



In silico predicting of pre- and pro- electrophilic
activation in the SS assessment
Simulating metabolic pathways through QSAR/SAR

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

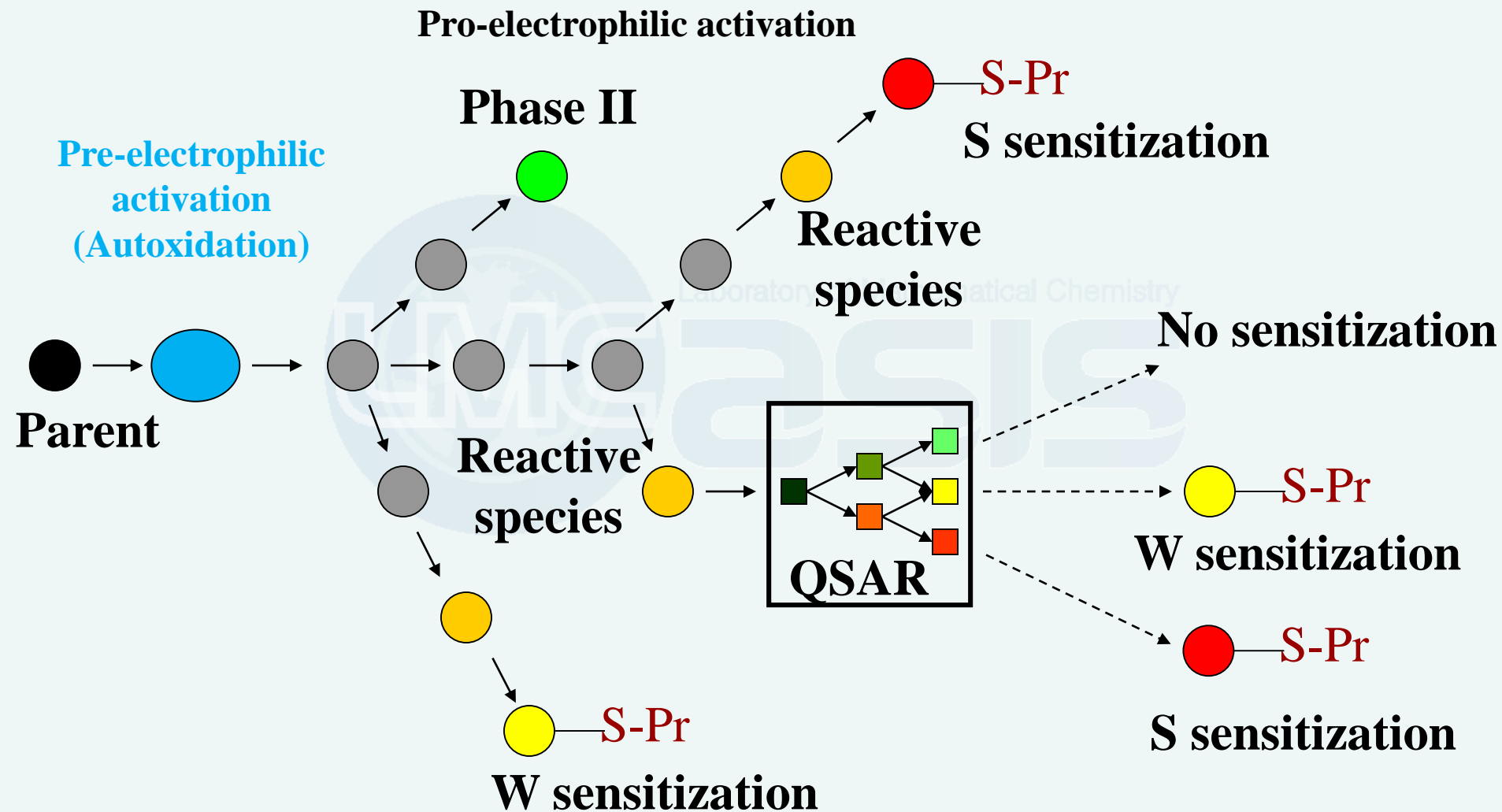
Short description

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

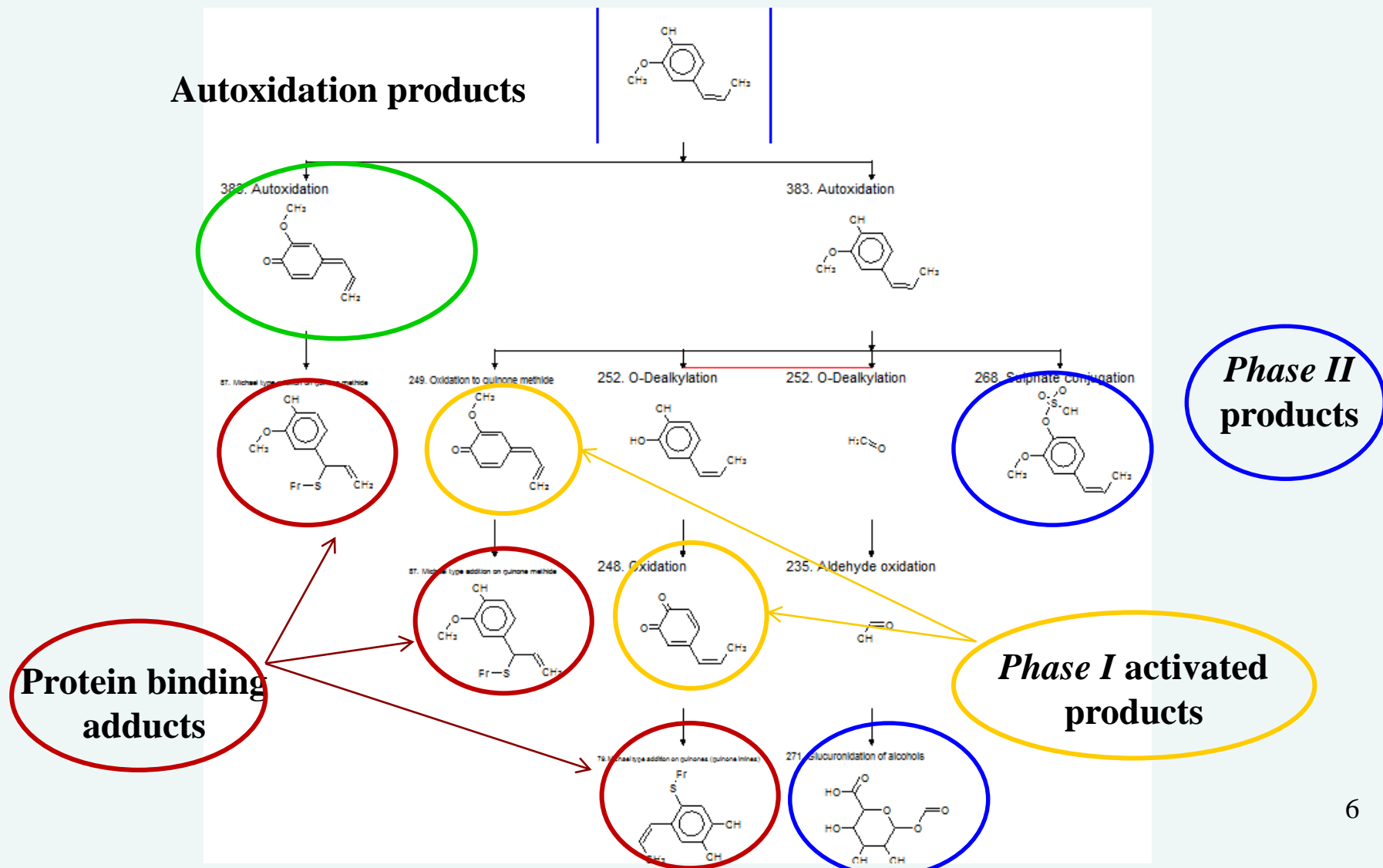
Predicting skin sensitization in TIMES

