Electrophilic and oxidative stress

Maja Aleksic

ERGECD Amsterdam 17th – 18th November 2022

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Assessing ingredient & product safety without animal testing

Next Generation Risk Assessment (NGRA)

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

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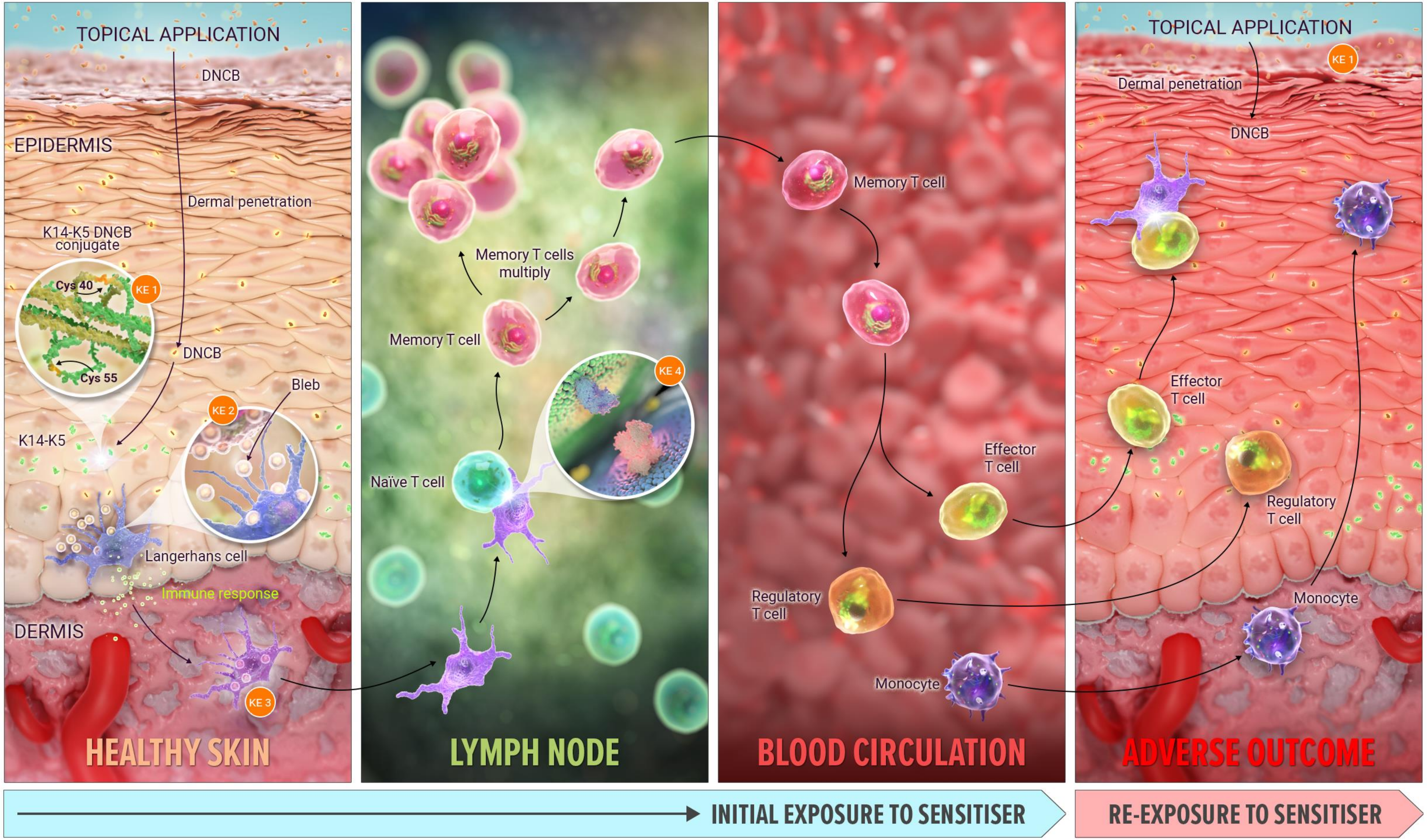
SKIN SENSITISATION OVERVIEW



