# *In silico* predicting of pre- and pro- electrophilic activation in the SS assessment

## Simulating metabolic pathways through QSAR/SAR

Laboratory of Mathematical Chemistry, Bourgas

2015

# Outlook

- TIMES-SS model (reminder from last presentation)
- Recent initiatives of improving TIMES-SS model
  - ✓ Alert reliability
  - ✓ (a)biotic activation pathways
- The role of pro-electrophilic activation
- Addition of kinetic experimental data for clearance
- Summary

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# TIMES Skin Sensitization model

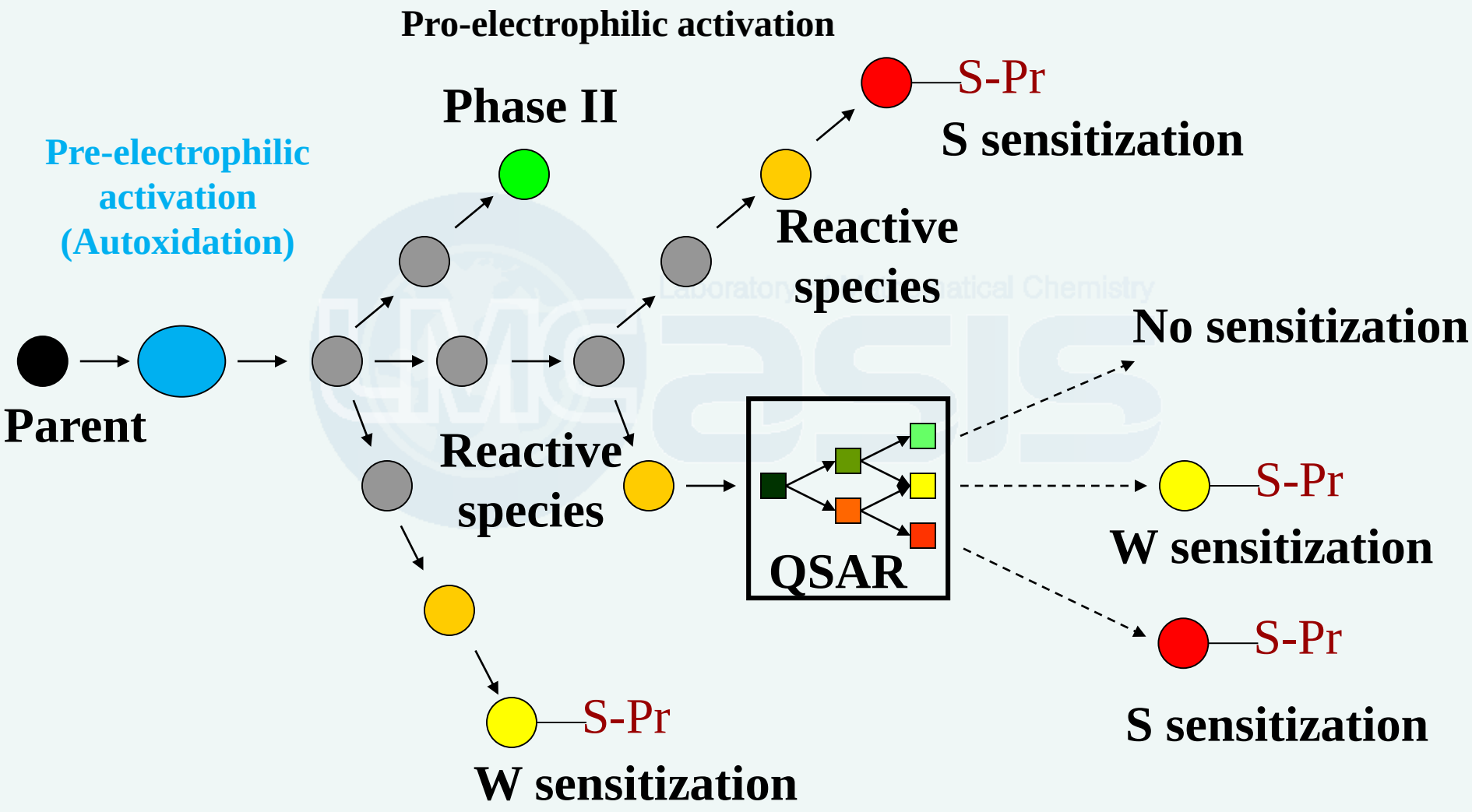
## Short description

**TIMES-SS model** includes:

- Application of Autoxidation simulator
- Simulation of Skin metabolism
- Specific molecular transformations describing the covalent interactions with proteins
- Mechanistic justification of the protein binding mechanism
- Accounting model applicability domain

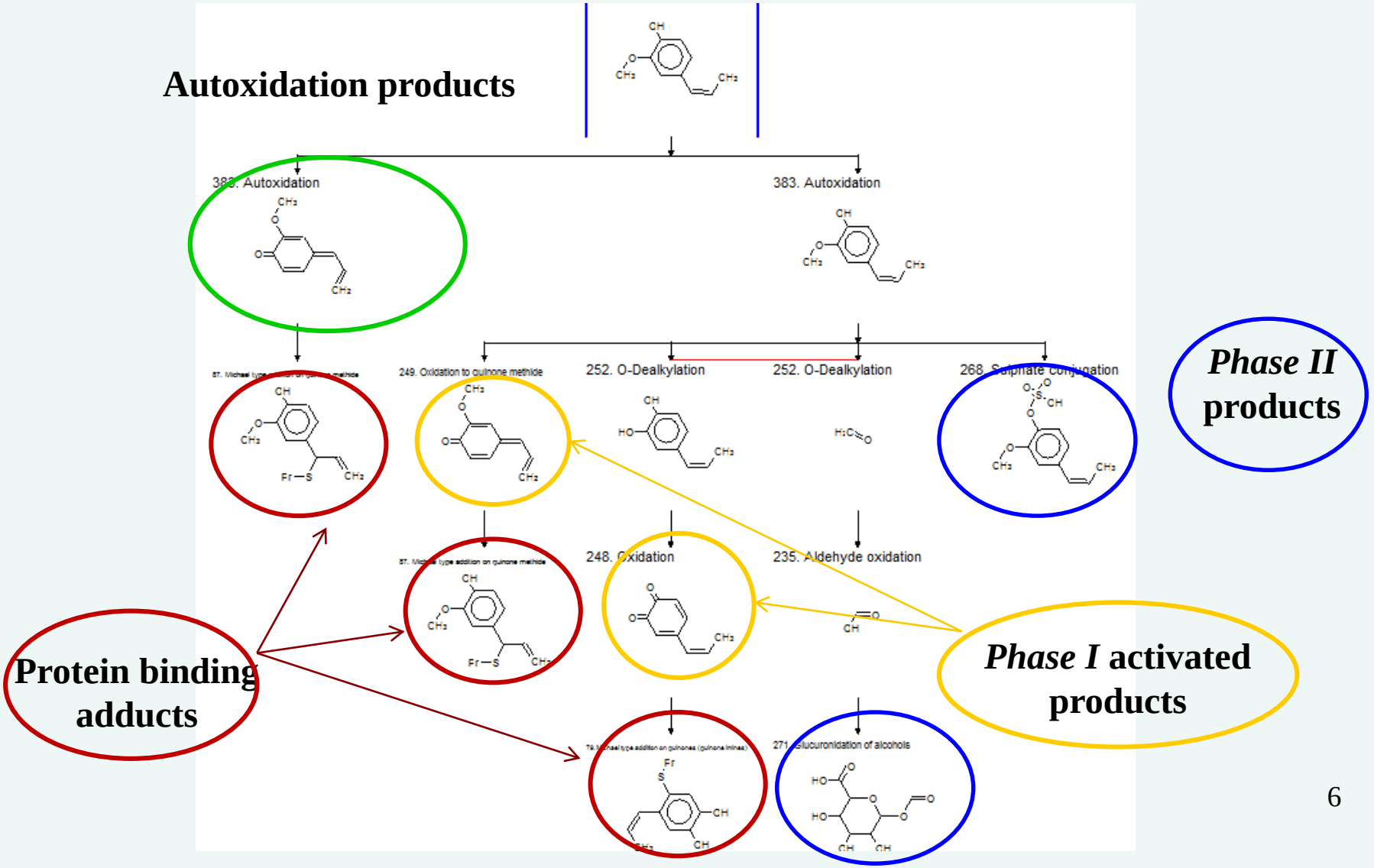
# Predicting skin sensitization in TIMES

