TOOLS FOR THE IDENTIFICATION OF STRUCTURAL ALERTS FOR BOTH PRE- AND PRO-HAPTENS

Rahul Parakhia, Ph.D.

Human Health Scientist, Dermatotoxicology

Email: rparakhia@rifm.org

IDEA

International Dialogue for the Evaluation of Allergens

13th December 2016

FLOW OF PRESENTATION

- Skin sensitization alerts
- Identification of Pre/Pro hapten mechanisms – In silico tools
- Examples of Pre/Pro hapten identification using TIMES-SS
- Experimental identification of Pre/Pro hapten
- Limitations
- Summary

SKIN SENSITIZATION

- Skin sensitization to chemicals, in most cases if not all, involves a *reaction with nucleophilic groups* on skin protein, leading to formation of antigens (Haptenation; OECD AOP Key Event 1).

- Degree of haptenation/alkylation could be predicted using Relative Alkylation Index (RAI) Model

  RAI = logD + Alogk +Blog P or pEC3 = alogk +blogP +C

- Hydrophobicity (log P), reactivity (log k) and dose (D)

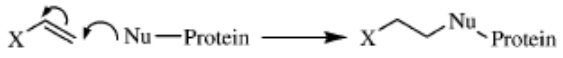
Roberts DW et. al. SAR and QSAR in Environmental research, (2007)18;343-365

ALERTS:

Scheme 1. Reaction Mechanistic Applicability Domains

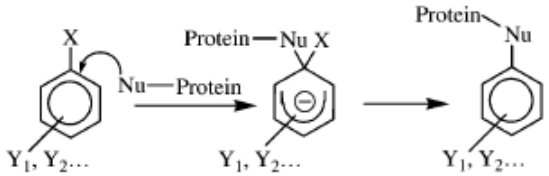
Mechanistic domain	Protein binding reaction	Modified protein

Michael acceptors



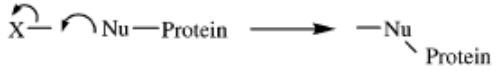
Identification characteristics. Double or triple bond with electron-withdrawing substituent X, such as -CHO, -COR, $-CO_2R$, -CN, $-SO_2R$, $-NO_2$...Includes para quinones and ortho quinones, often formed by oxidation of para and ortho di-hydroxy aromatics acting as pro-Michael acceptors. X can also be a heterocyclic group such as 2-pyridino or 4-pyridino.

S_NAr electrophiles



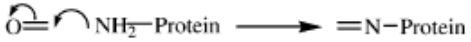
Identification characteristics. X = halogen or pseudohalogen, Y's are electron withdrawing groups (at least two) such as $-NO_2$, -CN, -CHO, $-CF_3$, -SOMe, $-SO_2Me$, ring fused nitrogen...One halogen is too weak to act as an X, but several halogens together can activate.

S_N2 electrophiles



Identification characteristics. X = halogen or other leaving group, e.g. OSO_2(R or Ar), OSO_2O(R or Ar) bonded to primary alkyl, benzylic, or allylic carbon. OR and NHR or NR_2 do not usually act as leaving groups, but can do so if part of a strained 3-membered ring (e.g. epoxides, ethylenimine and substituted derivatives).