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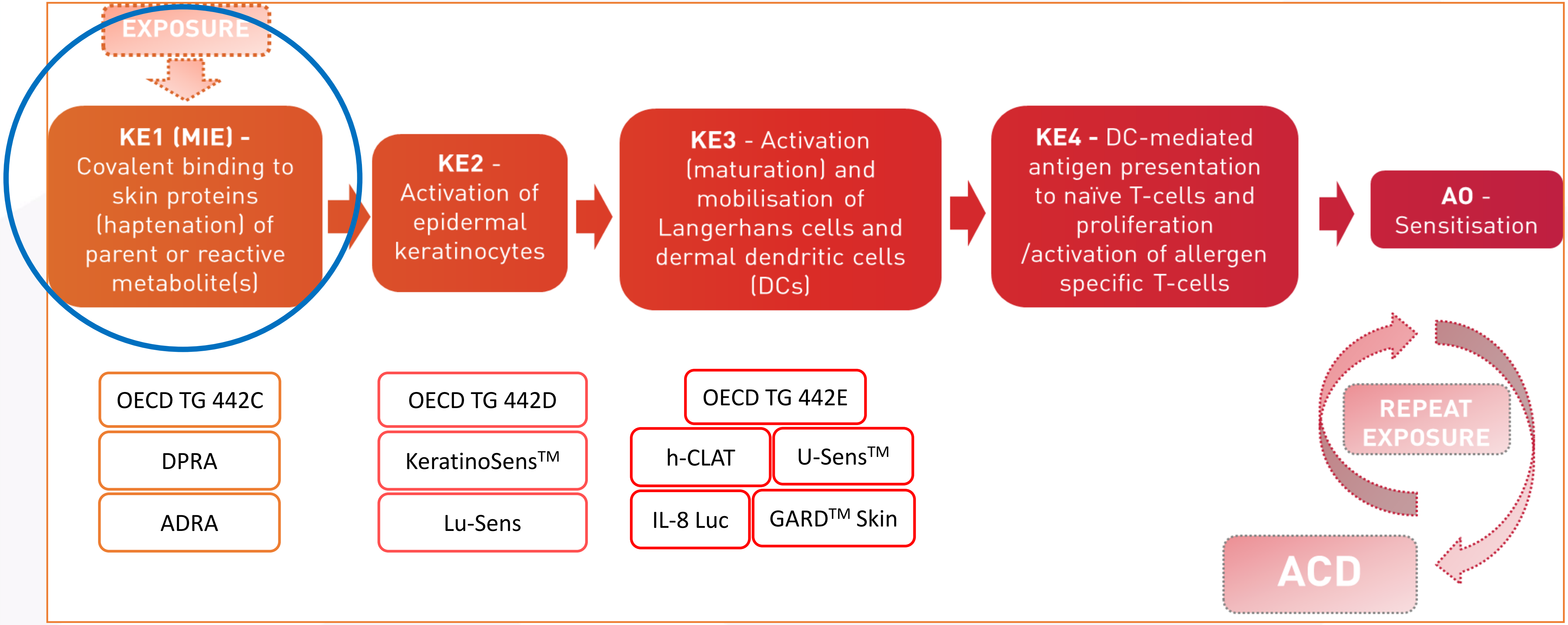
Imagery: NEXU Science Communication

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.

