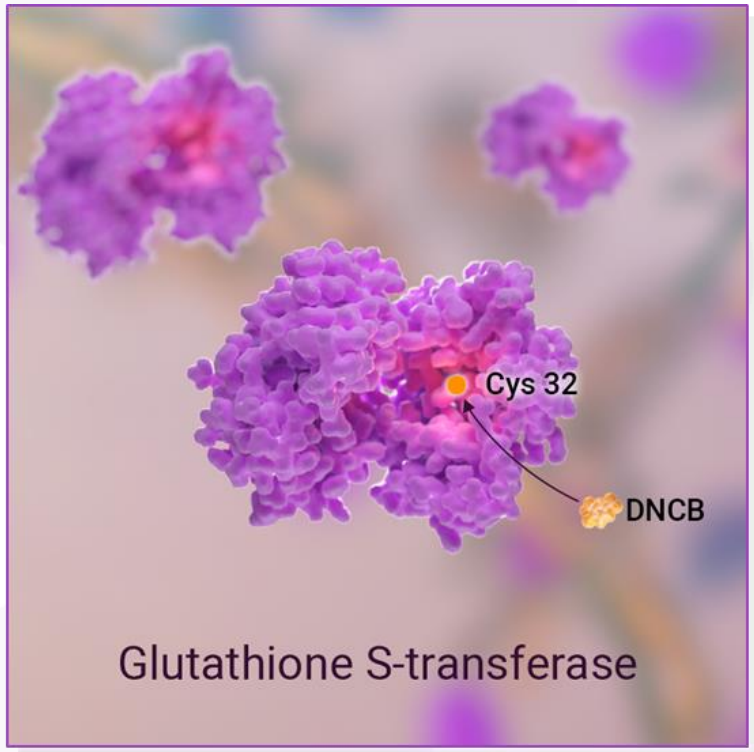


Parkinson E, Aleksic M, Kukic P, Bailey A, Cubberley R, Skipp PJ (2020), Proteomic analysis of the cellular response to a potent sensitiser unveils the dynamics of haptenation in living cells, Toxicology 445, pp1-10; 152603