Basic scheme illustrating the model concept



Predicting skin sensitization in TIMES

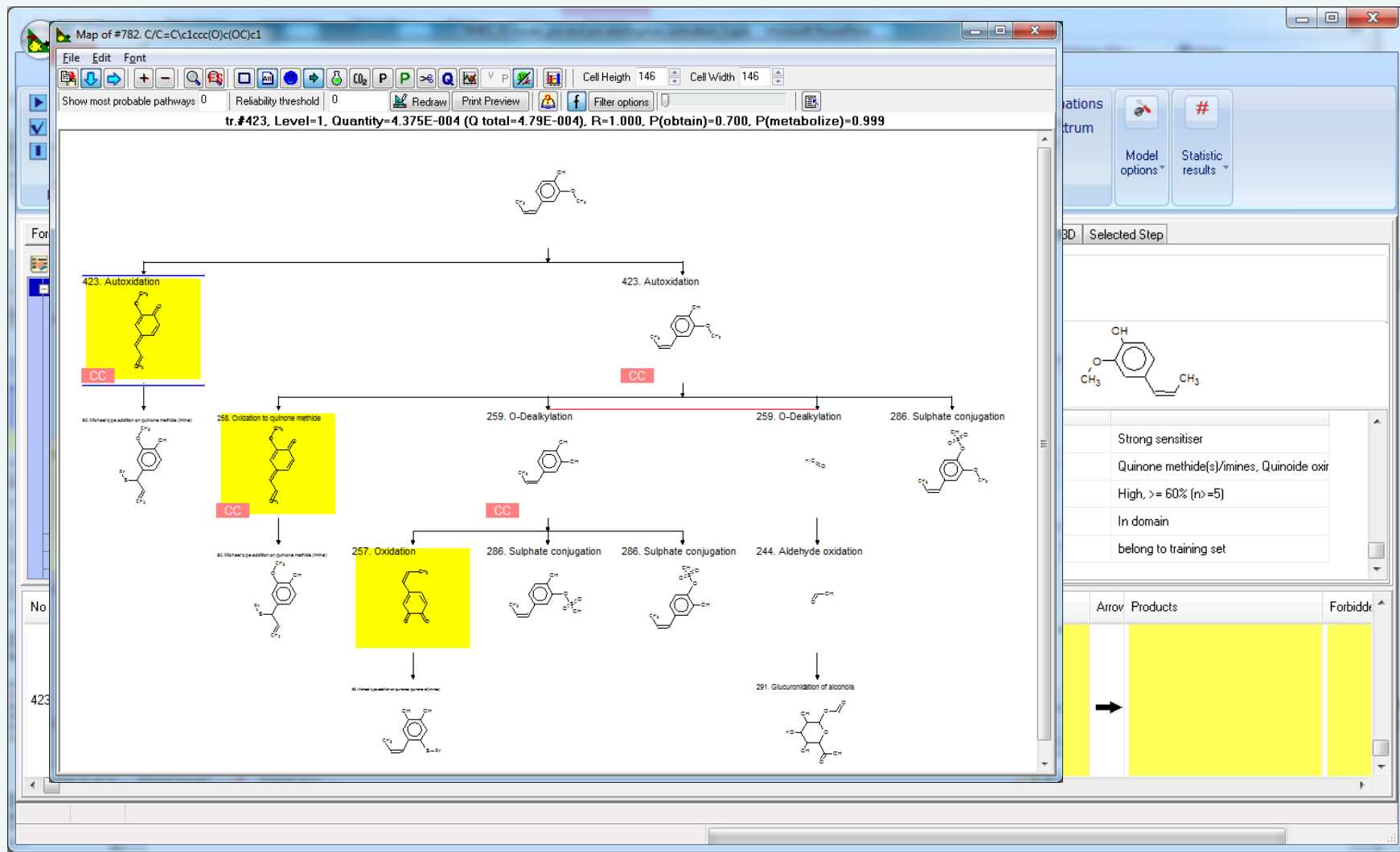
TIMES-SS Predicted metabolism of *Isoeugenol* accounting AU and SM activation



Predicting skin sensitization in TIMES

Results from TIMES-SS model

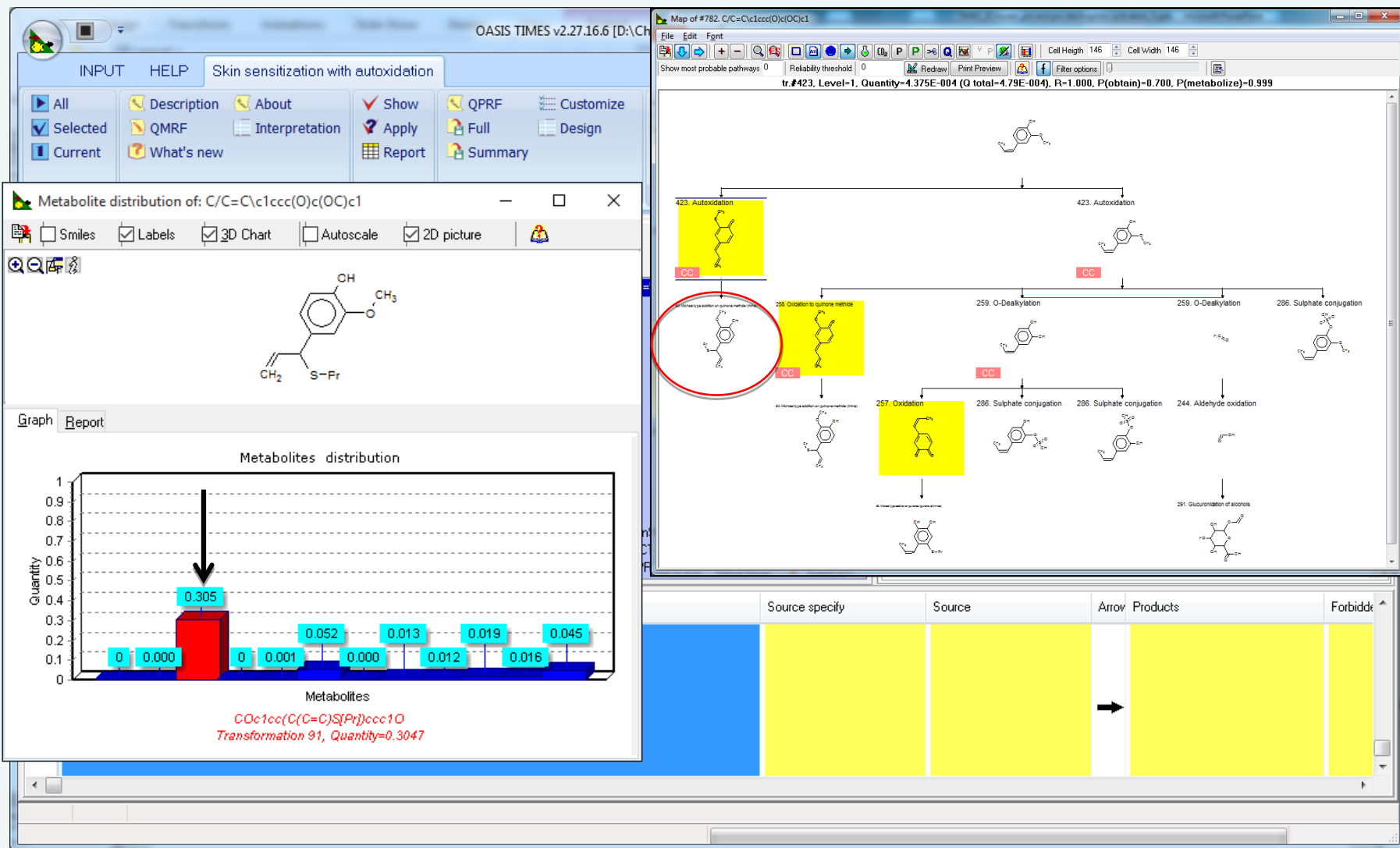
Generated metabolic map. Activated metabolites are highlighted