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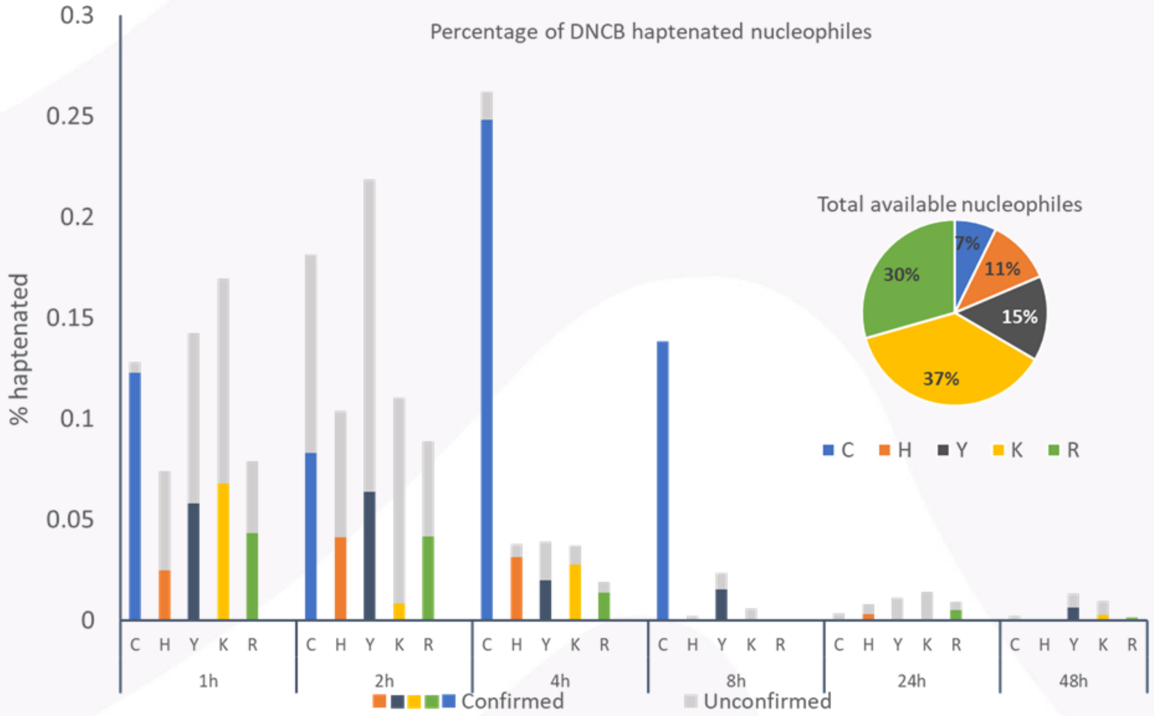
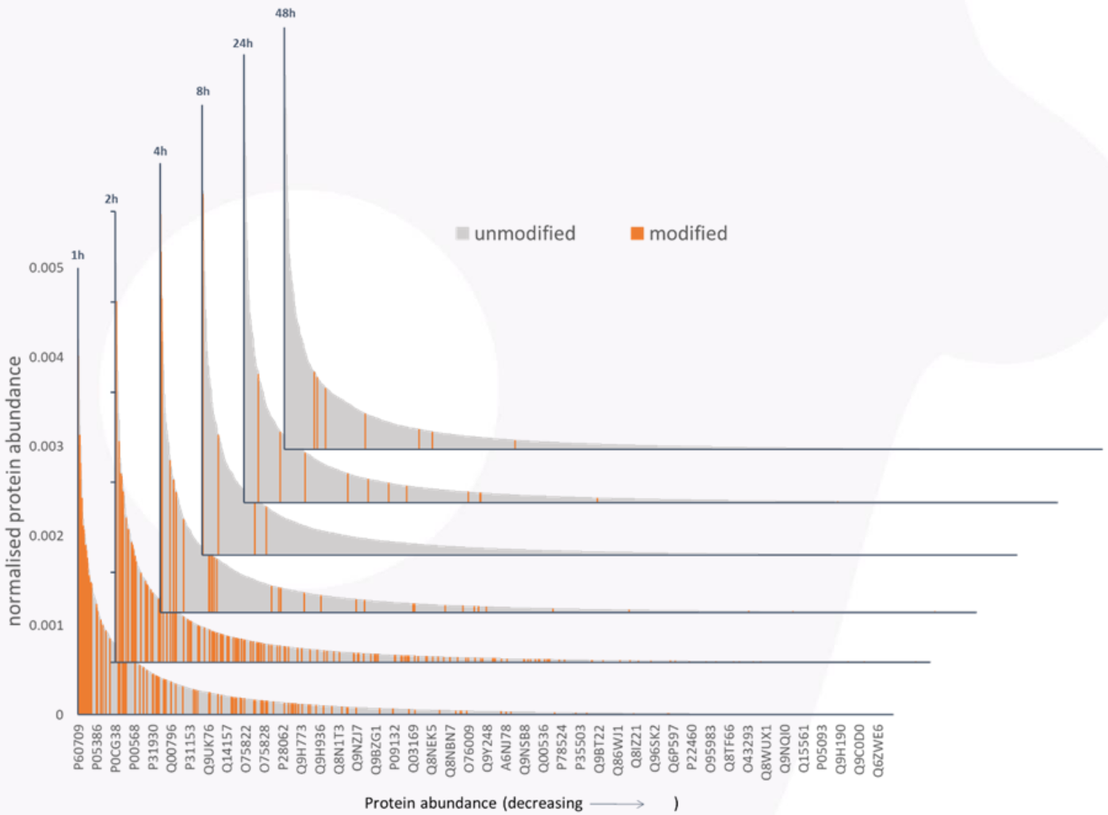
The Dynamics of Haptenation by DNCB in living HaCaT cells