## Basic scheme illustrating the model concept



# Predicting skin sensitization in TIMES

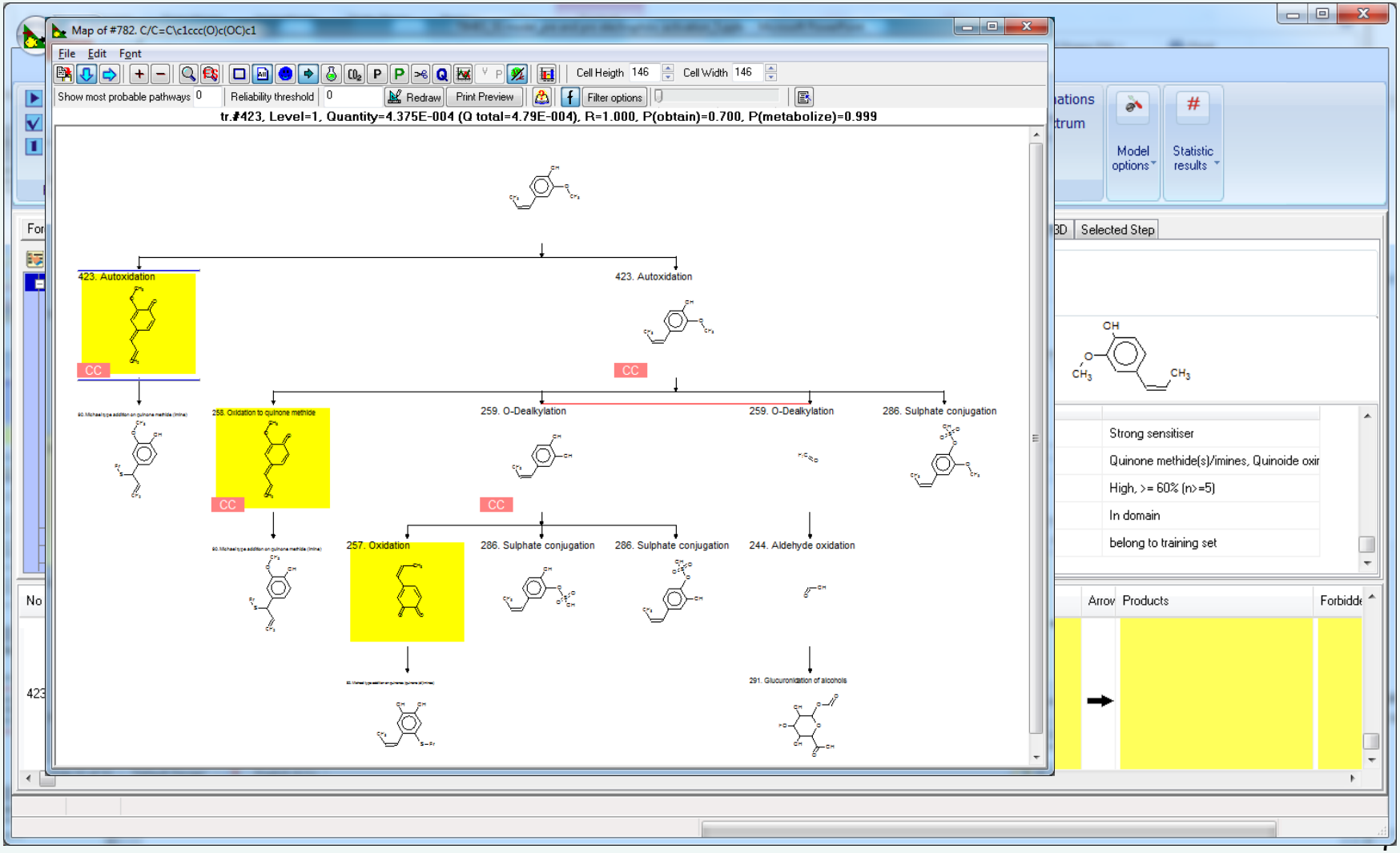
TIMES-SS Predicted metabolism of *Isoeugenol* accounting AU and SM activation



# Predicting skin sensitization in TIMES

## Results from TIMES-SS model

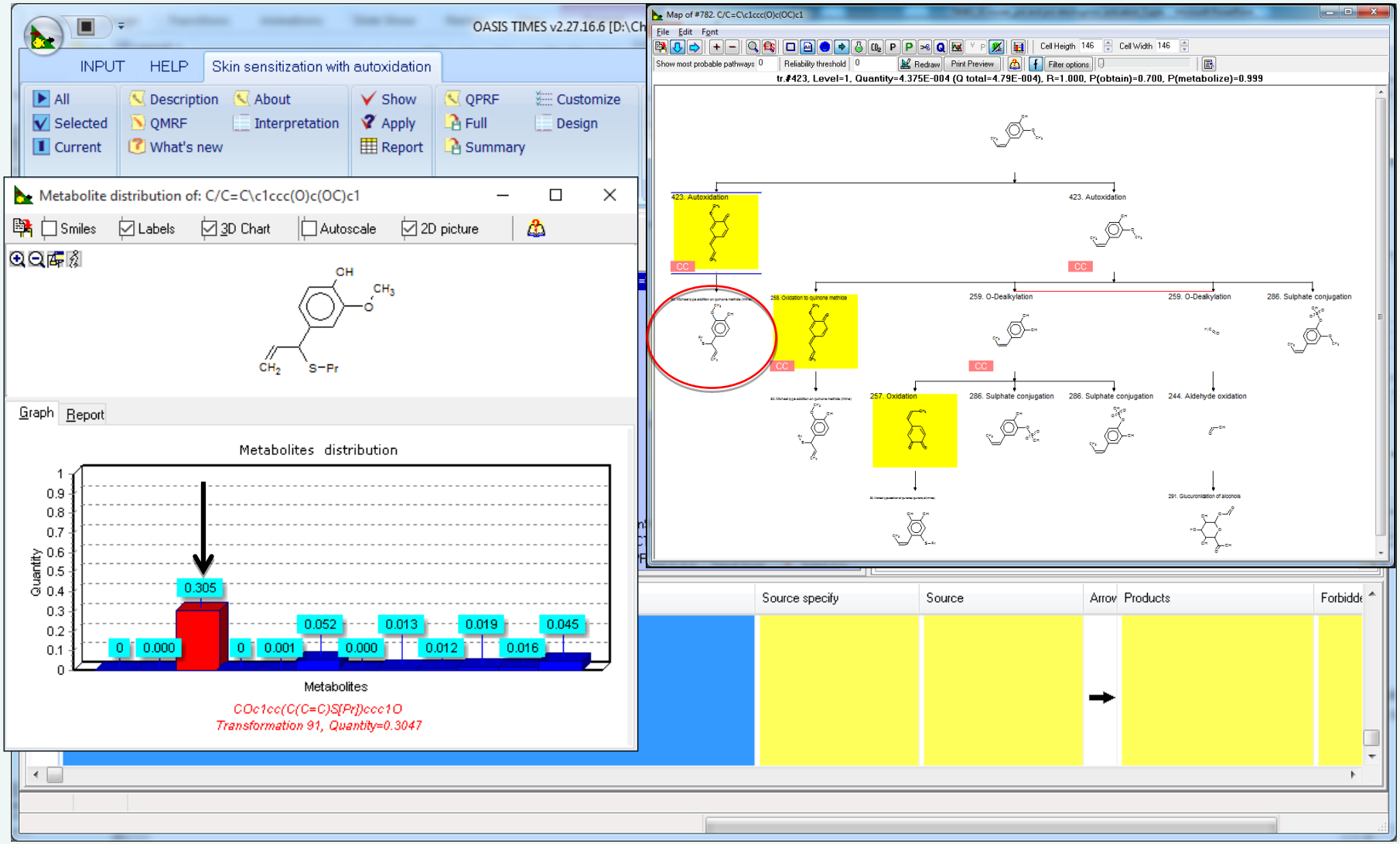
Generated metabolic map. Activated metabolites are highlighted



# Predicting skin sensitization in TIMES

## Results from TIMES-SS model

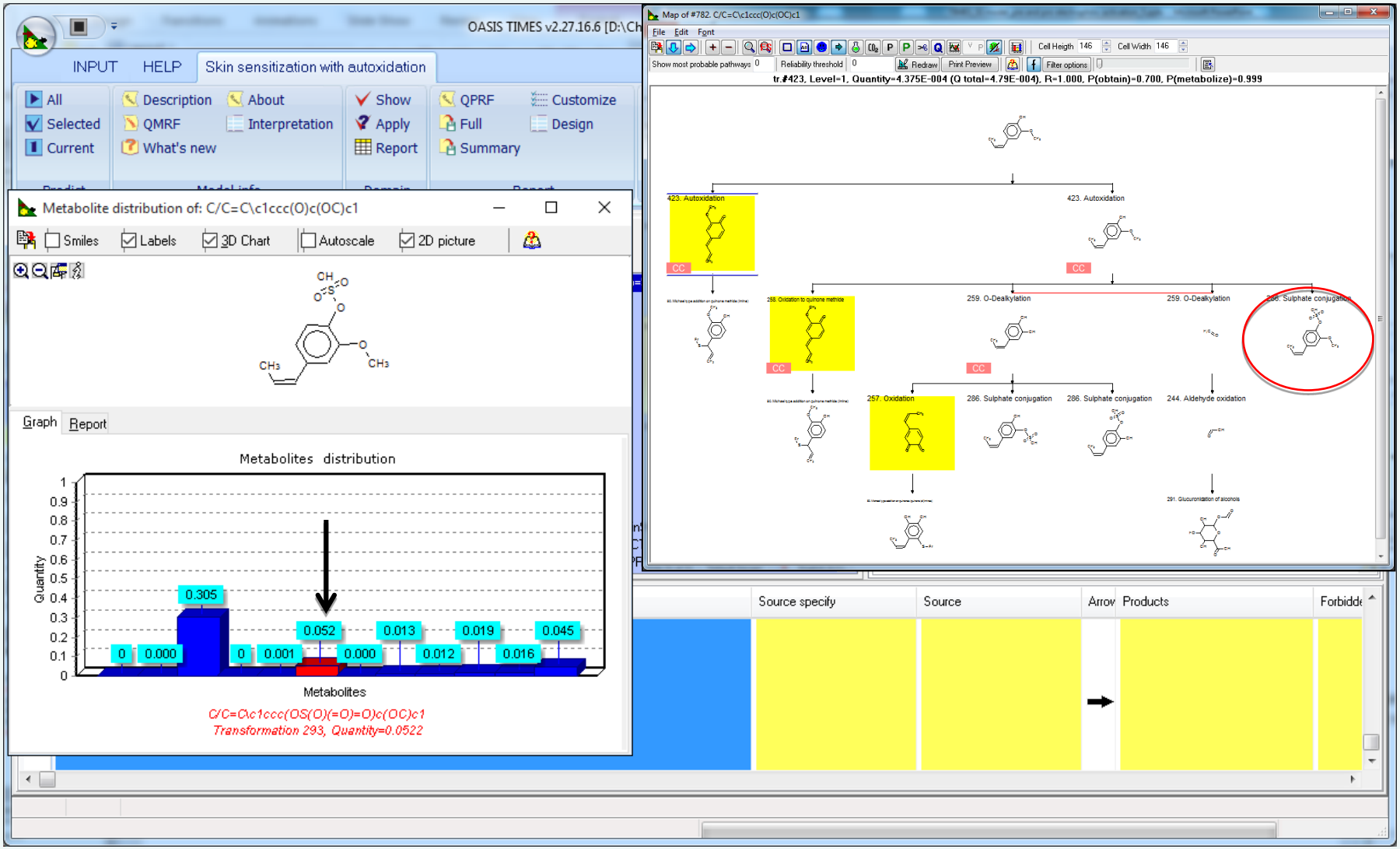
### Quantitative assessment of generated metabolites