Predicting skin sensitization in TIMES

Results from TIMES-SS model

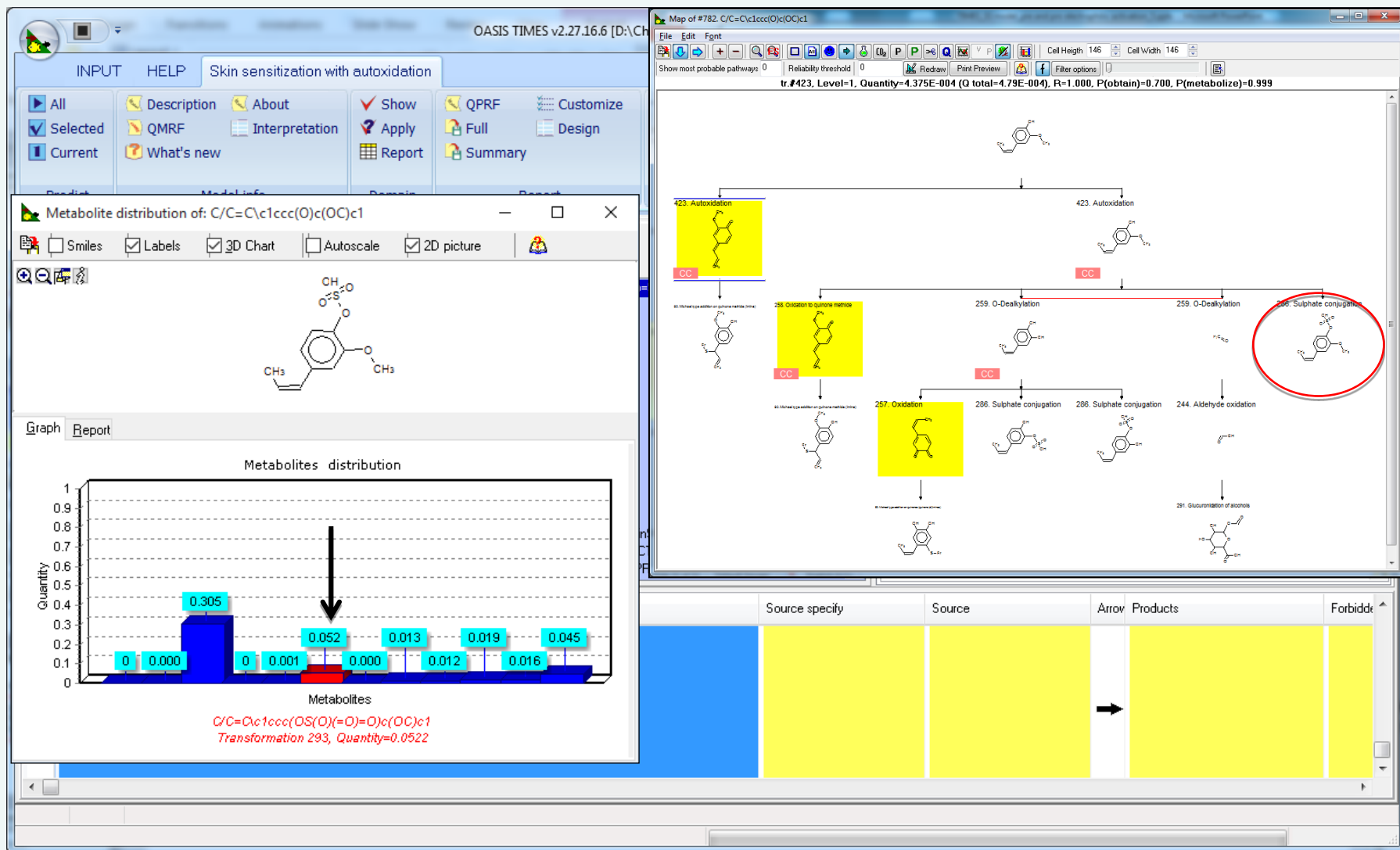
Quantitative assessment of generated metabolites



Predicting skin sensitization in TIMES

Results from TIMES-SS model

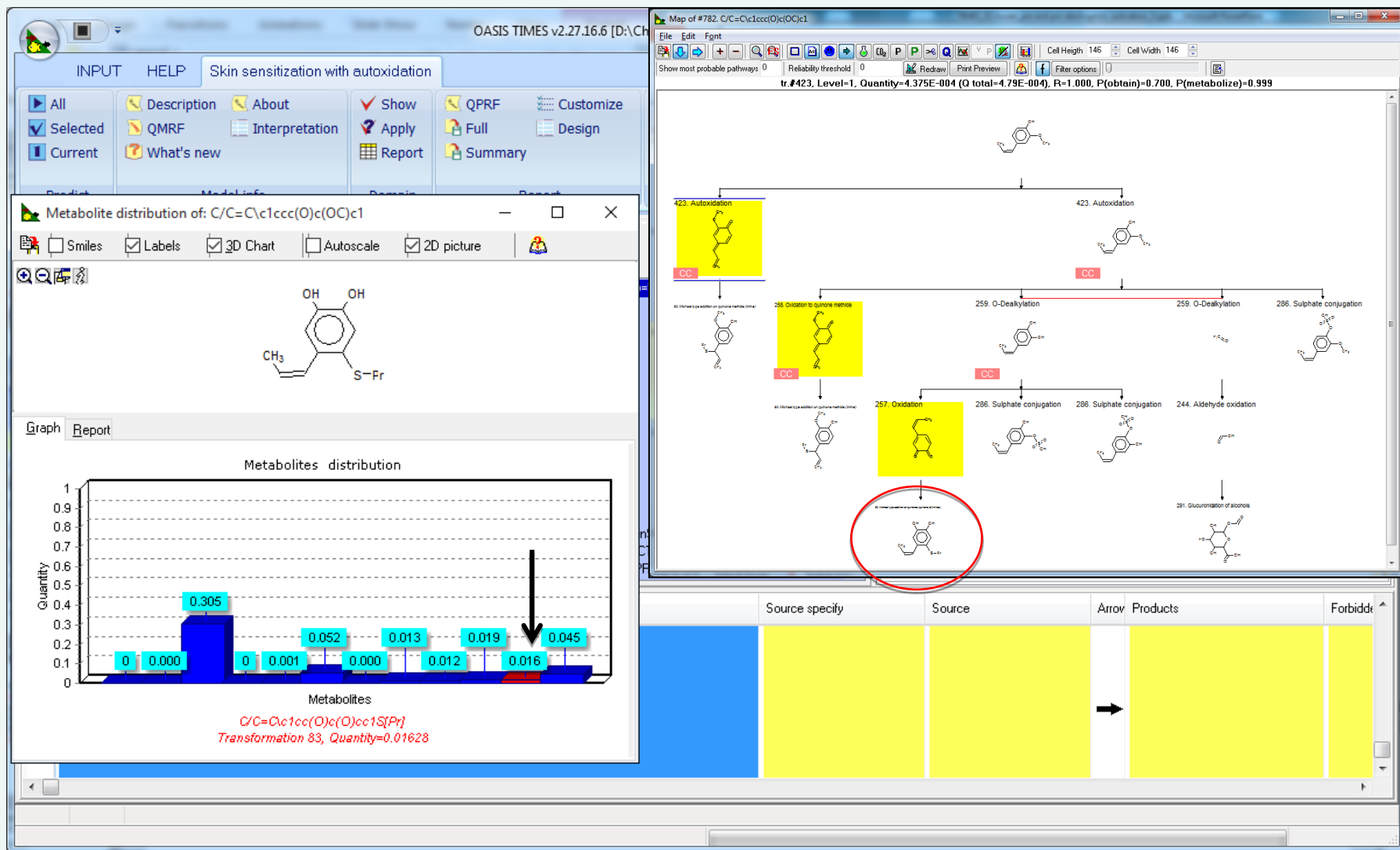
Quantitative assessment of generated metabolites