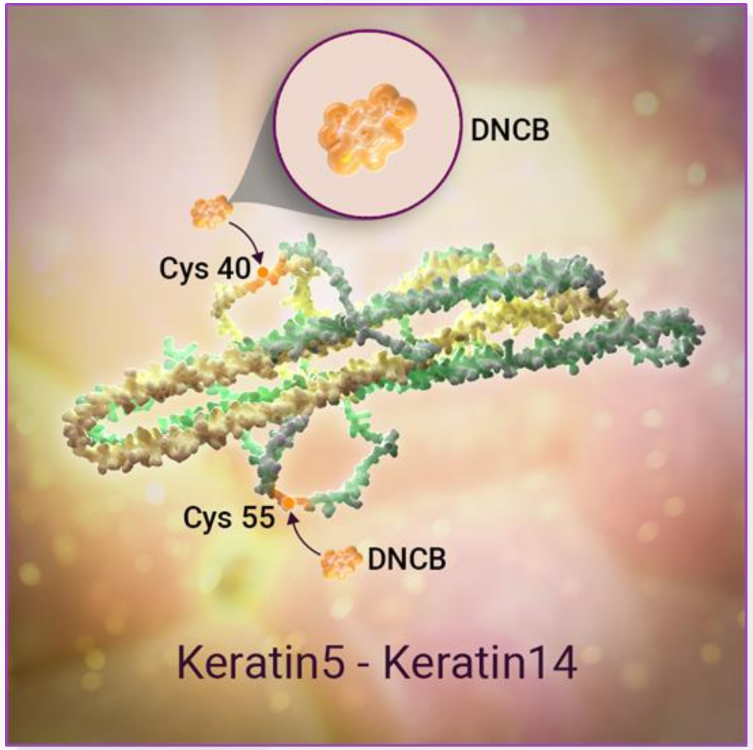


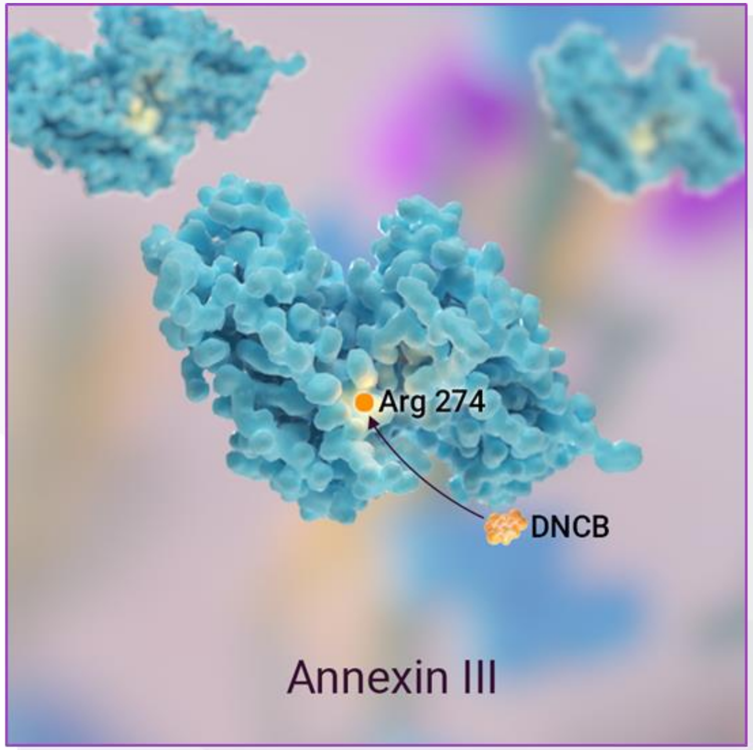
No change in protein expression throughout 48h experiment