# Predicting skin sensitization in TIMES

## Results from TIMES-SS model

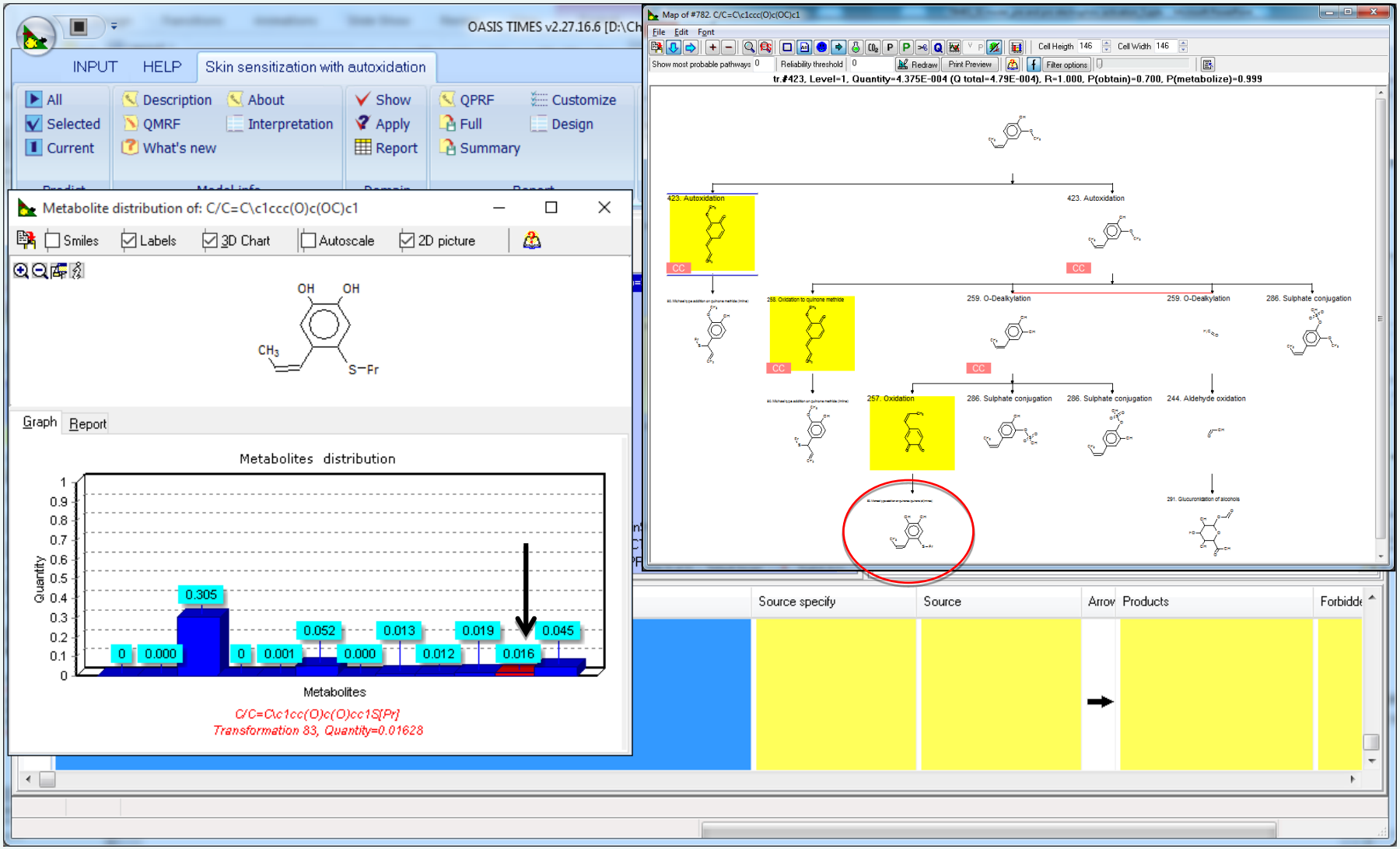
### Quantitative assessment of generated metabolites



# Predicting skin sensitization in TIMES

## Results from TIMES-SS model

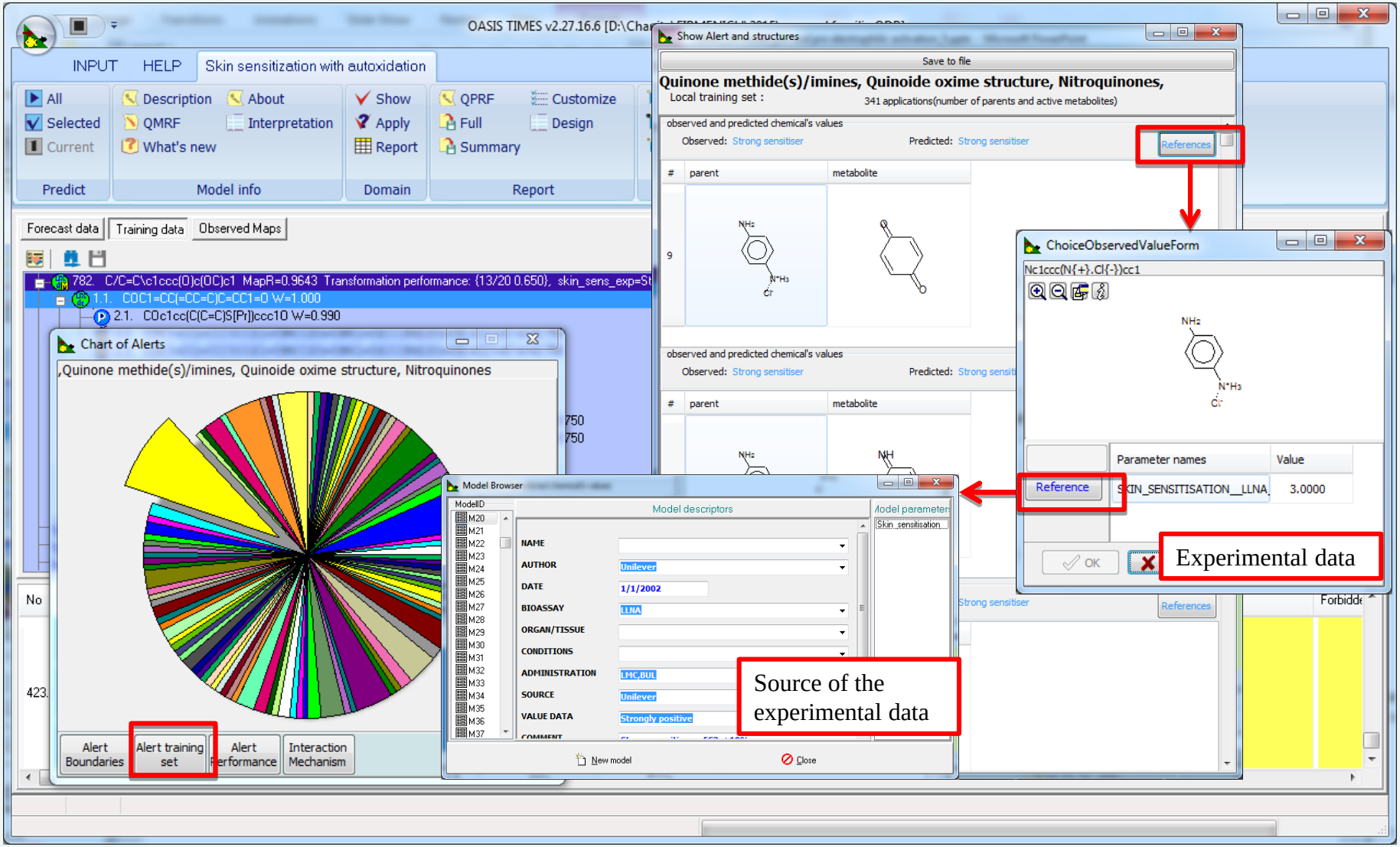
### Quantitative assessment of generated metabolites