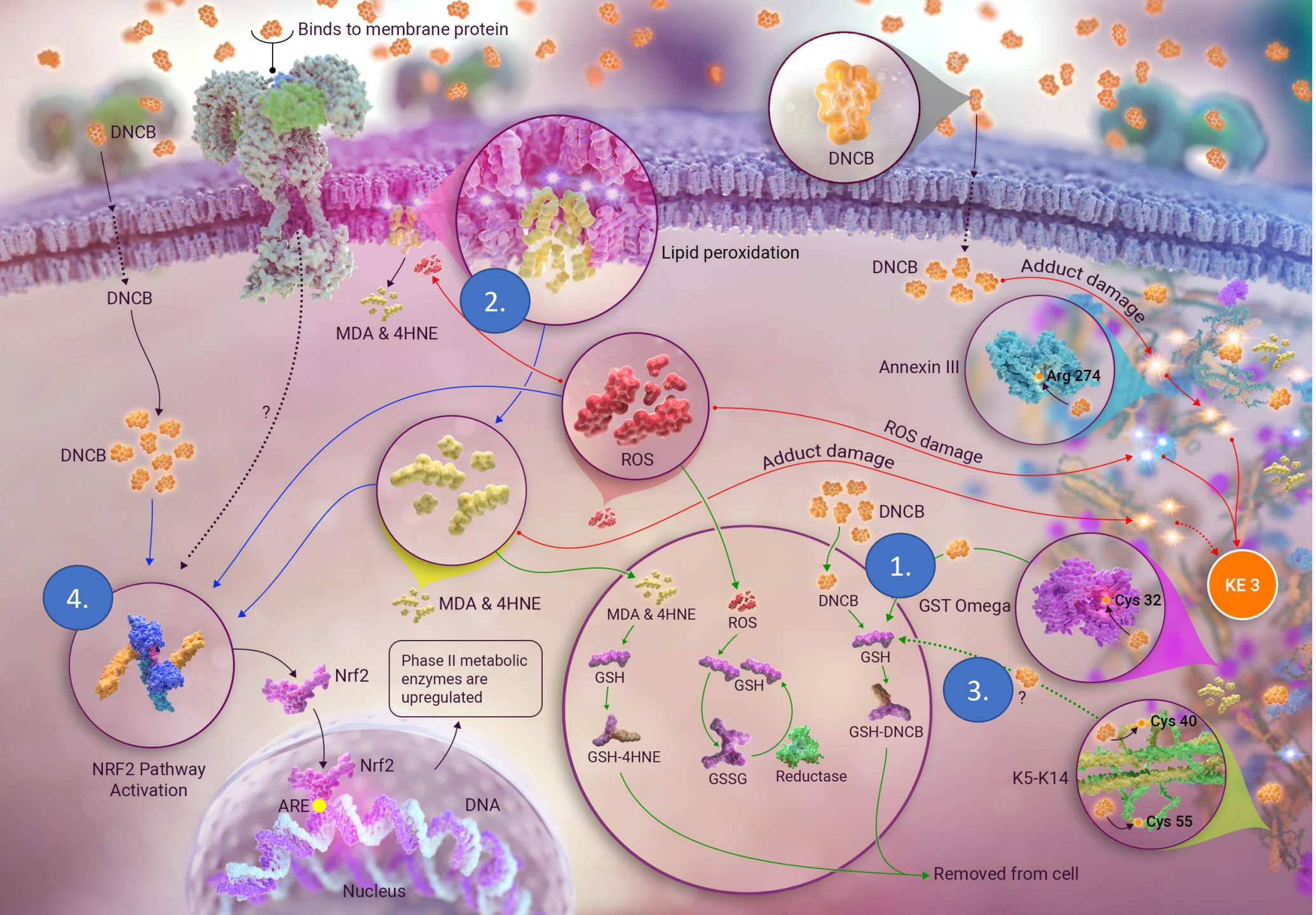
Typical DNCB haptenated proteins in HaCaT cells