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

The screenshot displays the OASIS TIMES v2.27.16.6 software interface. The main window shows a 'Chart of Alerts' for 'Quinone methide(s)/imines, Quinoide oxime structure, Nitroquinones'. The chart is a sunburst plot with many segments. Below the chart, there are buttons for 'Alert Boundaries', 'Alert training set', 'Alert performance', and 'Interaction Mechanism'. The 'Alert training set' button is highlighted with a red box.

Overlaid on the main window are several dialog boxes:

- Show Alert and structures:** This dialog shows the alert name 'Quinone methide(s)/imines, Quinoide oxime structure, Nitroquinones' and a local training set of 341 applications. It displays observed and predicted values as 'Strong sensitiser'. A 'References' button is highlighted with a red box, and a red arrow points from it to the 'ChoiceObservedValueForm' dialog.
- ChoiceObservedValueForm:** This dialog shows the chemical structure of the parent and metabolite. It contains a table with the following data:

Parameter names	Value
SKIN_SENSITISATION_LLNA	3.0000

A 'Reference' button is highlighted with a red box, and a red arrow points from it to the 'Experimental data' label.

The 'Experimental data' label is a red box containing the text 'Experimental data'. Below the 'ChoiceObservedValueForm' dialog, there is a 'Model Browser' dialog showing model descriptors for 'Skin_sensitisation', including fields for NAME, AUTHOR (Unilever), DATE (1/1/2002), BIOASSAY (LLNA), ORGAN/TISSUE, CONDITIONS, ADMINISTRATION (LMC,BUL), SOURCE (Unilever), and VALUE DATA (Strongly positive).

Predicting skin sensitization in TIMES

Results from TIMES-SS model

Alert Performance

The screenshot displays the OASIS TIMES v2.27.16.6 software interface. The main window shows a list of chemical structures with their respective SMILES strings and performance metrics. A pop-up window titled "Alert performance" is open, displaying the following information:

Alert performance

Quinone methide(s)/imines, Quinoide oxime structure

Transformation(s) included in this alert
81 82 83 84 85 86 87 88 89 90 91 92 93

Alert information

Alert performance : 0.908

Correct applications: 138 Incorrect applications: 14

save to file

The main window also features a "Chart of Alerts" showing a sunburst chart for "Quinone methide(s)/imines, Quinoide oxime structure, Nitroquinones". The "Alert Performance" button in the bottom left is highlighted with a red box.

Chemical structure shown in the pop-up window:

CC1=CC(=C(C=C1)C=O)C=C

Predicting skin sensitization in TIMES

Results from TIMES-SS model

Mechanistic justification

The screenshot displays the OASIS TIMES v2.27.16.6 software interface. The main window shows a list of chemical structures with their respective skin sensitization scores. The selected structure is 2.1, with the SMILES string COc1cc(C[C=S](Pr))ccc1O and a score of 0.990. A 'Chart of Alerts' window is open, showing a sunburst chart with the text 'Quinone methide(s)/imines, Quinoid oxime structure, Nitroquinones'. The 'Interaction Mechanism' button is highlighted with a red box.

Mechanistic Domain: Michael addition

Mechanistic Alert: Quinoid type compounds

Structural Alert: Quinone methide(s)/imines, Quinoid oxime structure, Nitroquinones, Naphthoquinone(s)/imines

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

Ortho-quinones and para-quinones react with proteins via 1,4-addition of lysine NH₂ or cysteine SH groups across the ring system. They often are formed by oxidation of para and ortho dihydroxy aromatics acting as pro-Michael acceptors. These acceptors are able to be converted to Michael acceptors by well-established transformations (metabolic or abiotic), for example, hydroquinone oxidized to benzoquinone. Michael-type addition provides a means of covalent adduct formation at an electrophilic center, without any leaving group. The groups comprising the Michael-type acceptor can be arranged either in an open chain or in the form of a ring such as quinone.

Ortho-aminophenols and para-aminophenols, after metabolic or abiotic transformations, form quinone imines that react with proteins via Michael type addition.

Nitrophenols, after metabolic or abiotic transformation (keto-acid tautomerism), form nitro quinones that react with proteins via Michael type addition.

For example, 2,4-dinitrophenol is a cellular metabolic poison. Allergic reactions can include hives, blisters, and/or inexplicable rashes.

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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