# Predicting skin sensitization in TIMES

## Results from TIMES-SS model

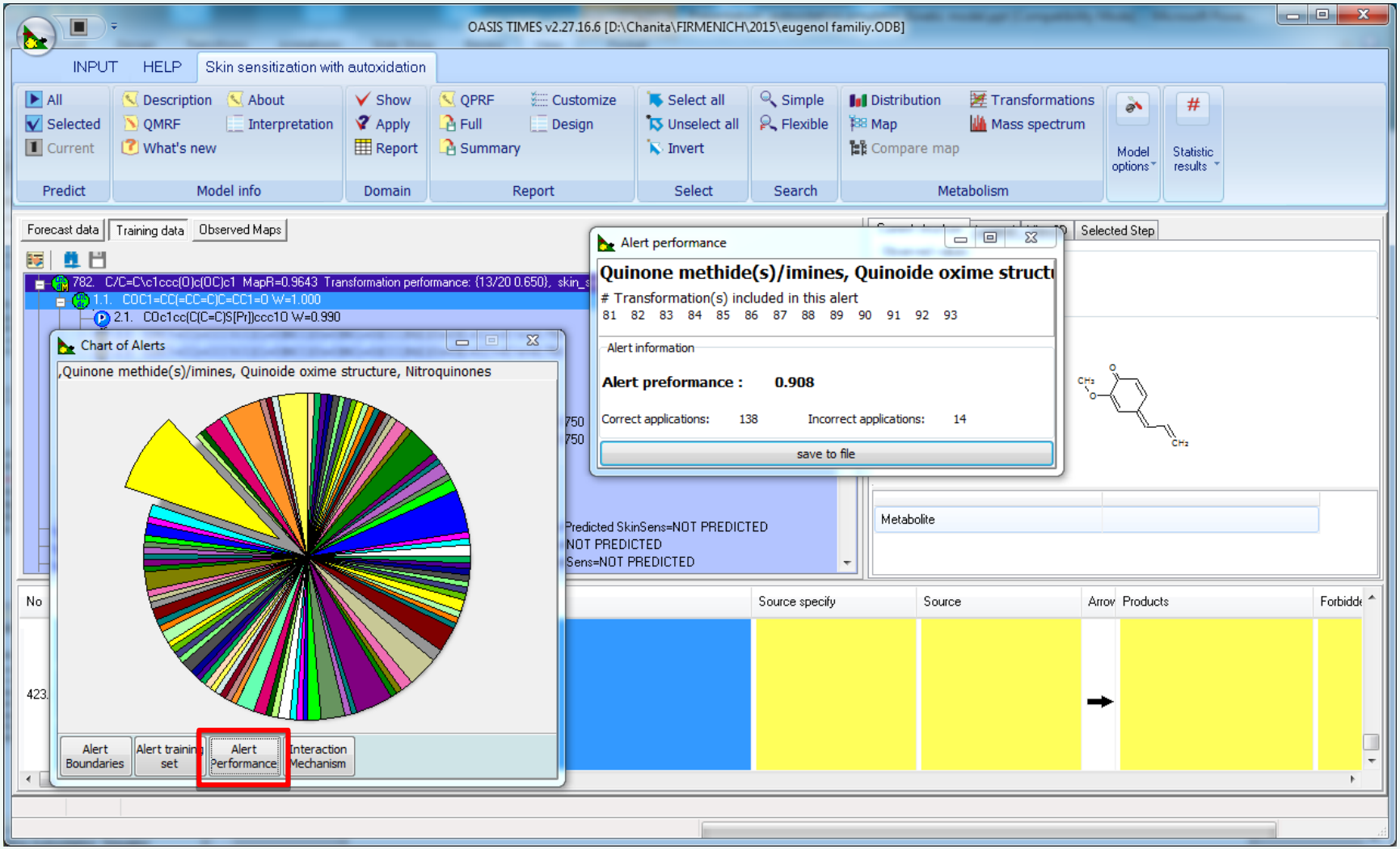
### Local Training Sets of alerts



# Predicting skin sensitization in TIMES

## Results from TIMES-SS model

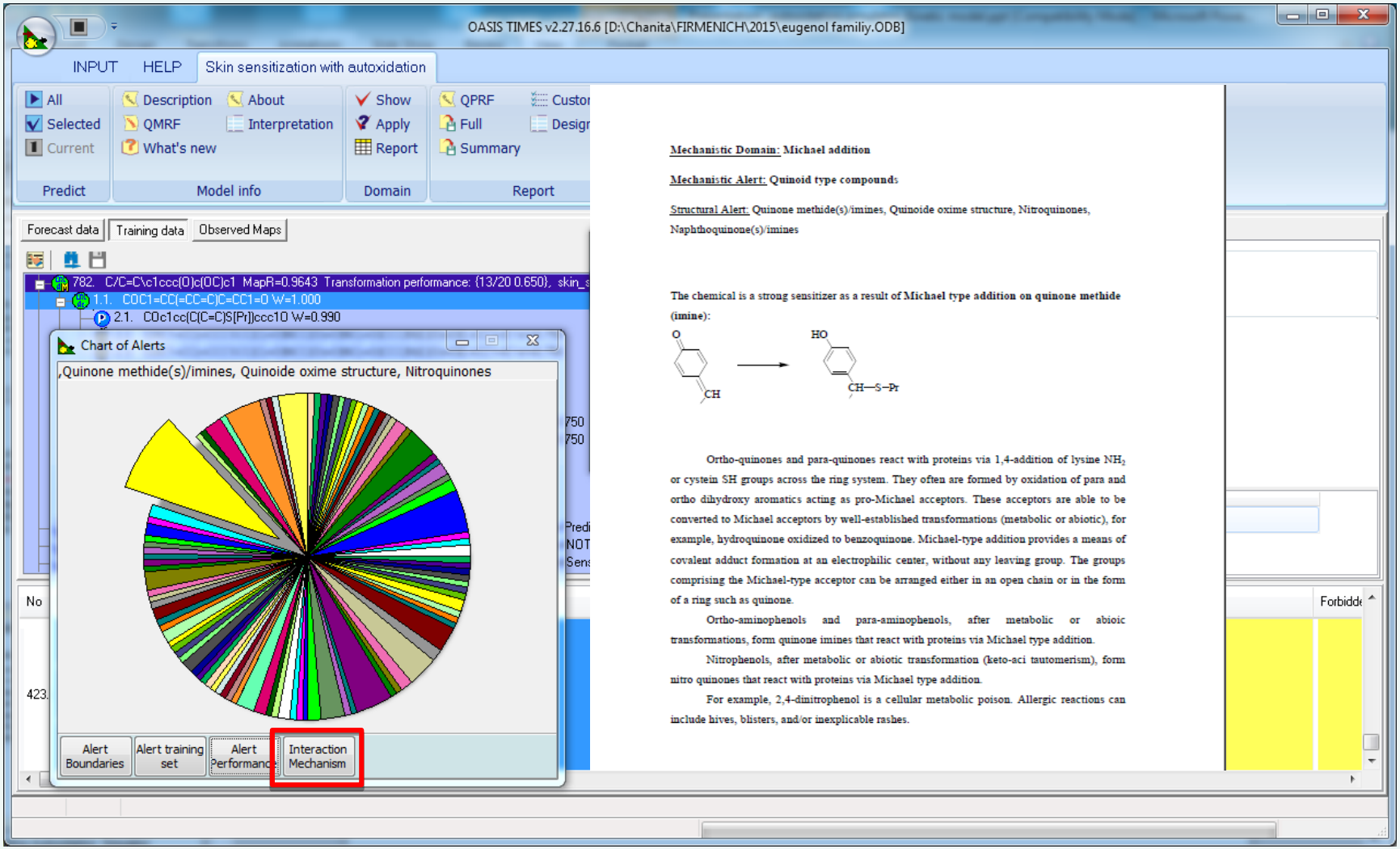
### Alert Performance



# Predicting skin sensitization in TIMES

## Results from TIMES-SS model

### Mechanistic justification



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# Recent initiatives of improving TIMES-SS model

**Alert reliability is assessed by:**

- ✓ ***Alert performance*** – it is defined as the ratio between the number of correctly classified chemicals vs. the total number of chemicals within the alert training set
- ✓ ***Number of chemicals in the alert training set*** is also used as a criteria for reliability (current threshold is set to five chemicals)

# Recent initiatives of improving TIMES-SS model

**Alert reliability - adopted thresholds**

Based on their performance protein binding alerts have been classified as having:

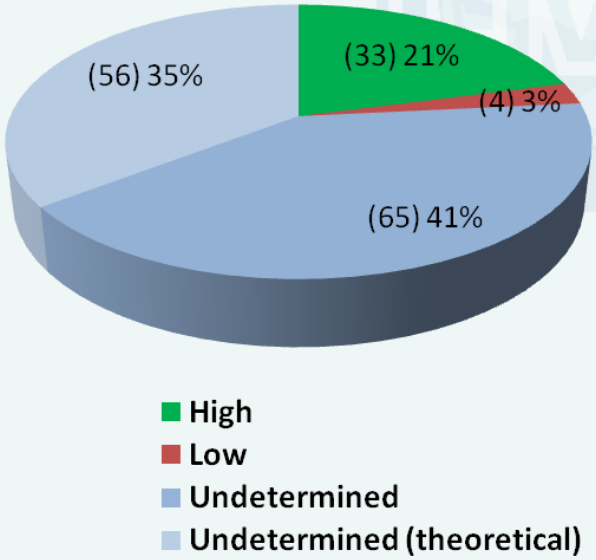
- ✓ **High** reliability (perf. ≥ 60% and n ≥ 5)
- ✓ **Low** reliability (perf. ≤ 60% and n ≥ 5)
- ✓ **Undetermined** reliability (1< n <5)
- ✓ **Undetermined theoretical** reliability (no support by exp. data)

# Recent initiatives of improving TIMES-SS model

**Alert reliability**

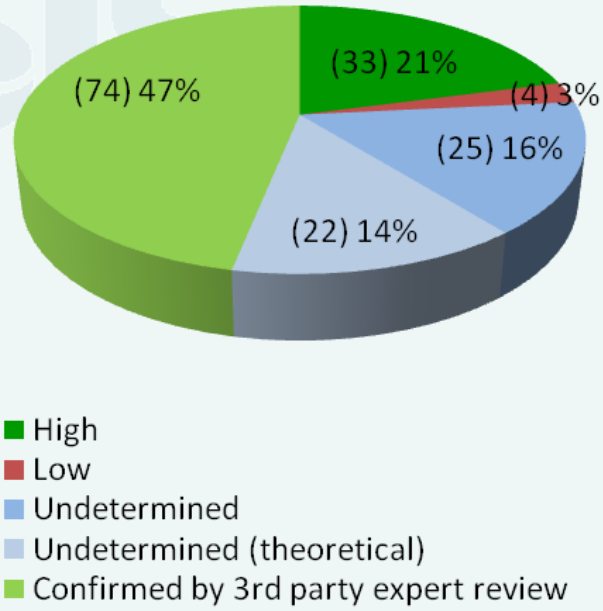
- ✓ Protein binding alerts in TIMES-SS model have been reviewed by **Dr. David Roberts**
- ✓ The following improvement have been reached:

**Before**




**After expert review reliable alerts increased up to 68%**

**After**



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# Recent initiatives of improving TIMES-SS model

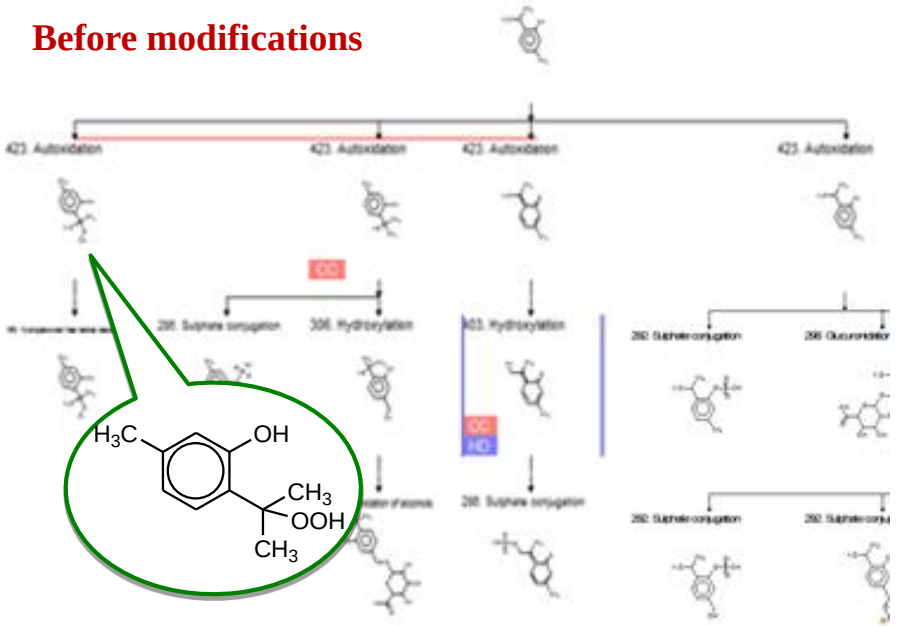
## Improving of (a)biotic pathways – current work

- Under a joint initiative of **RIFM** and **LMC** with participation of **Dr. David Roberts** as reviewing expert, curation of transformations leading to generation of protein binding alerts has been undertaken.
- All (a)biotic transformations that leads to generation of protein binding alerts are reviewed by Dr. D.Roberts and all modifications are currently implemented into the TIMES-SS model.

# Recent initiatives of improving TIMES-SS model

**Modifications addressing the abiotic (pre-electrophilic) activation to:**

- Hydroperoxides

| Metabolic path | Input from D. Roberts | Result |
|---|---|---|
| **Before modifications**   | Phenolic ortho OH group probably acts as an antioxidant, so rather than forming the benzylic radical (first stage in formation of the hydroperoxide) it forms the phenolic radical by abstraction of H from the OH group. This phenolic radical does not have a simple path to an electrophilic species, so the chemical is Non sensitizer. | |

# Recent initiatives of improving TIMES-SS model

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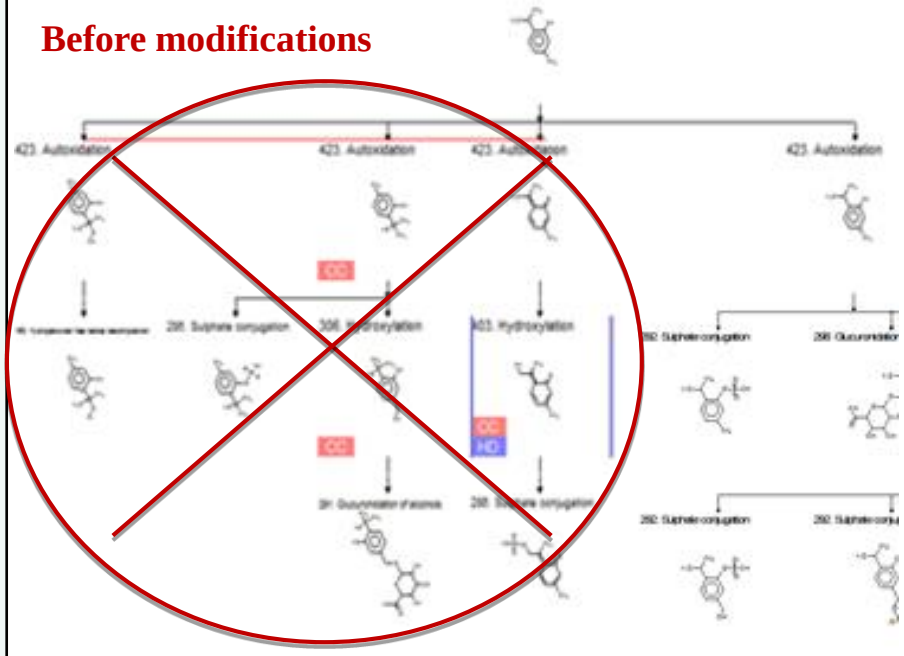
| **Before modifications**<br>  | Phenolic ortho OH group probably acts as an antioxidant, so rather than forming the benzylic radical (first stage in formation of the hydroperoxide) it forms the phenolic radical by abstraction of H from the OH group. This phenolic radical does not have a simple path to an electrophilic species, so the chemical is Non sensitizer.<br><br>**LMC action:**<br>Addition of a mask (phenolic ortho OH) that prevents the benzylic hydroperoxide formation. | |