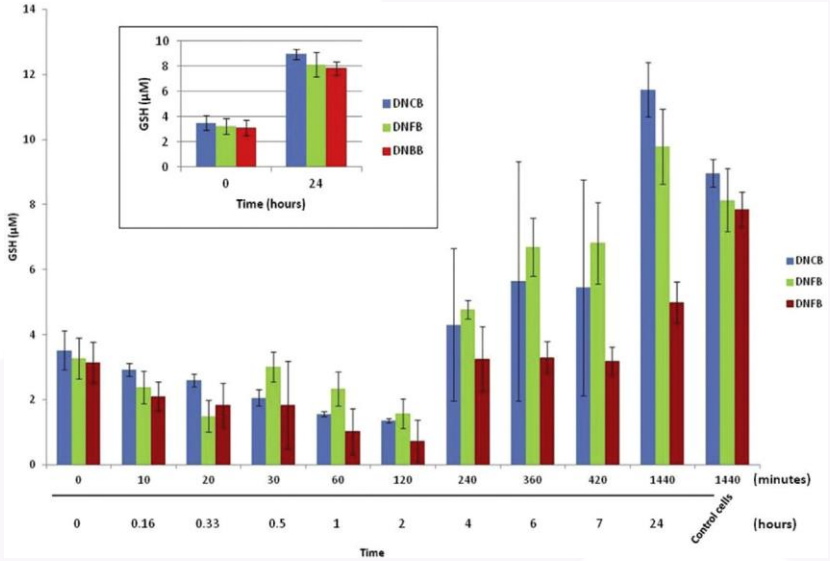
Imagery: NEXU Science Communication



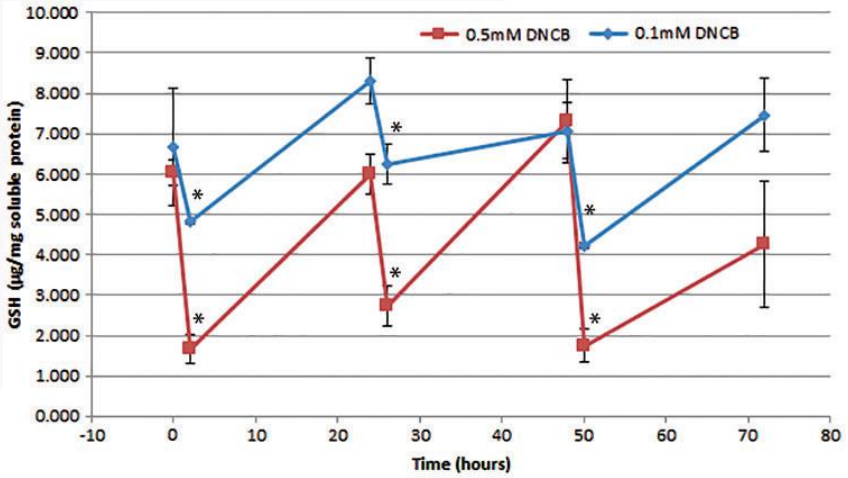
Worthy of investigation?

1. **Phase II metabolism**
2. **Lipid peroxidation**
3. Reversibility
4. Nrf2 activation

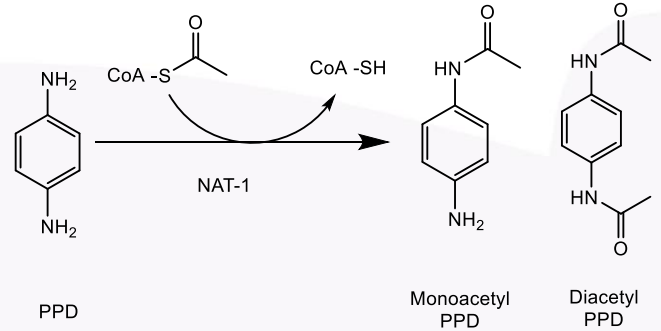
Phase II metabolism examples



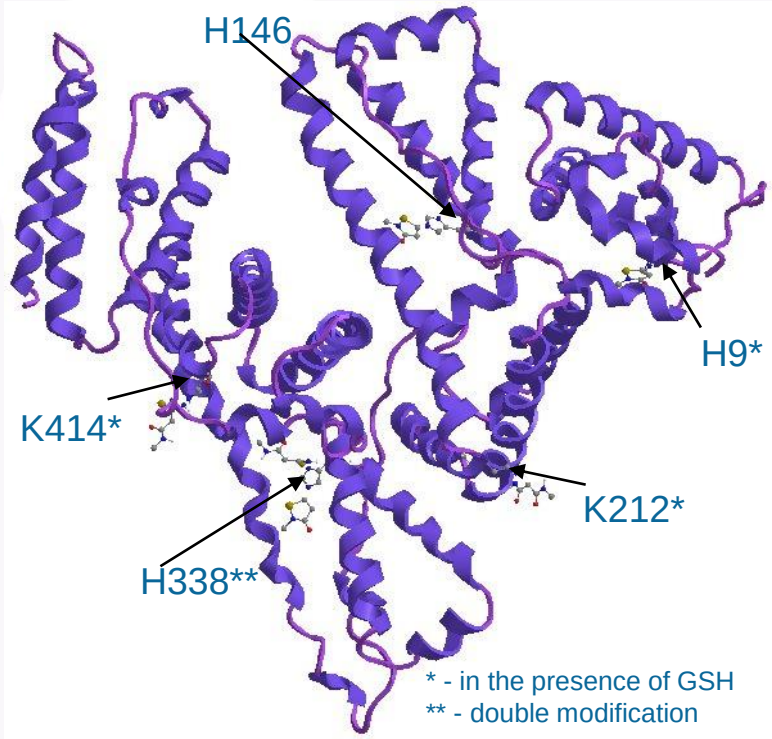
Jacquoilleot S et al, 2015, Tox Letters, 237(1):11-20