Schiff base formers



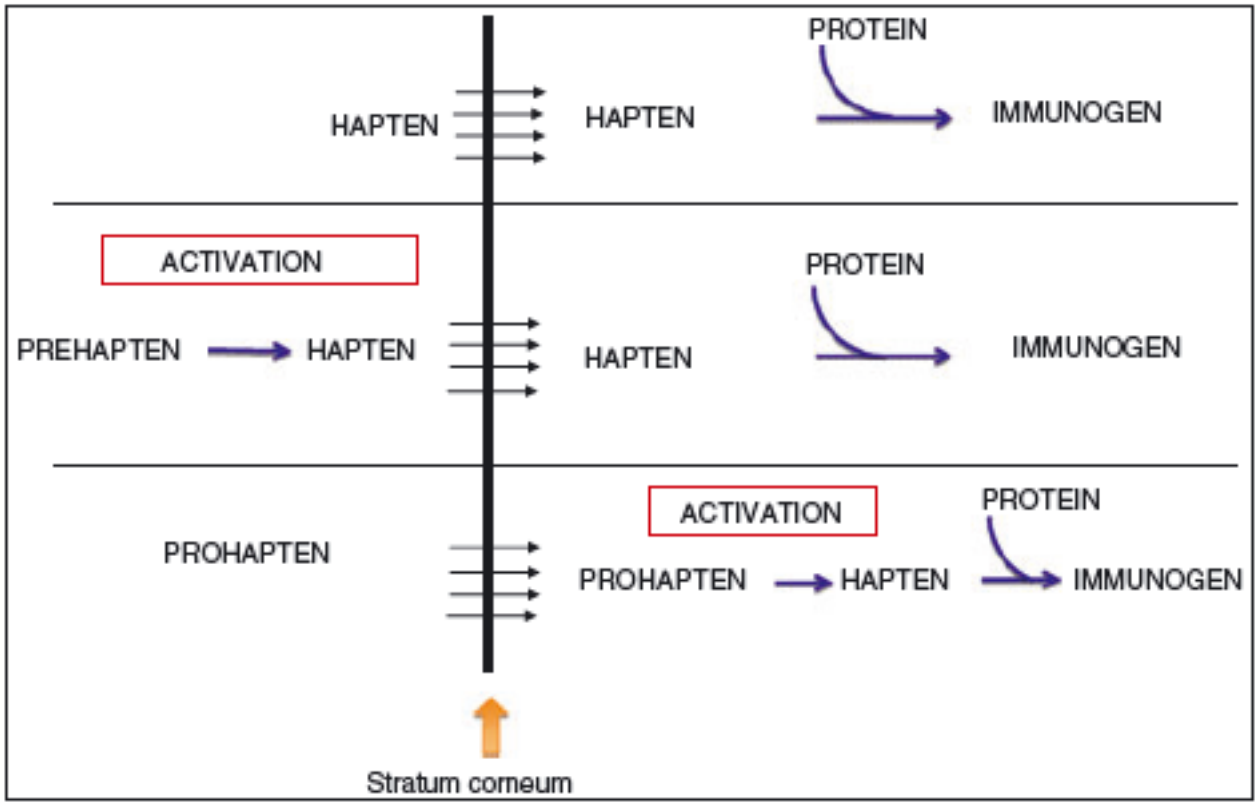
Identification characteristics. Reactive carbonyl compounds such as aliphatic aldehydes, some α,β- and α,γ-diketones, α-ketoesters. Not simple monoketones and aromatic aldehydes. Other hetero-unsaturated systems can behave analogously, e.g. C-nitroso compounds, thiocarbonyl compounds (C=S), cyanates and isocyanates, thiocyanates and isothiocyanates.

Acylating agents

Identification characteristics. X = halogen, or other group (e.g. $-OC_6H_5$) such that XH is acidic enough for X^- to act as a good leaving group. Includes anhydrides, cyclic or non-cyclic. X = -Oalkyl does not qualify, except when part of a strained lactone ring, e.g. β-propiolactone (but not γ-butyrolactone). Analogous reactions can occur with attack at sulfonyl S, phosphoryl P and thioacyl C.