# Recent initiatives of improving TIMES-SS model

**Modifications addressing the abiotic (pre-electrophilic) activation to:**

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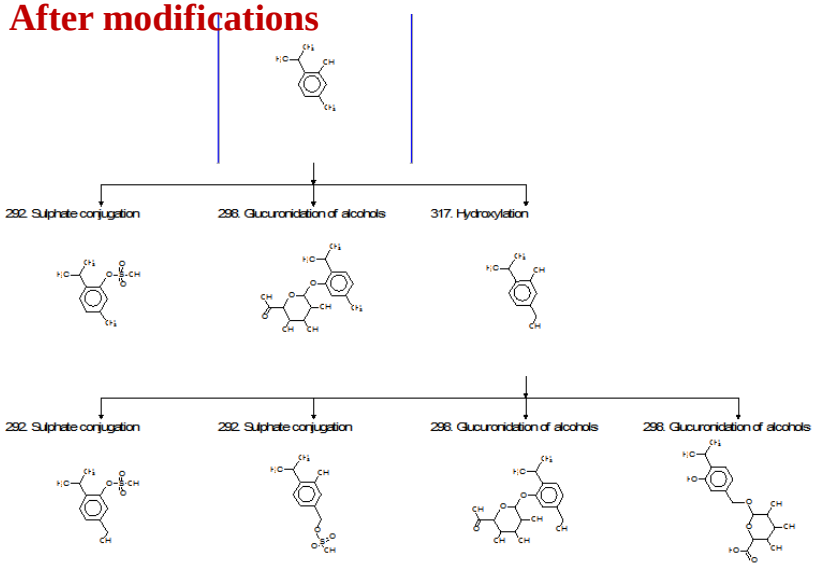
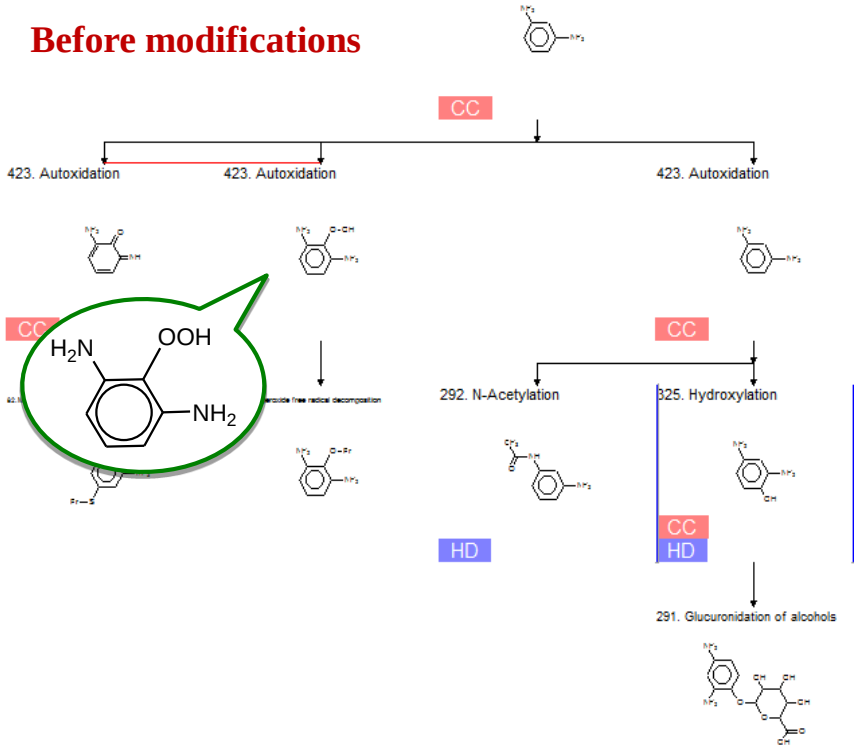
| **After modifications**<br>  | Phenolic ortho OH group probably acts as an antioxidant, so rather than forming the benzylic radical (first stage in formation of the hydroperoxide) it forms the phenolic radical by abstraction of H from the OH group. This phenolic radical does not have a simple path to an electrophilic species, so the chemical is Non sensitizer.<br><br>**LMC action:**<br>Addition of a mask (phenolic ortho OH) that prevents the benzylic hydroperoxide formation. | Correctly predicted as Non sensitizer – no autoxidation products are generated. |

# Recent initiatives of improving TIMES-SS model

**Modifications addressing the abiotic (pre-electrophilic) activation to:**

- Quinones

| Metabolic path | Input from D. Roberts | Result |
|---|---|---|
| **Before modifications**   | This chemistry is unlikely. Compound has a repeatable EC3, and probably does not sensitize via the hydroperoxide shown. It is most susceptible to oxidation (enzymatic or abiotic) by attack at the carbon ortho to both NH2 groups, or at a carbon para to one and ortho to the other, resulting in a highly electrophilic quinone-imine as the ultimate sensitizer. | |

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**Modifications addressing the abiotic (pre-electrophilic) activation to:**

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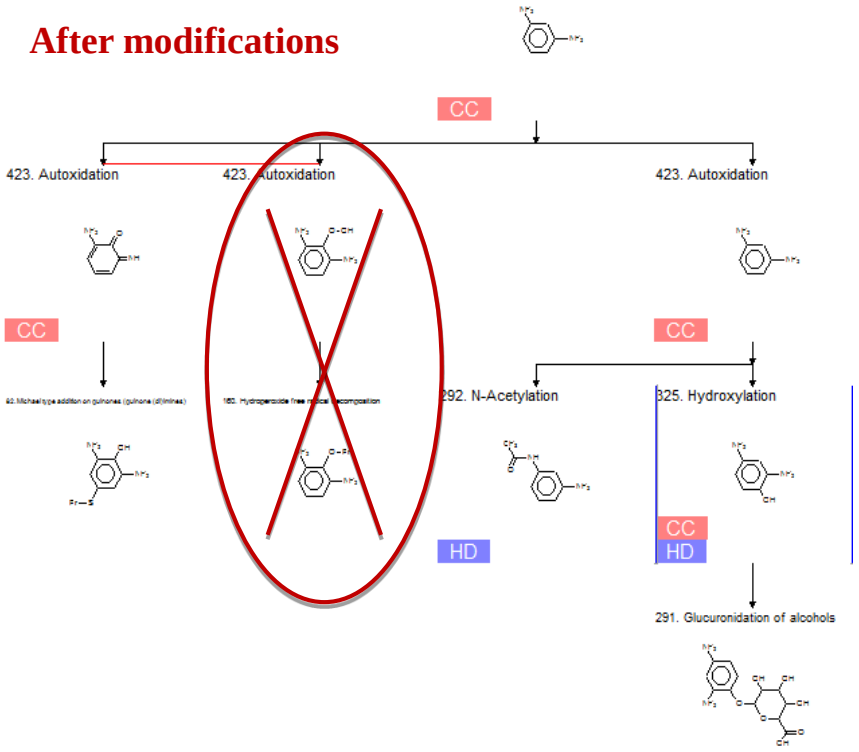
|  | This chemistry is unlikely. Compound has a repeatable EC3, and probably does not sensitize via the hydroperoxide shown. It is most susceptible to oxidation (enzymatic or abiotic) by attack at the carbon ortho to both NH2 groups, or at a carbon para to one and ortho to the other, resulting in a highly electrophilic quinone-imine as the ultimate sensitizer.<br><br>**LMC action:**<br>Addition of spontaneous conversion of hydroperoxide to quinone imine in order to prevent the interaction with proteins of the intermediate hydroperoxide.<br>Generation of an additional para quinone imine. | |

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**Modifications addressing the abiotic (pre-electrophilic) activation to:**

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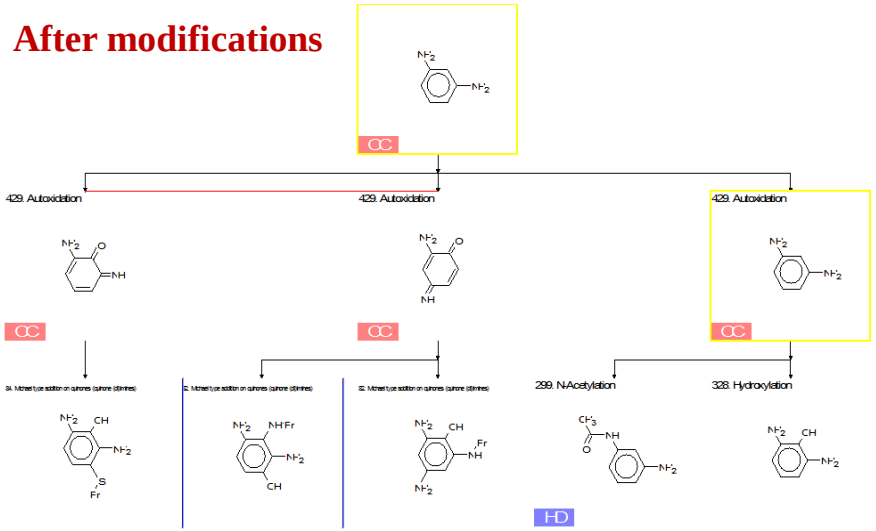
| **After modifications**<br>  | This chemistry is unlikely. Compound has a repeatable EC3, and probably does not sensitize via the hydroperoxide shown. It is most susceptible to oxidation (enzymatic or abiotic) by attack at the carbon ortho to both NH2 groups, or at a carbon para to one and ortho to the other, resulting in a highly electrophilic quinone-imine as the ultimate sensitizer.<br><br>**LMC action:**<br>Addition of spontaneous conversion of hydroperoxide to quinone imine in order to prevent the interaction with proteins of the intermediate hydroperoxide.<br>Generation of an additional para quinone imine. | Autoxidation products are more scientifically reliable. |

# Recent initiatives of improving TIMES-SS model

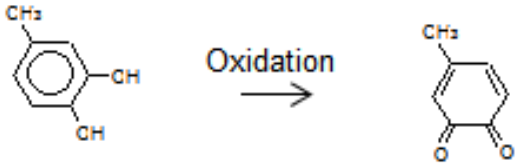
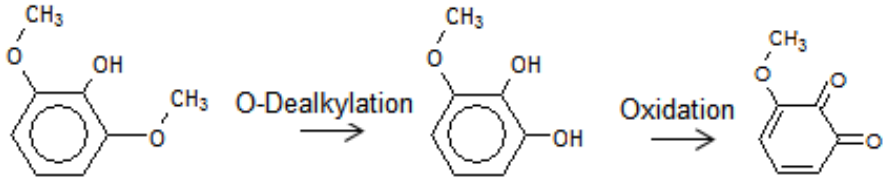
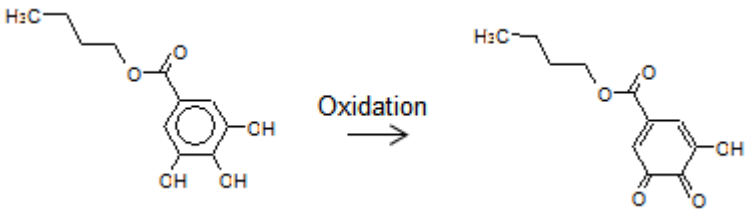
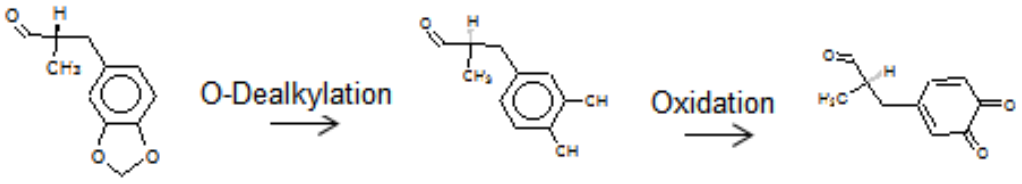
**Modifications addressing the biotic (pro-electrophilic) activation of chemicals**

- It has been found that skin sensitization potential is reduced if the chemicals are activated by consecutive transformation steps. This is probably due to participation of more than one enzymes catalyzing each of the consecutive transformations.