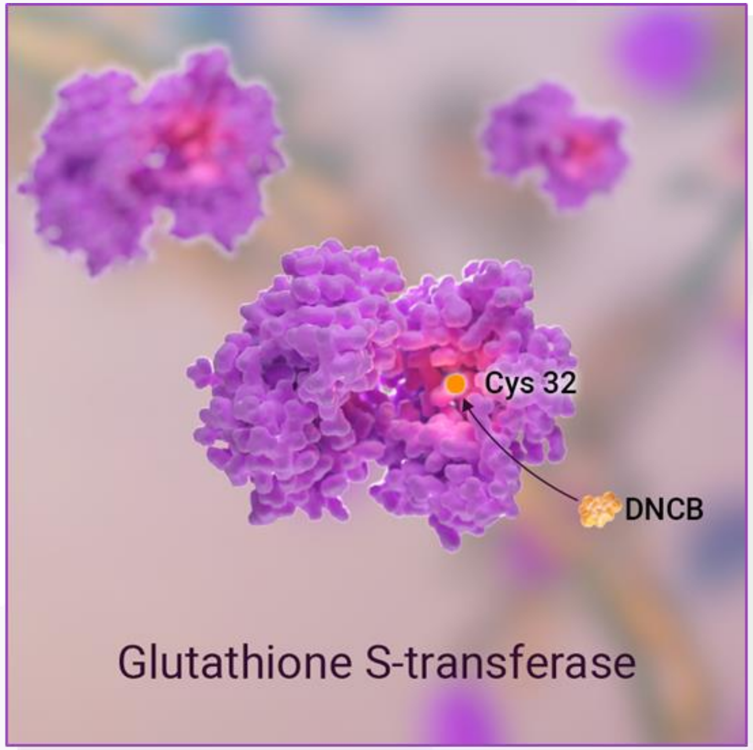


Parkinson E, Aleksic M, Kukic P, Bailey A, Cubberley R, Skipp PJ (2020), Proteomic analysis of the cellular response to a potent sensitiser unveils the dynamics of haptenation in living cells, Toxicology 445, pp1-10; 152603

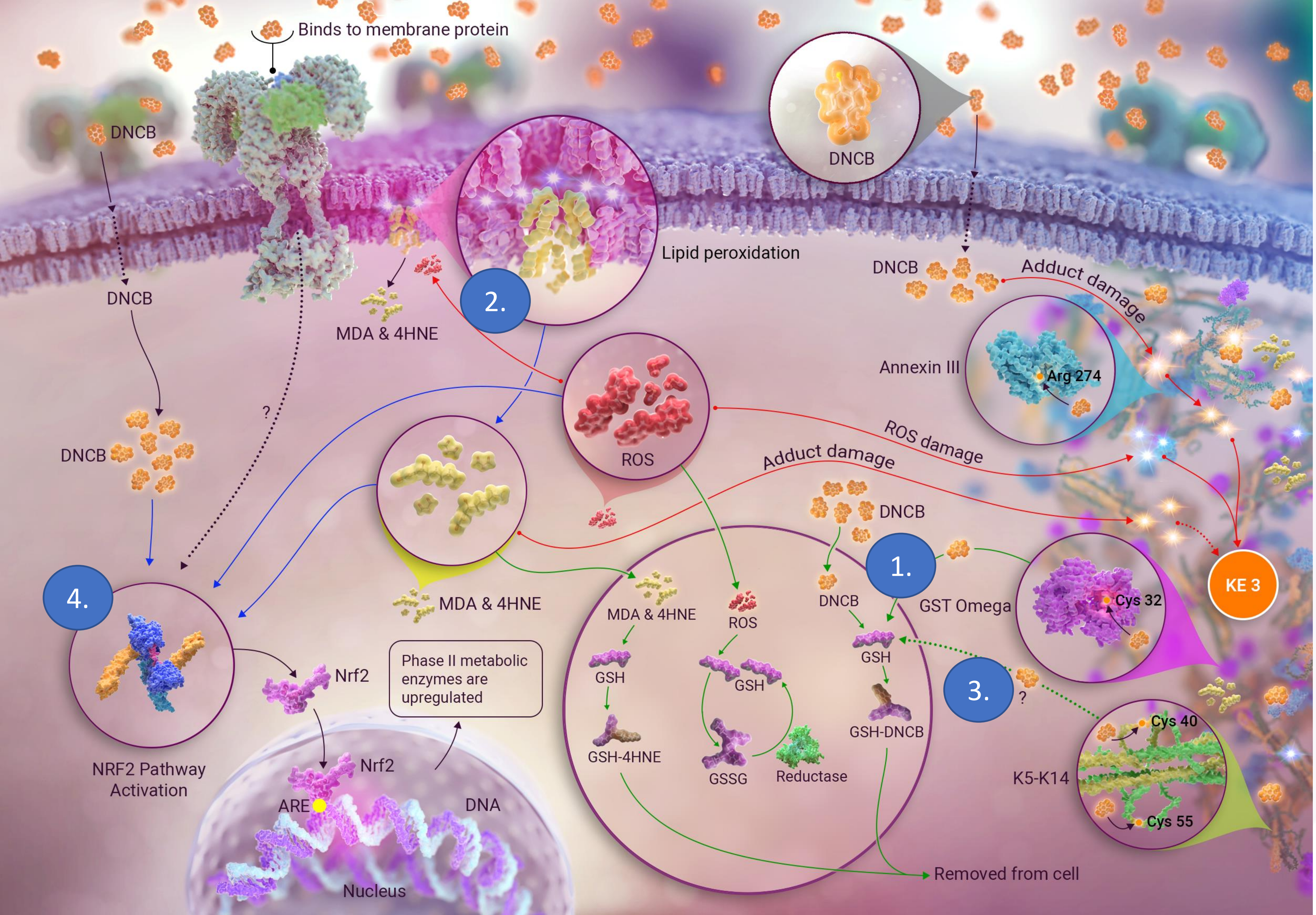
Typical DNCB haptenated proteins in HaCaT cells