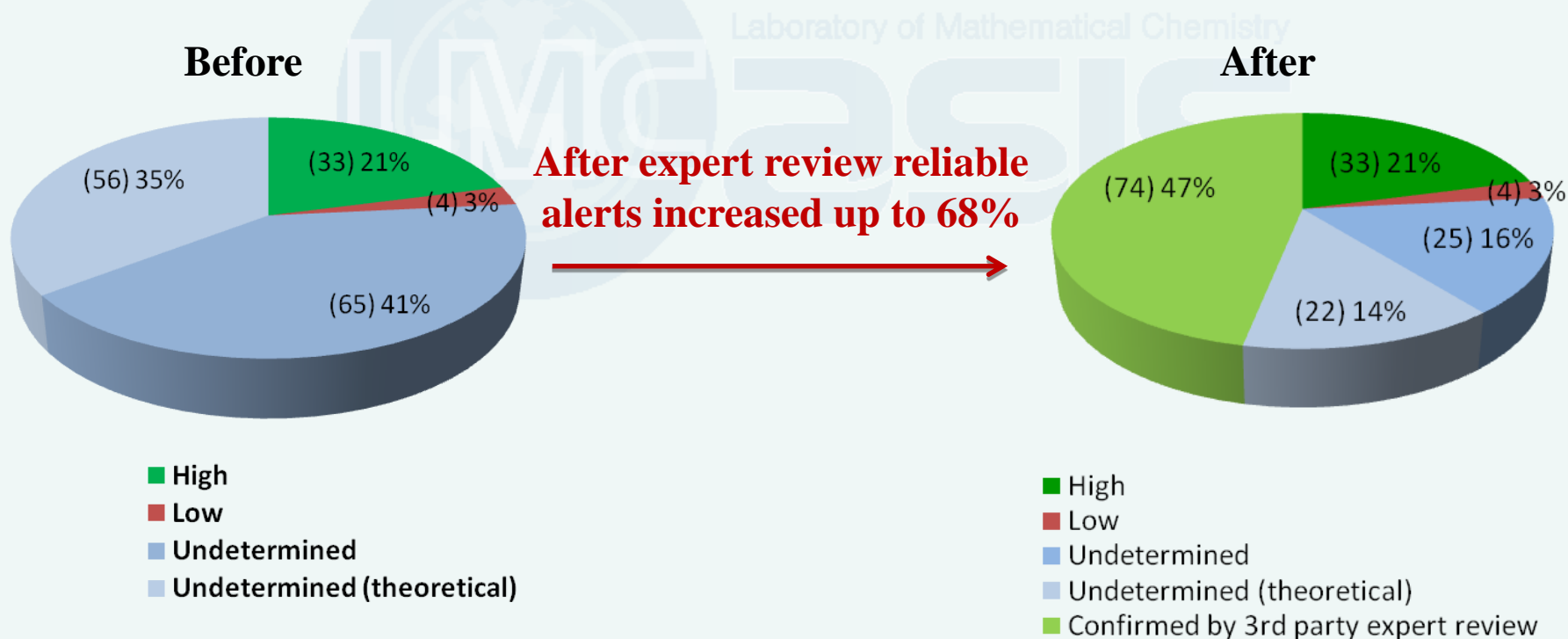
Based on their performance protein binding alerts have been classified as having:

- ✓ **High** reliability (perf. $\geq 60\%$ and $n \geq 5$)
- ✓ **Low** reliability (perf. $\leq 60\%$ and $n \geq 5$)
- ✓ **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:



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Recent initiatives of improving TIMES-SS model

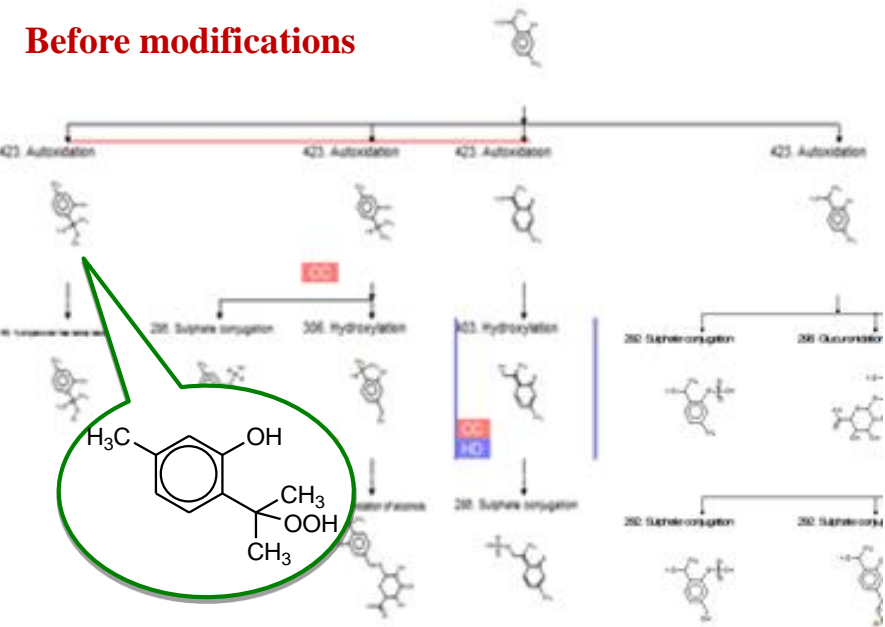
Improving of (a)biotic pathways – current work

- 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:

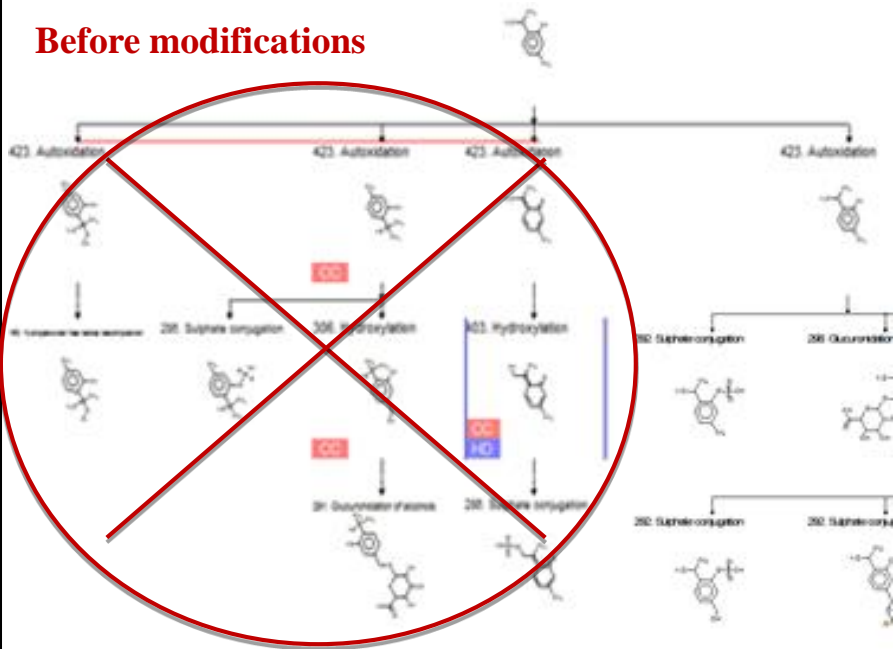
- Hydroperoxides

Metabolic path	Input from D. Roberts	Result
<p>Before modifications</p>  <p>The diagram illustrates the metabolic pathways for a phenolic compound before modifications. It shows a central phenolic structure with a methyl group (H₃C) and a hydroperoxide group (OOH) on the benzene ring. The pathway branches into several routes, including '423 Autoxidation' and '200 Sulfate conjugation'. A green callout bubble highlights the chemical structure of the starting material.</p>	<p>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.</p>	

Recent initiatives of improving TIMES-SS model

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

- Hydroperoxides

Metabolic path	Input from D. Roberts	Result
<p>Before modifications</p> 	<p>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.</p> <p>LMC action: Addition of a mask (phenolic ortho OH) that prevents the benzylic hydroperoxide formation.</p>	