# Recent initiatives of improving TIMES-SS model

## Modifications addressing the biotic (pro-electrophilic) activation of chemicals

- Examples of pro-electrophilic activation to ortho-quinones

| Chemicals generating o-quinone at: | |
|---|---|
| **One transformation step** | **More than one transformation steps** |
|   **Obs. SS – Pred. SS** |   **Obs. WS – Pred. WS** |
|   **Obs. SS – Pred. SS** |   **Obs. WS – Pred. WS** |

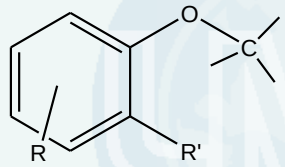
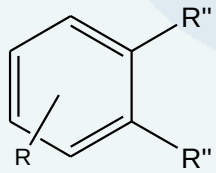


* WS – Weak sensitizers; SS – Strong sensitizers

# Recent initiatives of improving TIMES-SS model

**Modifications addressing the biotic (pro-electrophilic) activation of chemicals**

- Experimental support

| 2D fragment | Number of observed* | | | Note |
|---|---|---|---|---|
| | NS | WS | SS | |
|   R' = OH or OC | 3 | 3 | 3 | Chemicals requiring consecutive steps of activation |
|   R" = OH | 2 | 1 | 31 | Chemicals requiring one step of activation |

* NS – Non sensitizers; WS – Weak sensitizers; SS – Strong sensitizers

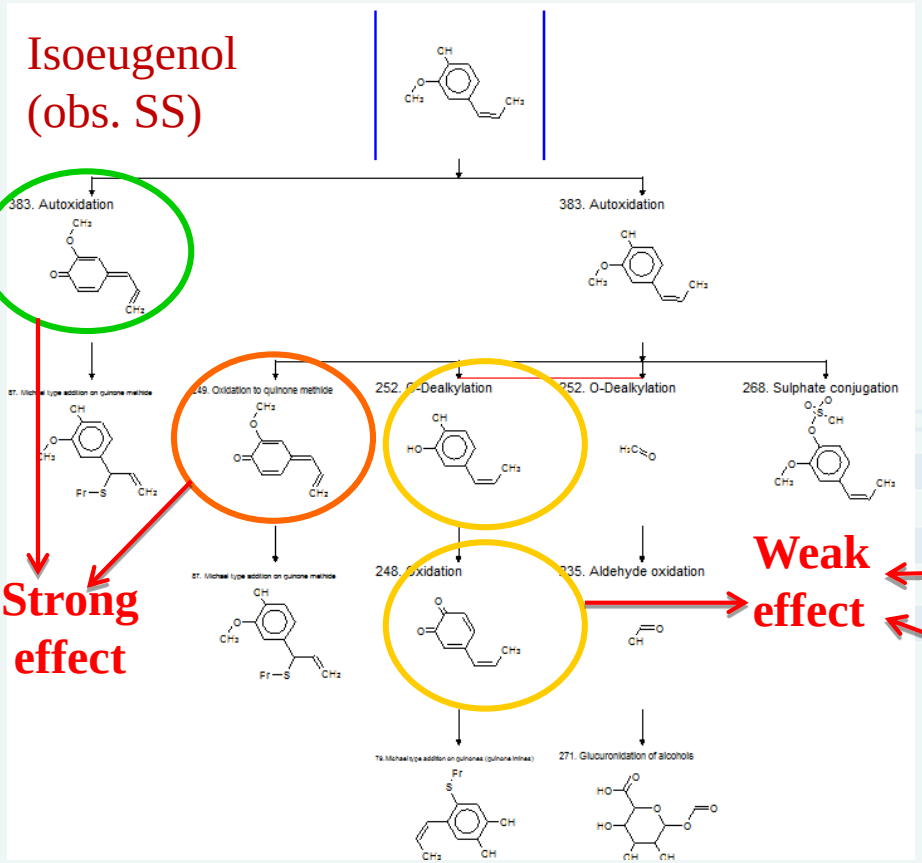
# Recent initiatives of improving TIMES-SS model

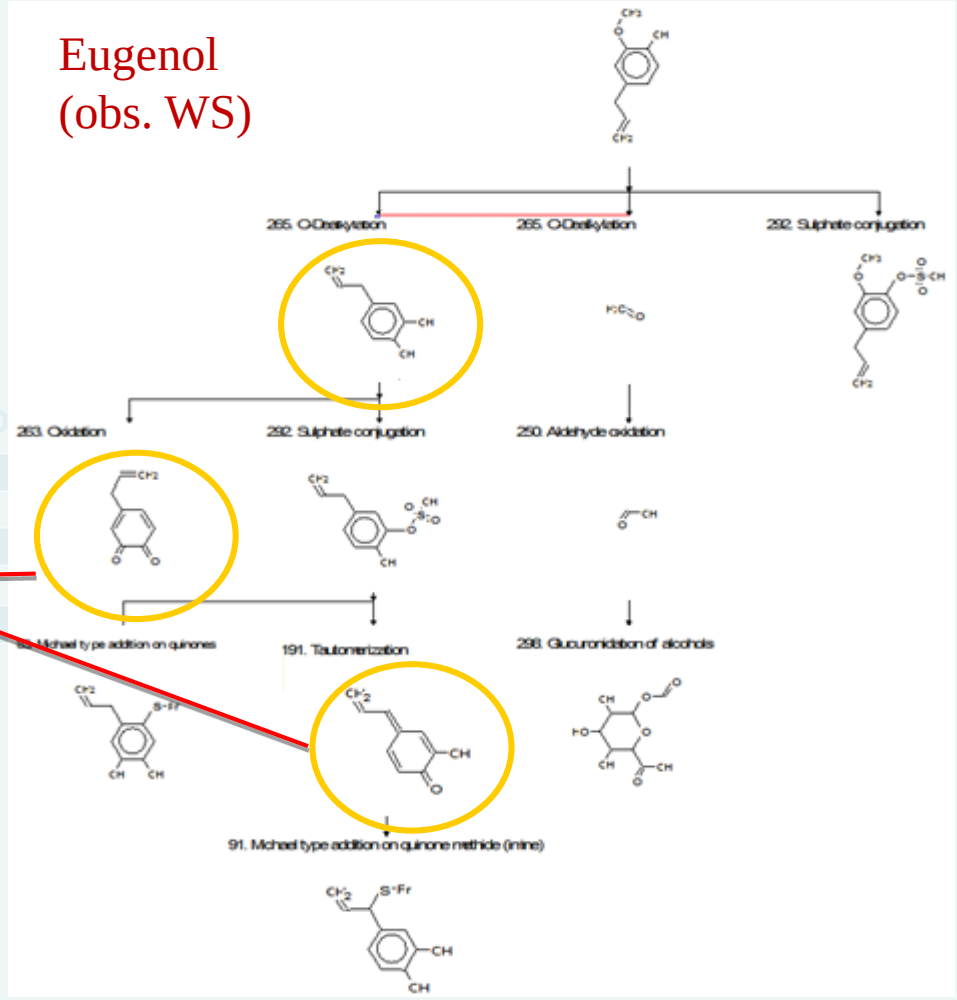
**Modifications addressing the biotic (pro-electrophilic) activation of chemicals**

- It has been found that skin sensitization potential is reduced if the chemicals are activated by consecutive transformation steps. This is probably due to participation of more than one enzymes catalyzing each of the consecutive transformations.
- This relation was used to discriminate the observed skin sensitization effect of **Isoeugenol** (obs. *Strong sensitizer*) and **Eugenol** (obs. *Weak sensitizer*)

# Recent initiatives of TIMES-SS model upgrade

**TIMES-SS Predicted metabolism of *Isoeugenol* and *Eugenol***




**Metabolism of:**

Isoeugenol

- Autoxidation to quinone methide
- Biotic oxidation to quinone methide (single step activation)
- Biotic dealkylation and subsequent oxidation (consecutive steps of activation)