Imagery: NEXU Science Communication

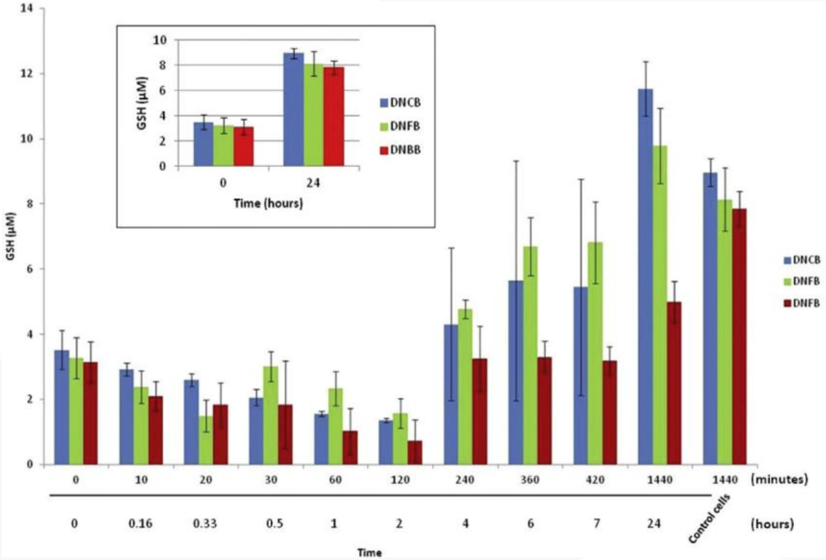


Worthy of investigation?

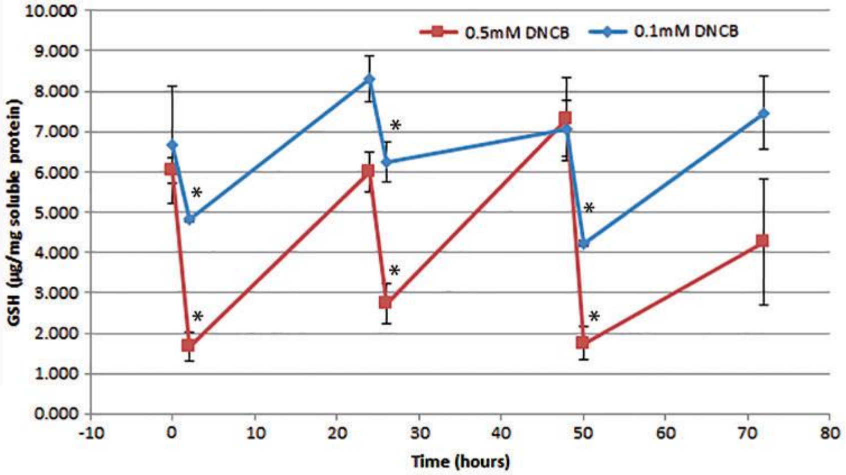
1. **Phase II metabolism**
2. **Lipid peroxidation**
3. Reversibility
4. Nrf2 activation