Roberts DW et. al. SAR and QSAR in Environmental research, (2007)18;343-365

PRE/PRO HAPTENS



Karlberg AT et. al. Contact Dermatitis, 69 (2013) 323-334

IN SILICO TOOLS FOR PRE/PRO HAPTENS

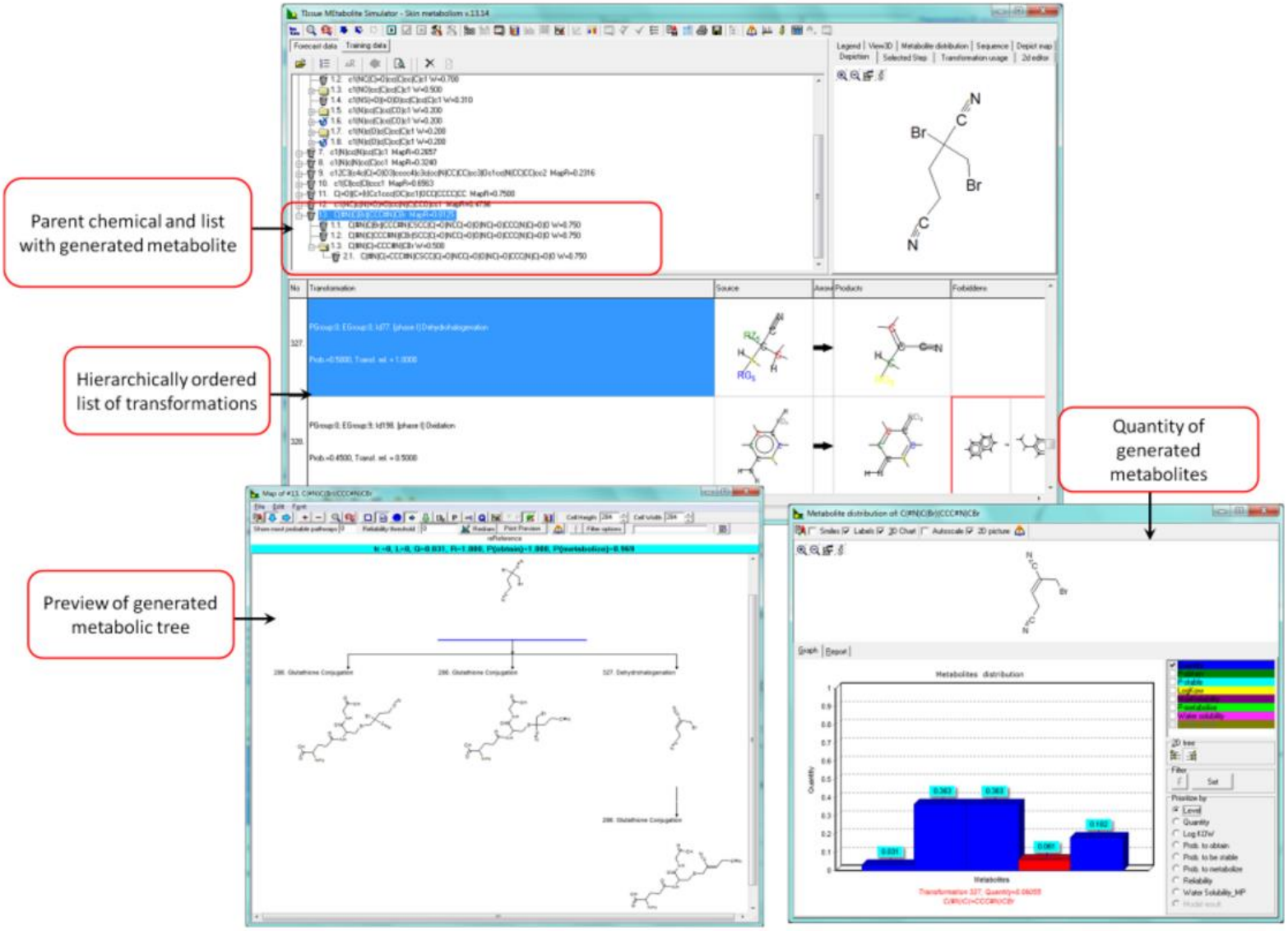
- OECD (Q)SAR toolbox (Developed by LMC OASIS, Bourgas, Bulgaria)
  - Metabolism simulators – Autooxidation simulator and Skin metabolism simulator
  - List of metabolites
- TIMES-SS (Developed by LMC OASIS, Bourgas, Bulgaria)
  - Hybrid system ((Q)SAR with Metabolism including Autooxidation)
  - Simulates in-vivo skin metabolism with auto-oxidation
  - Effect of metabolism on skin sensitization potential
- Meteor Nexus (Developed by Lhasa, Leeds, UK)
  - All biotransformation reactions
  - How likely a reaction will occur
  - Metabolite toxicity prediction from Derek Nexus

TISSUE METABOLISM SIMULATOR-SKIN SENS.