Recent initiatives of improving TIMES-SS model

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

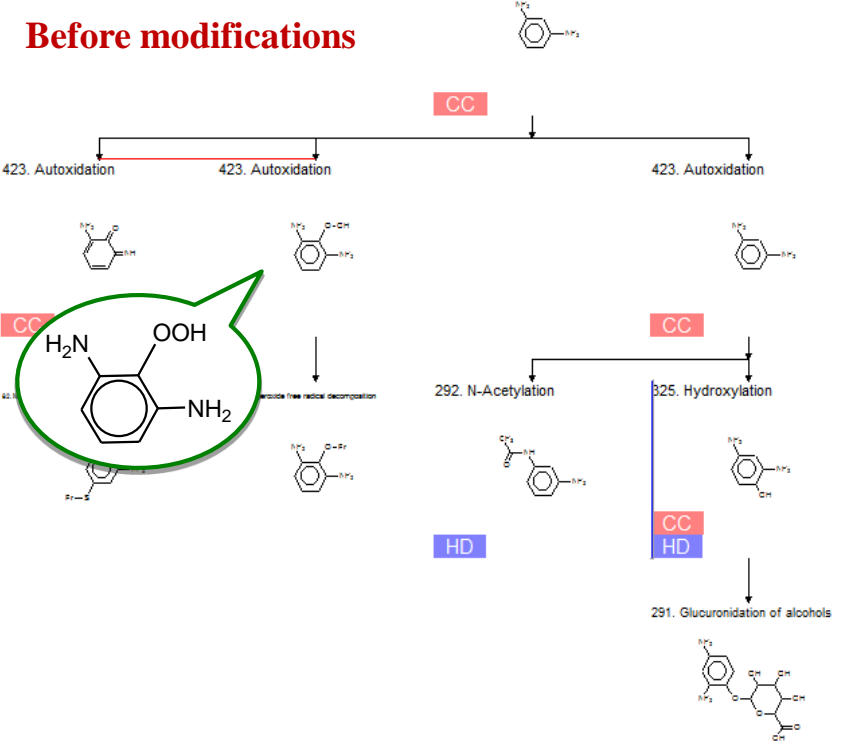
- Hydroperoxides

Metabolic path	Input from D. Roberts	Result
<p>After modifications</p> <p>The diagram illustrates the metabolic pathways for a phenolic compound with an ortho-methyl group. The starting material is 2-methylphenol. Three primary metabolic routes are shown:</p> <ul style="list-style-type: none"> 292 Sulphate conjugation: The phenolic oxygen is conjugated with a sulfate group. 298 Glucuronidation of alcohols: The methyl group is oxidized to a primary alcohol, which is then conjugated with glucuronic acid. 317 Hydroxylation: The methyl group is oxidized to a primary alcohol. <p>The hydroxylation product (2-methylphenol-1-ol) further undergoes metabolism:</p> <ul style="list-style-type: none"> 292 Sulphate conjugation: The primary alcohol is conjugated with a sulfate group. 298 Glucuronidation of alcohols: The primary alcohol is conjugated with glucuronic acid. 298 Glucuronidation of alcohols: The primary alcohol is conjugated with glucuronic acid. 	<p>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.</p> <p>LMC action: Addition of a mask (phenolic ortho OH) that prevents the benzylic hydroperoxide formation.</p>	<p>Correctly predicted as Non sensitizer – no autoxidation products are generated.</p>

Recent initiatives of improving TIMES-SS model

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

- Quinones

Metabolic path	Input from D. Roberts	Result
<p data-bbox="92 411 426 444">Before modifications</p> 	<p data-bbox="967 392 1605 778">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.</p>	

Recent initiatives of improving TIMES-SS model

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

- Quinones

Metabolic path	Input from D. Roberts	Result
<p>After modifications</p> <p>The diagram illustrates a metabolic pathway starting from a quinone derivative. Three autoxidation pathways (423) are shown, with the central one being crossed out. Other pathways include Michael-type addition (42), N-acetylation (292), hydroxylation (325), and glucuronidation (291). Classification codes CC and HD are used throughout the diagram.</p>	<p>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.</p> <p>LMC action: Addition of spontaneous conversion of hydroperoxide to quinone imine in order to prevent the interaction with proteins of the intermediate hydroperoxide. Generation of an additional para quinone imine.</p>	

Recent initiatives of improving TIMES-SS model

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

- Quinones

Metabolic path	Input from D. Roberts	Result
<p>After modifications</p>	<p>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.</p> <p>LMC action: Addition of spontaneous conversion of hydroperoxide to quinone imine in order to prevent the interaction with proteins of the intermediate hydroperoxide. Generation of an additional para quinone imine.</p>	<p>Autoxidation products are more scientifically reliable.</p>

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.



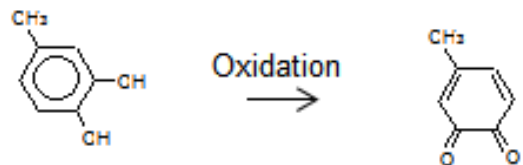
Recent initiatives of improving TIMES-SS model

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

- Examples of pro-electrophilic activation to ortho-quinones

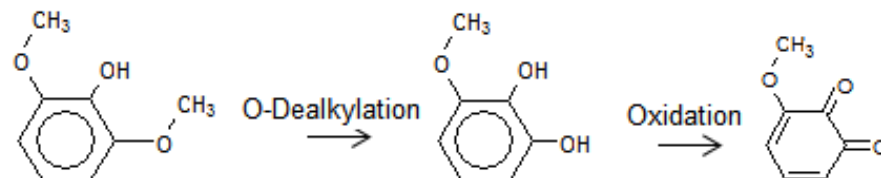
Chemicals generating o-quinone at:

One transformation step

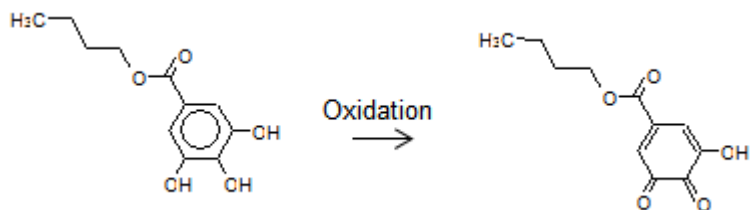


Obs. SS – Pred. SS

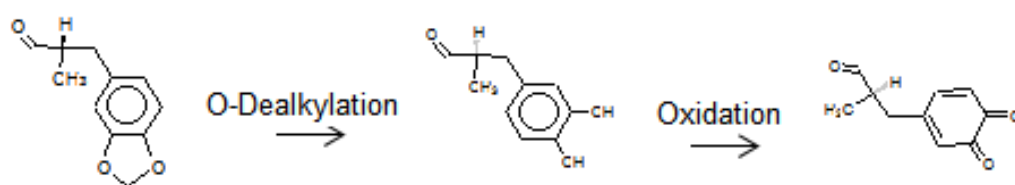
More than one transformation steps



Obs. WS – Pred. WS



Obs. SS – Pred. SS

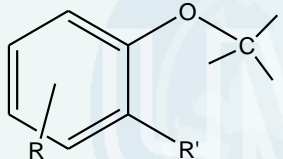
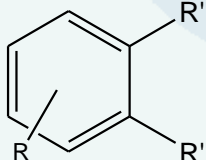


Obs. WS – Pred. WS

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	
 <p>R' = OH or OC</p>	3	3	3	Chemicals requiring consecutive steps of activation
 <p>R'' = OH</p>	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