Imagery: NEXU Science Communication

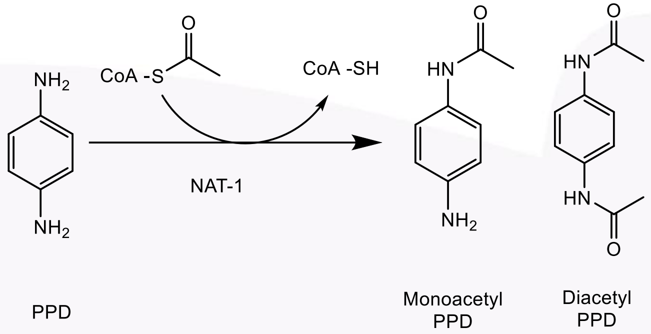
Phase II metabolism examples



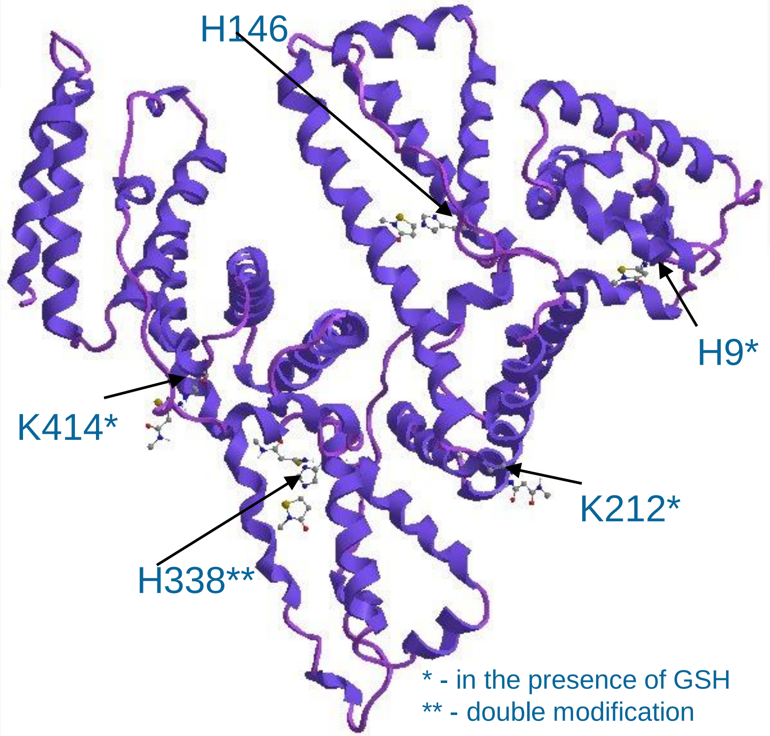
Jacquoilleot S et al, 2015, Tox Letters, 237(1):11-20