- **TIMES-SS:** Collaboration with Prof. Mekenyan and Dr. Dave Roberts
- **Assumptions:**
  - 1. Chemicals always penetrate stratum corneum
  - 2. Formation of protein conjugates is a premise for ultimate effect
  - 3. Metabolism may play significant role in skin sensitization
- **Training set:** 875 chemicals with experimental data from three sources (436 LLNA, 568 GPMT and 171 BfR).
- 380 hierarchically ordered transformations
  - Non-enzymatic transformations e.g. Hydrolysis of salts, Autoxidation reactions
  - Enzyme-mediated reactions (Phase I and Phase II) e.g. C-hydroxylation, Glucoronidation
  - Covalent interactions of chemicals/metabolites with skin proteins are described by almost 160 protein binding reactions

http://oasis-lmc.org/products/models/human-health-endpoints/skin-sensitization.aspx

TIMES-SS



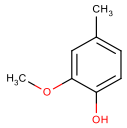
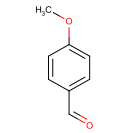
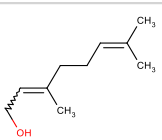
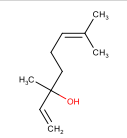
http://oasis-lmc.org/products/models/human-health-endpoints/skin-sensitization.aspx

TRANSFORMATION OF FRAGRANCE CHEMICALS

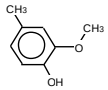
- Transformation alerts resulting in weak sensitizers
  - Hydroperoxides
  - Ketones
  - Quinone methide(s)/imines, Quinoide oxime structure, Nitroquinones

- Transformation alerts resulting in strong sensitizers
  - Epoxides, Aziridines and Sulfuranes
  - alpha, beta-Aldehydes
  - Di-substituted a,b-unsaturated aldehydes
  - Quinone methide(s)/imines, Quinoide oxime structure, Nitroquinones

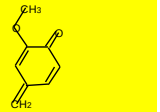
EXAMPLES FROM TIMES-SS V2.27.19

#	Parent Cas#	Parent Chem. Name	Parent Smiles	Parent skin_sens_exp	Parent Predicted SkinSens	Parent Total Domain	Metabolite Predicted SkinSens	Metabolite Active alert	Summary Predicted SkinSens
1	93-51-6	2-Methoxy-4-methyl phenol		Strong sensitizer	Non sensitizer	In domain	Strong sensitizer	Quinone methide(s)/imines, Quinoide oxime structure, Nitroquinones,	Strong sensitizer
2	123-11-5	p-Methoxybenaldehyde		Non sensitiser	Non sensitizer	In domain			Non sensitizer
3	106-24-1	Geraniol		No data	Non sensitizer	In domain	Strong sensitizer	Di-substituted a,b-unsaturated aldehydes	Strong sensitizer
4	78-70-6	Linalool		Weak sensitizer	Non sensitizer	In domain	Weak sensitizer	Hydroperoxides	Weak sensitizer

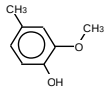
2-METHOXY-4-METHYL PHENOL



423. Autoxidation

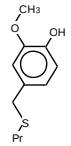


423. Autoxidation

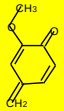


CC

99. Michael type addition on quinone methide (imine)



264. Oxidation

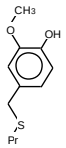


Phase 1 oxidation

266. O-Dealkylation

CC

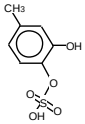
99. Michael type addition on quinone methide (imine)