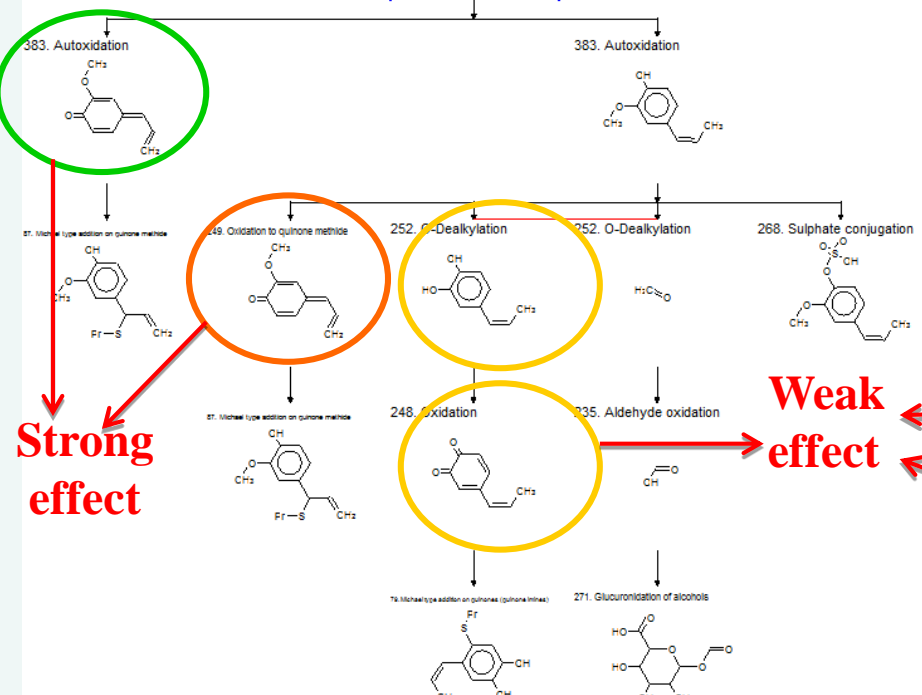
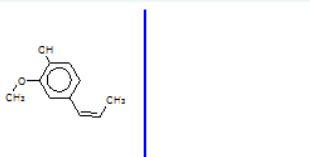
Modifications addressing the biotic (pro-electrophilic) activation of chemicals

- 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*)

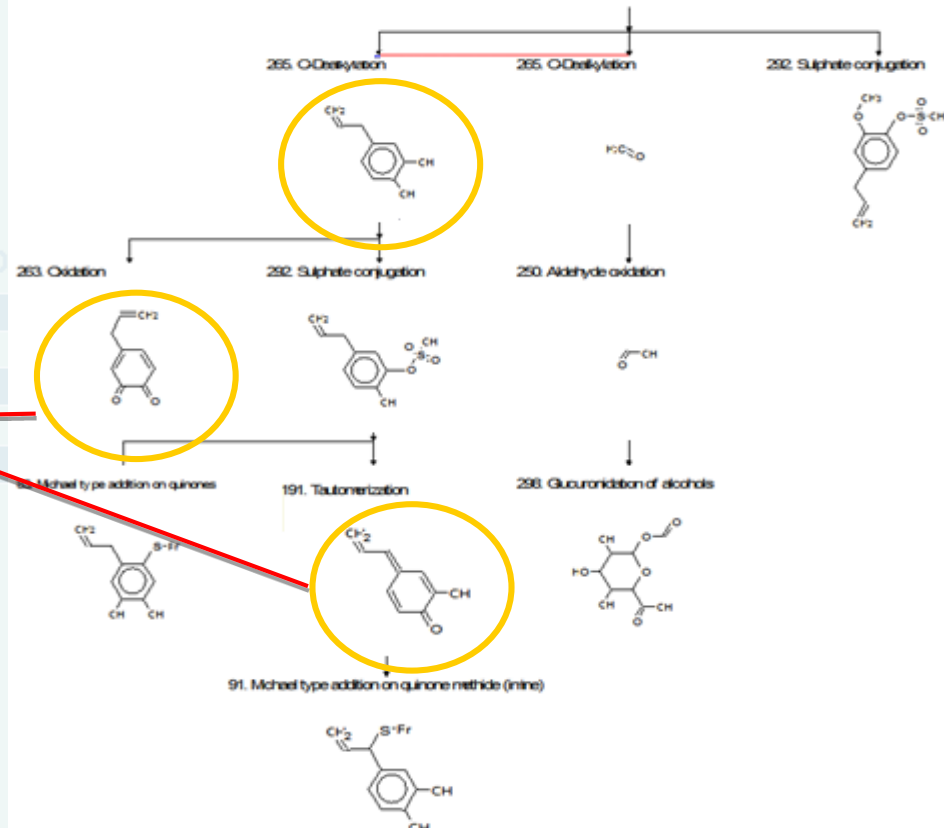
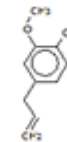
Recent initiatives of TIMES-SS model upgrade

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

Isoeugenol
(obs. SS)



Eugenol
(obs. WS)



Metabolism of:

Isoeugenol

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

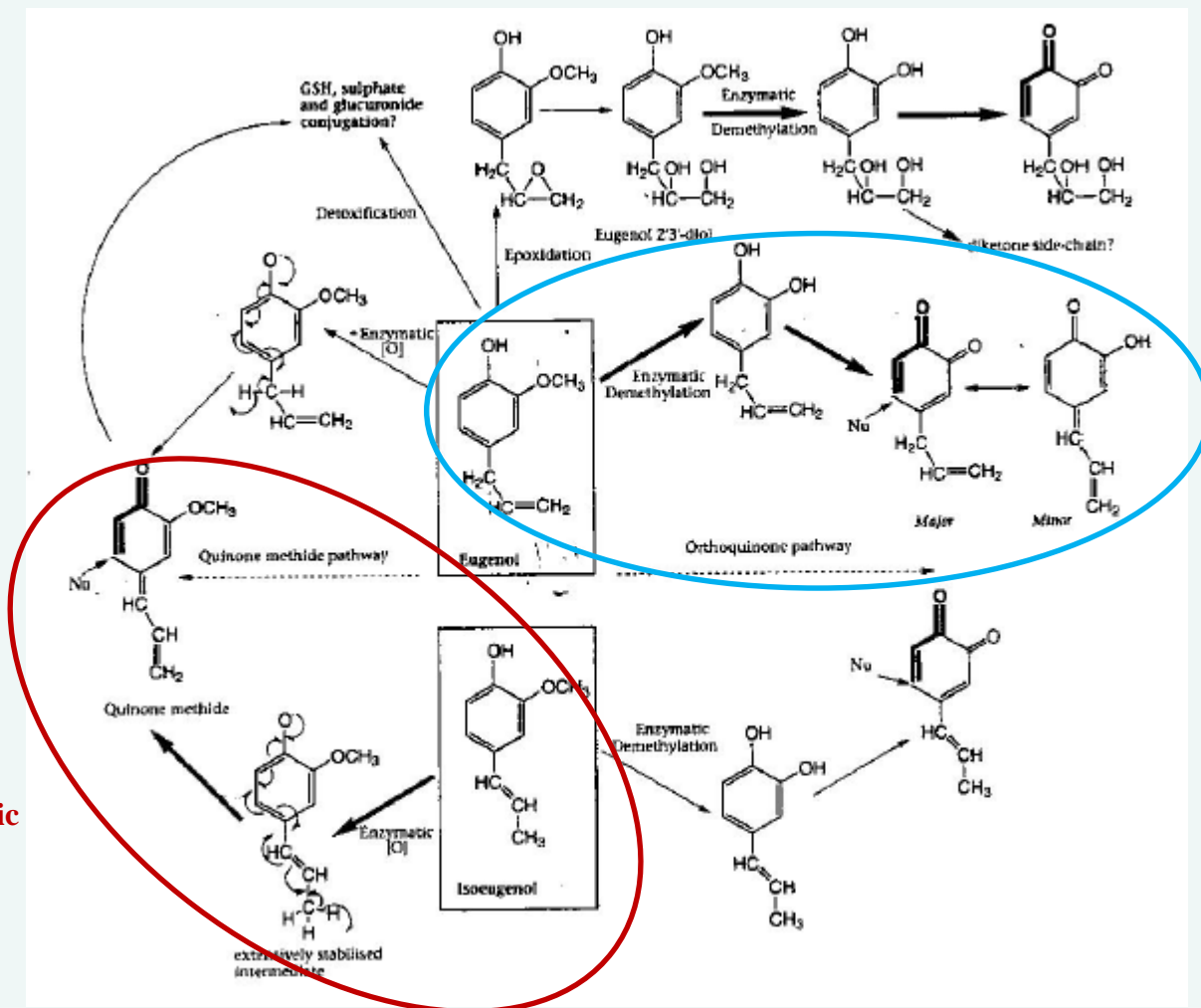
Eugenol

- 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

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The role of pro-electrophilic activation in skin