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



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

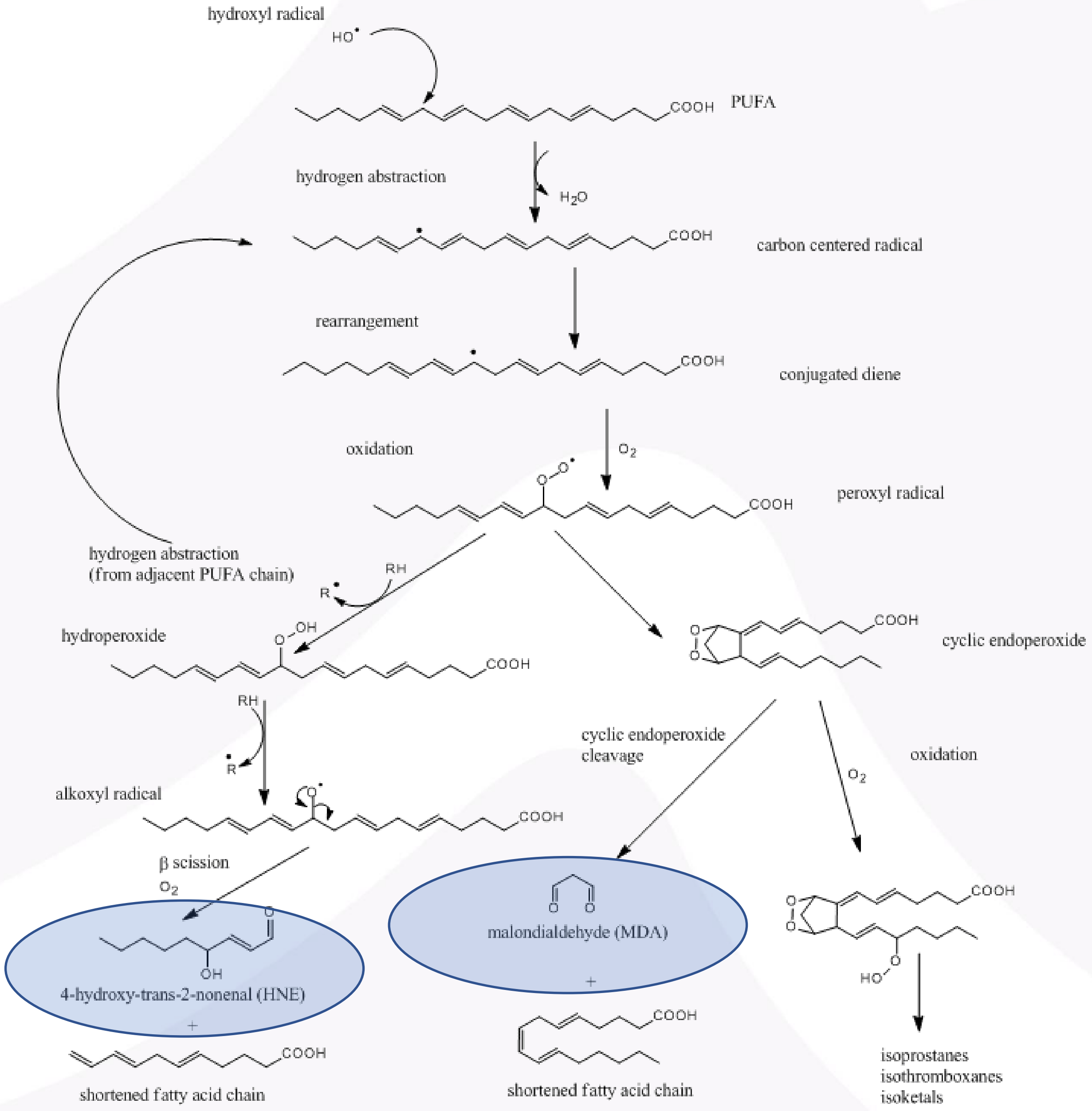


Alvarez-Sanchez, R. et al, 2004, CRT, 17 (9) 1280-1288

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

ROS and Lipid peroxidation end products



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Advanced lipoxidation end-products

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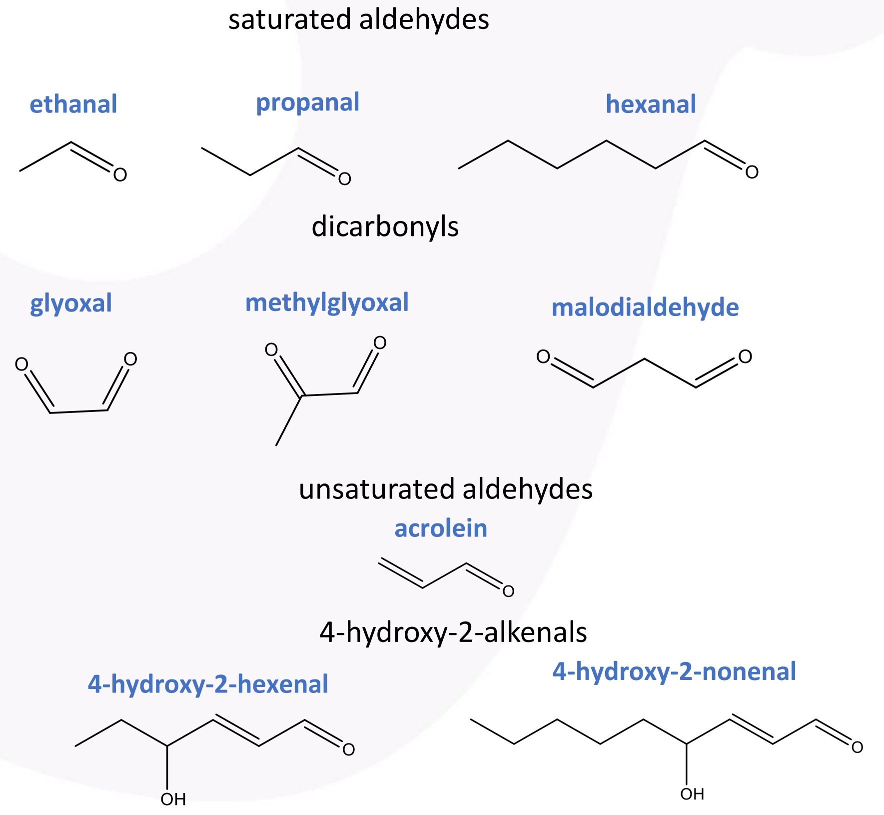

Conclusions, future work in research and potential use in RA

- **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 electrophilic end products from lipid peroxidation

- **Assays do not have to be complicated to be useful in risk assessment!**

Unilever

Thank you:

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Thank you for your attention!

Questions?

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