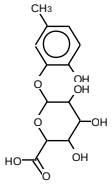
249. Oxidation

Phase 1 oxidation

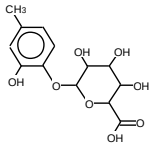
303. Sulphate conjugation



309. Glucuronidation of alcohols



309. Glucuronidation of alcohols



91. Michael type addition on quinones

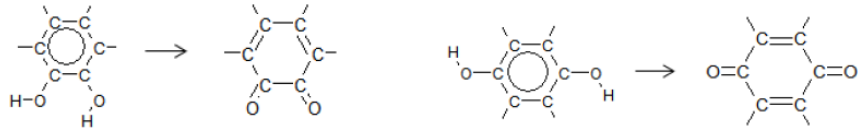
QUINONE METHIDE(S)

Oxidation

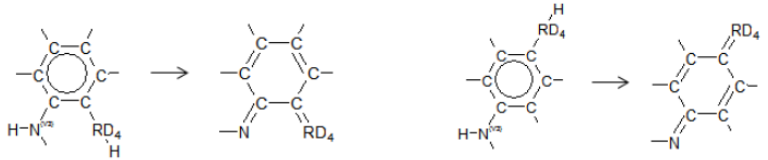
The transformation is confirmed by 3rd party expert (Dr. D. Roberts)

Oxidation transformations used in TIMES SS model are illustrated bellow:

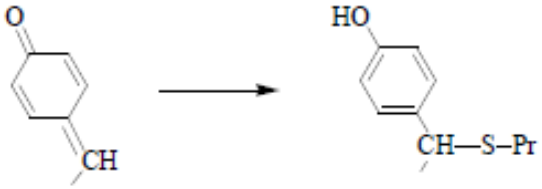
- o- and p-quinones



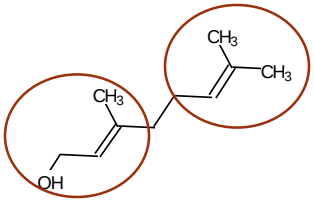
- o- and p-quinone (di)imines



The chemical is a strong sensitizer as a result of Michael type addition on quinone methide (imine):

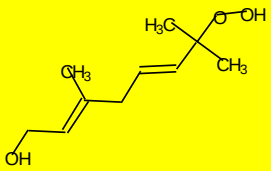


GERANIOL

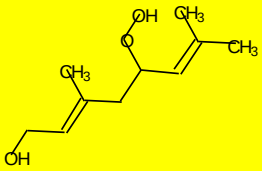


CC

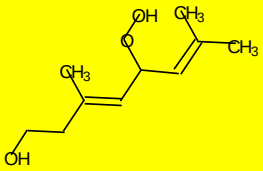
423. Autoxidation



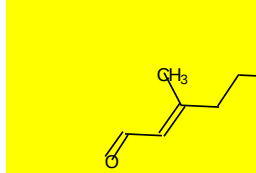
423. Autoxidation



423. Autoxidation



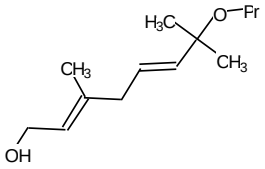
423. Autoxidation



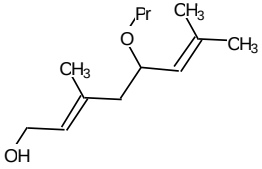
Strong sensitizer

CC

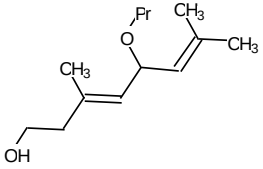
171. Hydroperoxide free radical decomposition



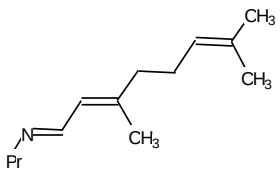
171. Hydroperoxide free radical decomposition



171. Hydroperoxide free radical decomposition



78. Schiff base formation with disubstituted a,b-aldehydes



HD

HD

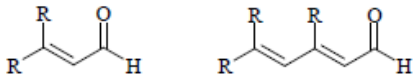
DI-SUBSTITUTED A,B-UNSATURATED ALDEHYDES

Mechanistic Domain: Schiff base formation

Mechanistic Alert: Direct Acting Schiff Base Formers

Structural Alert: Di-substituted α,β-unsaturated aldehydes

The chemical is a strong sensitizer as a result of **Schiff base formation with disubstituted α,β-aldehydes:**



R = alkyl or aromatic carbon (including carbons in heterocyclic rings)

Mechanism

These chemicals have been suggested to act via Schiff base formation due to the steric hindrance at the β-carbon atom preventing Michael addition (Roberts *et al.*, 2006).