Spriggs S et al, 2016, Tox Sci 154 (1), 5-15



Venkatesan, Lim et al., 2022, Archives of Toxicology 96 (2)



Alvarez-Sanchez, R. et al, 2004, CRT, 17 (9) 1280-1288

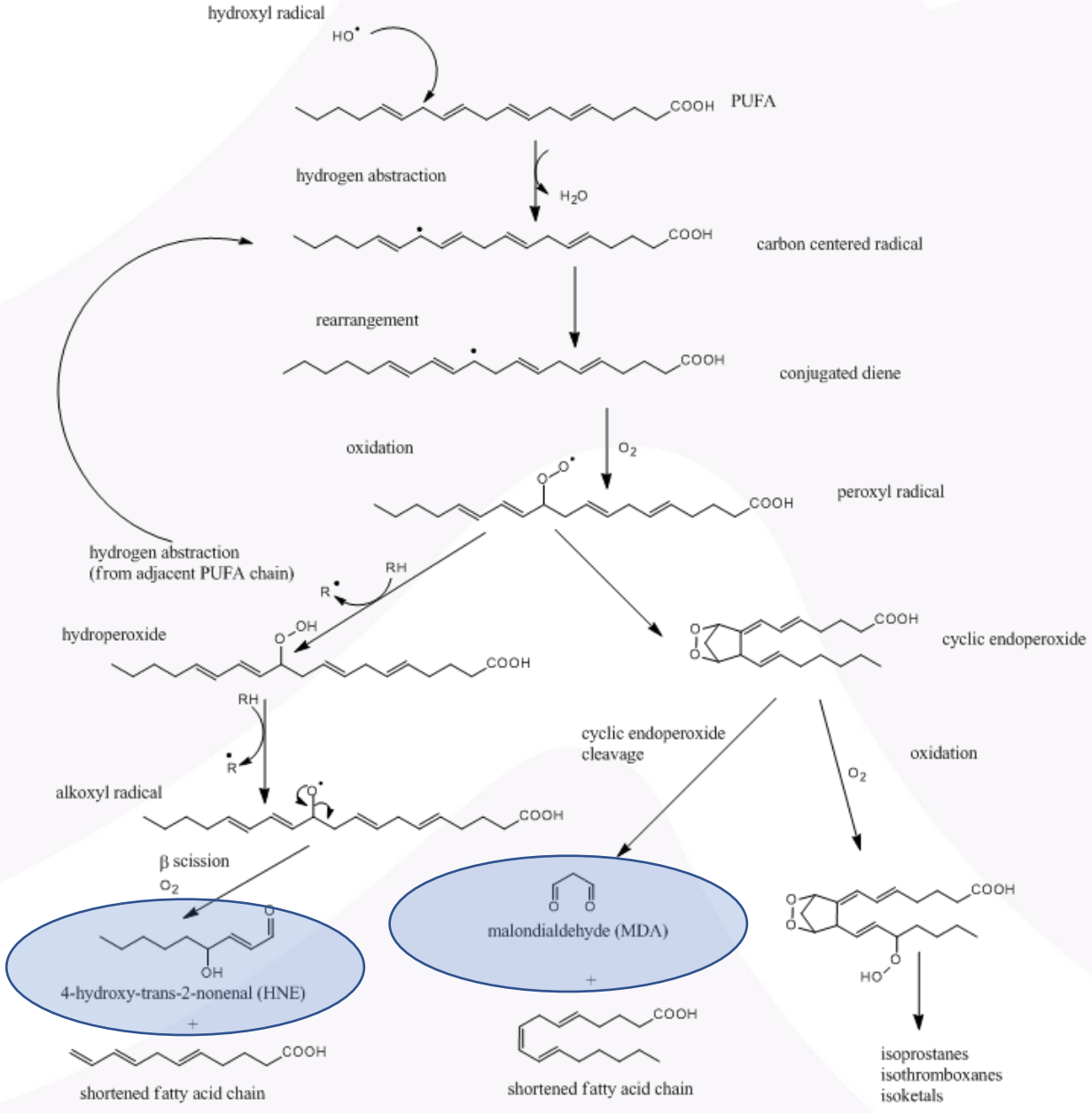
Potential phase II metabolism mechanisms