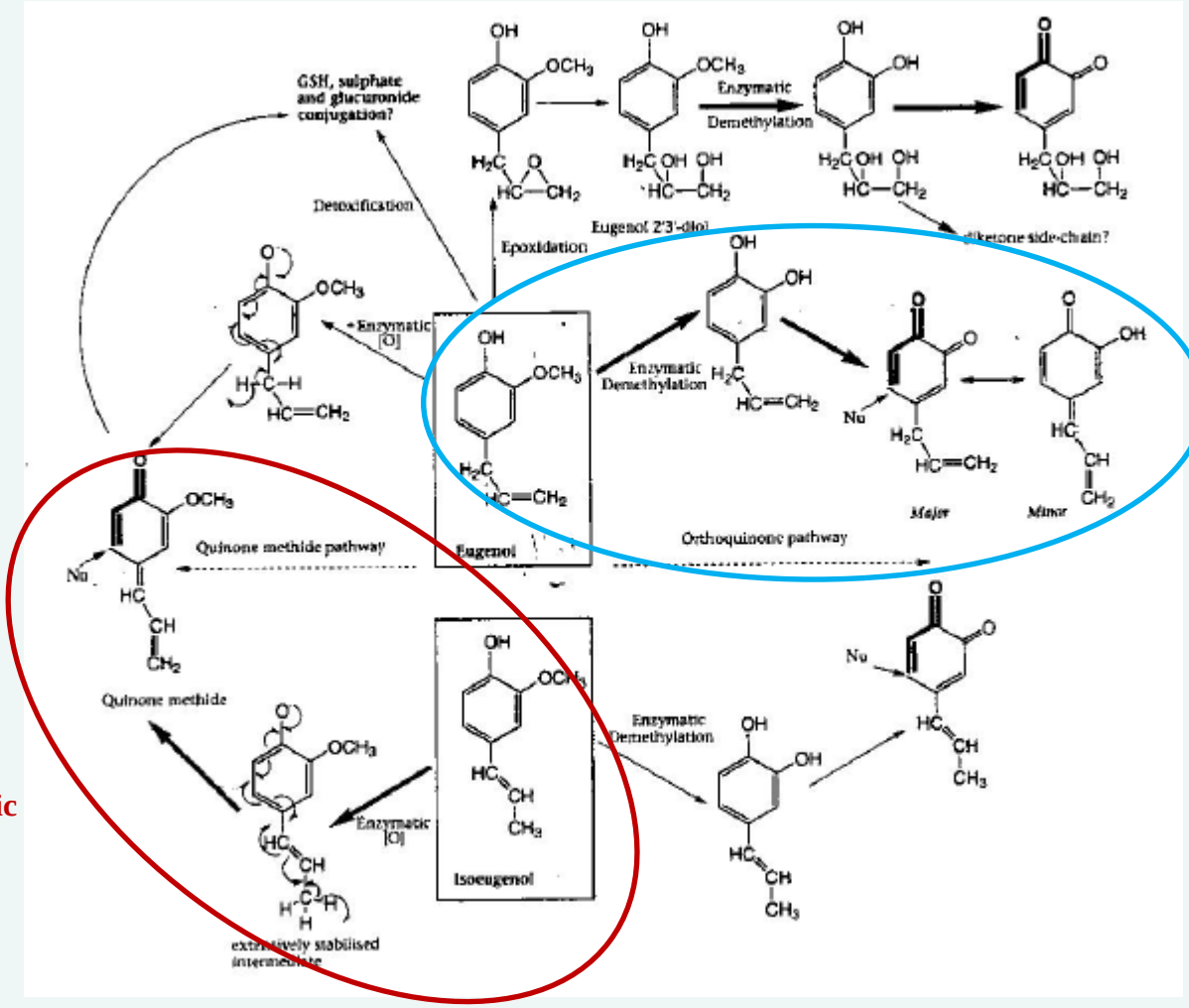
Eugenol

- Biotic dealkylation; subsequent oxidation (and/or tautomerism) (consecutive steps of activation)

# Recent initiatives of improving TIMES-SS model

**Potential metabolism of *Isoeugenol* and *Eugenol***

Simulation of metabolism is in agreement with cited in the literature enzymatic metabolism of Isoeugenol and Eugenol*




**Consecutive steps of enzymatic activation**

**Single step of enzymatic activation**



*Camilla K. Smith and Sharon A.M. Hotchkiss, Allergic Contact Dermatitis: Chemical and Metabolic Mechanisms, 2001, CRC Press, London (Taylor&Francis)

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# The role of pro-electrophilic activation in skin

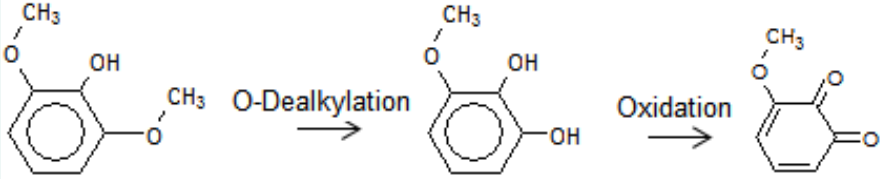
## List of not feasible abiotical reactions

- Dealkylation
- Oxidative deamination
- Azo-reduction
- …..

# The role of pro-electrophilic activation in skin

## List of not feasible abiotical reactions

- Dealkylation – Example



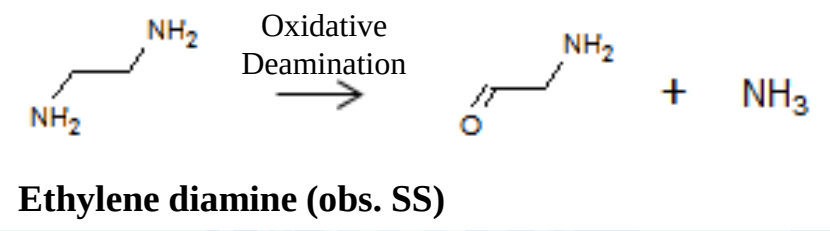

**2,6 – Dimethoxyphenol (obs. WS)**

- Oxidative deamination
- Azo-reduction

# The role of pro-electrophilic activation in skin

## List of not feasible abiotical reactions

- Dealkylation
- Oxidative deamination – Example



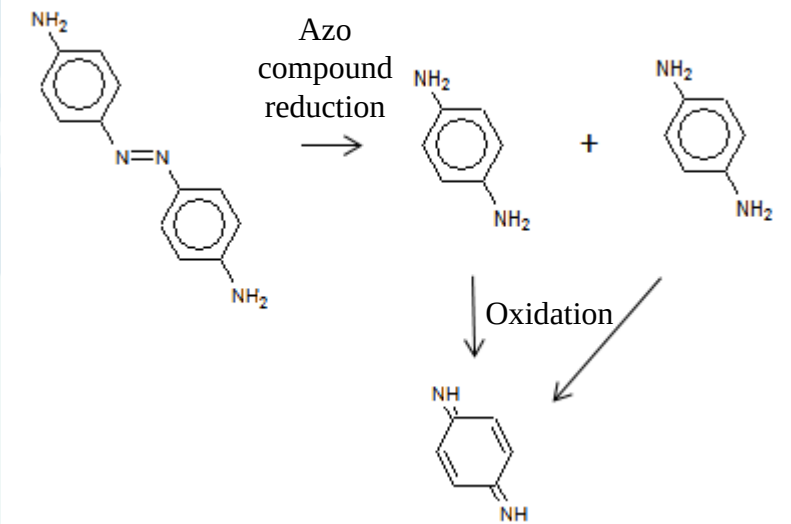

**Ethylene diamine (obs. SS)**

- Azo-reduction

# The role of pro-electrophilic activation in skin

## List of not feasible abiotical reactions

- Dealkylation
- Oxidative deamination
- Azo-reduction – Example




**4,4'-diaminoazobenzene (obs. SS)**

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# Addition of kinetic experimental data for clearance

- The aims under this initiative are:
  - ✓ To build a database with observed metabolic pathways in skin including different enzymatic parameters.
  - ✓ To improve skin metabolism simulator by using experimental data for clearance.
  - ✓ Currently data for clearance has been found for 150 chemicals.

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# Summary

- TIMES-SS model for predicting skin sensitization effect accounting for (a)biotic activation of chemicals was demonstrated
- TIMES-SS model provides qualitative and quantitative distribution of generated metabolites
- Curation of the model has been illustrated by:
  - ✓ Improving alert reliabilities
  - ✓ Adjusting (a)biotic activation pathways
  - ✓ Using kinetic data

# LMC team working on Skin sensitization endpoint

Chanita Kuseva

Gergana Poryazova

Aycel Mehmed

Stefan Kotov

Stoyanka Stoeva

Hristiana Ivanova

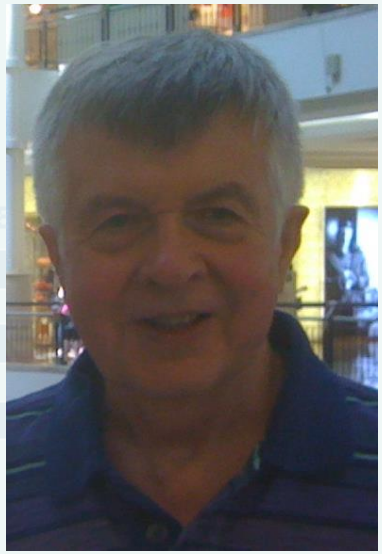
Ovanes Mekenyan, *Head of LMC*

Saby Dimitrov, *Deputy Head of LMC*

# LMC partners involved in the improvement of TIMES-SS

**Anne Marie Api**

*Vice President, Human Health Sciences of RIFM*



**David Roberts**

*Toxicological Chemistry Consultant*

# Thank you.

More information for LMC scientific activities and projects you could find visiting our web site here:
http://oasis-lmc.org/