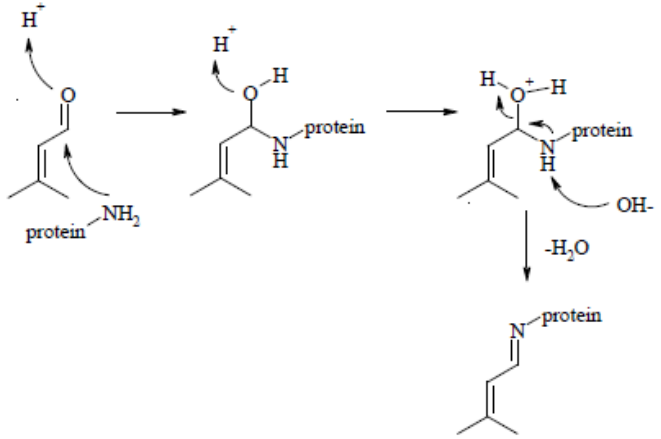
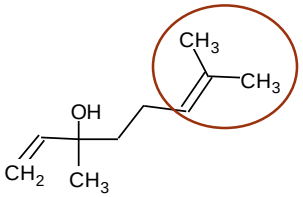


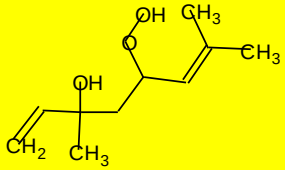
Figure 1: Mechanism for Schiff base formation for di-substituted α,β-unsaturated aldehydes

LINALOOL

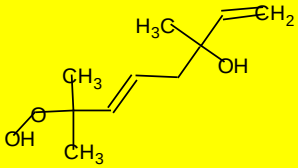


CC

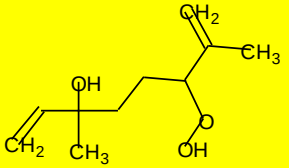
423. Autoxidation



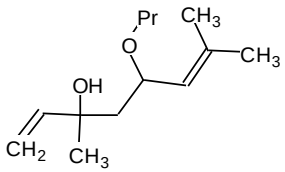
423. Autoxidation



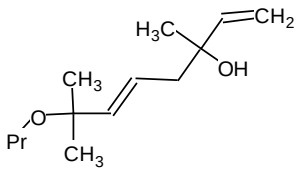
423. Autoxidation



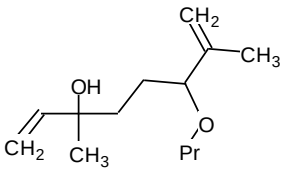
171. Hydroperoxide free radical decomposition



171. Hydroperoxide free radical decomposition



171. Hydroperoxide free radical decomposition



HD

HYDROPEROXIDES

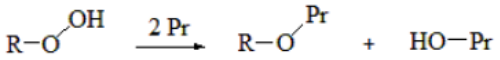
The reliability of the transformation is supported by Dr D. Roberts,
School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University,
Liverpool, England L3 3AF

Mechanistic Domain: **Radical reactions**

Mechanistic Alert: **Free Radical formation**

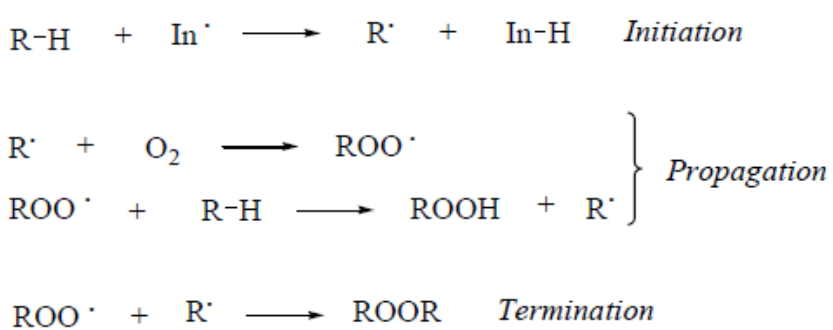
Structural Alert: Hydroperoxides

The chemical has an assumptive weak sensitization effect as a result of **Hydroperoxide free radical decomposition:**