Reaction mechanism	Proposed associated detoxification mechanism	Proposed enzyme(s) involved	Case study
Michael addition	glutathione conjugation	glutathione-s-transferases	α,β unsaturated compounds
Schiff base formation	conversion of aldehyde to corresponding carboxylic acid	aldehyde oxidase/dehydrogenase(s)	aldehydes
Acylation	conversion of aldehyde to corresponding carboxylic acid	aldehyde oxidase/dehydrogenase(s)	aldehydes
SN2/SNAr	glutathione conjugation	glutathione-s-transferases	dinitrohalobenzenes
Other examples	N-acetylation	N-acetyl transferase(s)	PPD
	hydrolysis	carboxylesterases	esters

Some sensitisers have more than one reaction mechanism for haptenating proteins and are likely to have more than one mechanism for phase II metabolism

Unilever

ROS and Lipid peroxidation endproducts



Chemico-Biological Interactions 192 (2011) 14–20

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Advanced lipoxidation end-products

Reinald Pamplona*

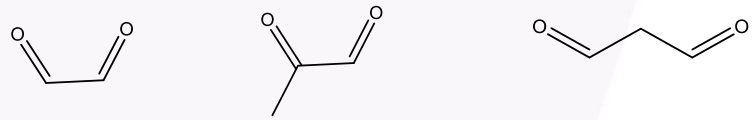
Department of Experimental Medicine, Faculty of Medicine, University of Lleida-IRBLleida, c/Montserrat Roig-2, E-25008 Lleida, Spain

saturated aldehydes

ethanal **propanal** **hexanal**

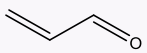
dicarbonyls

glyoxal **methylglyoxal** **malodialdehyde**



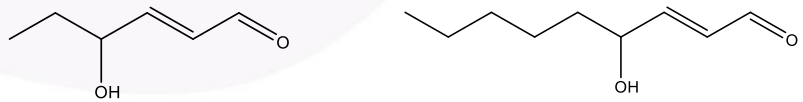
unsaturated aldehydes

acrolein



4-hydroxy-2-alkenals

4-hydroxy-2-hexenal **4-hydroxy-2-nonenal**



Conclusions and future work in research and potential use in RA

- **Phase II metabolism – concomitant and likely faster than haptenation**
  - Can simple assays be developed to be used in addition to reactivity assays and improve our prediction of sensitising potency?
- **Are all haptenation events reversible?**
  - To what extent and can this be measured?
- **ROS increase results from disturbance of redox balance by sensitisers**
  - Does protein damage resulting from ROS and lipid peroxidation speed up processing and presentation of haptenated epidermal proteins (antigens)?
  - Do ROS and lipid peroxidation endproducts compete with hapten for detoxification (phase II metabolism)?
  - Can we measure the effect of ROS and levels of lipid peroxidation endproducts?
- **Do any of the above events hold the key to interindividual variability in susceptibility to sensitisation?**
  - Individuals have different levels and activity of metabolic enzymes and can therefore process sensitisers at different pace
  - Individuals have different PUFA make up of cell membrane and could produce different levels of endproducts from lipid peroxidation
- **Assays do not have to be complicated to be useful in risk assessment!**

Unilever

Thank you:

SEAC, Unilever:

Nicola Gilmour
Ramya Rajagopal
Sandrine Spriggs
Richard Cubberley
Gavin Maxwell

University of Southampton:

Erika Parkinson
Scott Adams
Alex Lester
Paul Skipp

University Louis Pasteur, Strasbourg:

Marie Betou
Jean-Pierre Lepoittevin

NexuCreative, Dublin:

Eoin Winston
Frank Munnelly

Thank you for your attention!

Questions?

maja.aleksic@unilever.com

Unilever