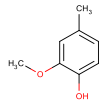
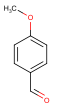
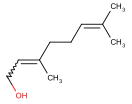
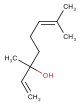


R = -C(C)C=C

Autoxidation can be defined as insertion of oxygen into a C-H bond forming a hydroperoxide (ROOH). Autoxidation is a free radical chain reaction:



EXPERIMENTAL IDENTIFICATION OF PRE/PRO HAPTENS

							KE-1		KE-2	KE-3		KE-4	ADVERSE OUTCOME	
#	Parent Cas#	Parent Chem. Name	Parent chemical	Parent Predicted SkinSens	Summary Predicted SkinSens	Parent Total Domain	DPRA	PPRA	KeratinoSens™	hCLAT	U-Sens™	LLNA (%) EC3	LOEL ($\mu g/cm^2$)	NOEL ($\mu g/cm^2$)
1#	93-51-6	2-Methoxy-4-methyl phenol		Non sensitiser	Strong sensitiser	In domain	Neg	Pos	Neg	Pos	Pos	5.80%	NA	118
2	123-11-5	p-Metoxybenaldehyde		Non sensitiser	Non sensitiser	In domain	Neg/Pos	Pos	Neg§	Pos	Pos	>25%	4700	3500
3#	106-24-1	Geraniol		Non sensitiser	Strong sensitiser	In domain	Neg	NA	Neg	Pos	Pos	22.4%*	NA	11800
4#	78-70-6	Linalool		Non sensitiser	Weak sensitiser	In domain	Neg	NA	Neg	Pos	Pos	46.2%*	NA	15000

* EC3 value of pure material as reviewed by Uter W et. al Contact Dermatitis, 69 (2013) 196-230

\# In-vitro data, except PPRA, are reported in Urbisch et. al Regulatory Toxicology and Pharmacology, 71 (2015) 337–351

§ KeratinoSens™ data provided by Andreas Natsch, Givaudan Inc.

- Literature reports of activating a chemical using Air exposure/CYP before testing in one or more of the above methods
  - A-T Karlberg group e.g. Hagvall et. al Toxicology and Applied Pharmacology 233 (2008) 308–313
  - Andreas Natsch Rat liver S9 fraction in KeratinoSens™ e.g. Toxicological Sciences 135(2) (2013) 356-368
- Patlewicz G. et. al. Regulatory Toxicology and Pharmacology 82 (2016) 147e155
  - EURL ECVAM dataset of 127 indirect acting sensitizers (J-P Lepoittevin, DW Roberts, A-T Karlberg and G Patlewicz)
  - Concluded that "sensitizers requiring activation could be identified correctly using one or more of the current non-animal methods"

LIMITATIONS OF THE TOOLS

- TIMES-SS is not a perfect in silico tool, it is most advanced –
  - Strong sens. 91%
  - Weak sens. 52%
  - Non sens. 70%
  - Overall performance: 78%
- Relevance of all alerts/transformations to positive outcomes in humans in real life scenario ?
- DPRA – One concentration, One time point
- KeratinoSens™ – Limited metabolic capacity
- hCLAT/U-Sens™ – Limited metabolic capacity

SUMMARY

- Skin sensitization is a reactivity driven endpoint (Molecular Initiating Event)
- Chemicals, including fragrance ingredients, could be divided into various reaction mechanistic domains "Alerts"
- Chemicals that are non-sensitizers could be activated by (a)biotic transformations
- TIMES-SS can identify Pre/Pro haptens
- Validated In-chemico and In-vitro methods can identify indirect acting sensitizers

THANK YOU!