List of not feasible abiological reactions

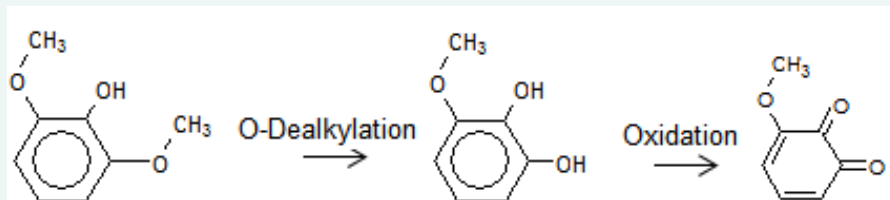
- Dealkylation
- Oxidative deamination
- Azo-reduction
-



The role of pro-electrophilic activation in skin

List of not feasible abiotical reactions

- **Dealkylation – Example**



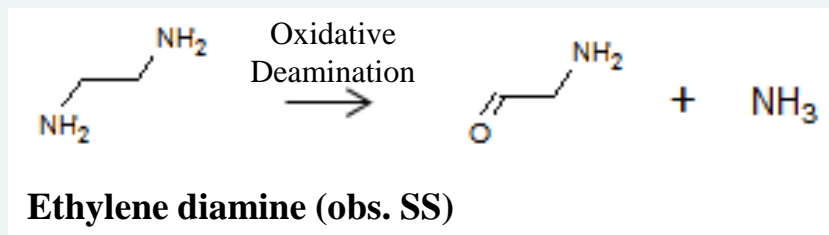
2,6 – Dimethoxyphenol (obs. WS)

- Oxidative deamination
- Azo-reduction

The role of pro-electrophilic activation in skin

List of not feasible abiological reactions

- Dealkylation
- Oxidative deamination – Example

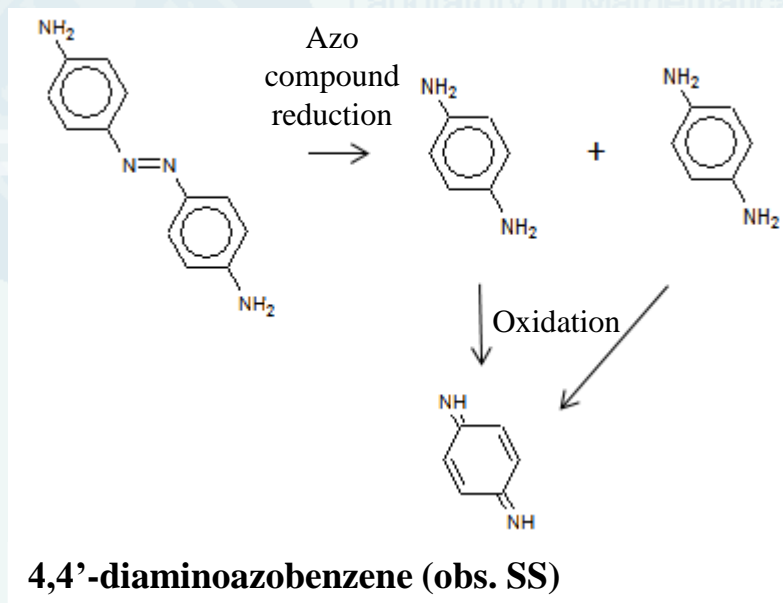


- Azo-reduction

The role of pro-electrophilic activation in skin

List of not feasible abiotical reactions

- Dealkylation
- Oxidative deamination
- Azo-reduction – Example



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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



Aysel
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*

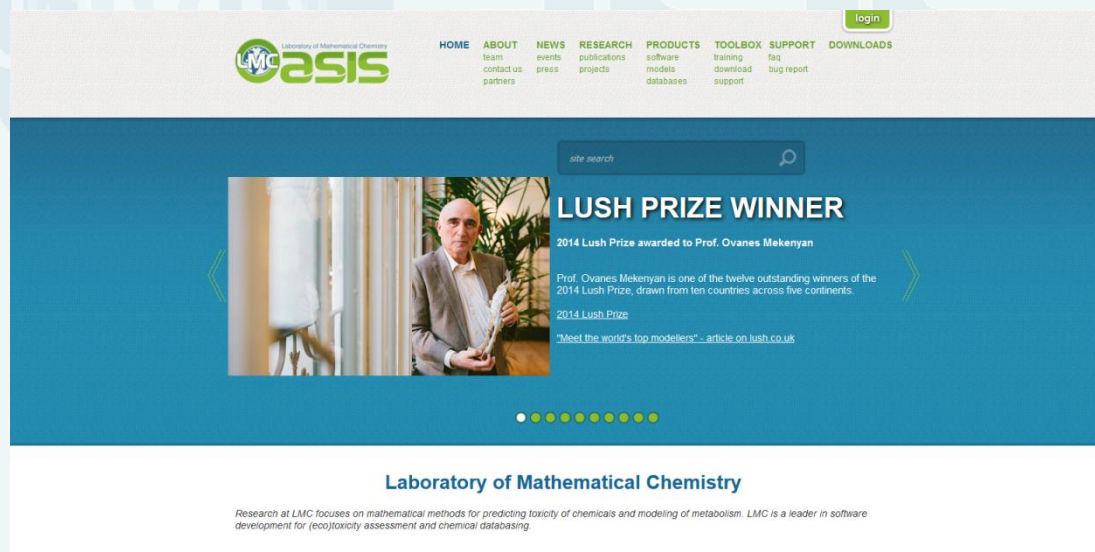


David Roberts
*Toxicological
Chemistry Consultant*

Thank you.

More information for LMC scientific activities and projects you could find visiting our web site here:

<http://oasis-lmc.org/>



The screenshot shows the homepage of the Laboratory of Mathematical Chemistry (LMC) Oasis website. The header includes the LMC Oasis logo and a navigation menu with categories: HOME, ABOUT (team, contact us, partners), NEWS (events, press), RESEARCH (publications, projects), PRODUCTS (software, models, databases), TOOLBOX (training, download, support), SUPPORT (faq, bug report), and DOWNLOADS. A 'login' button is also present. The main content area features a blue banner with a search bar and a featured article titled 'LUSH PRIZE WINNER'. The article text reads: '2014 Lush Prize awarded to Prof. Ovanes Mekenyan. Prof. Ovanes Mekenyan is one of the twelve outstanding winners of the 2014 Lush Prize, drawn from ten countries across five continents. 2014 Lush Prize. "Meet the world's top modelers" article on lush.co.uk'. Below the banner is a row of seven colored dots, with the first one being white and the others green. The footer contains the text 'Laboratory of Mathematical Chemistry' and a short description of the research focus: 'Research at LMC focuses on mathematical methods for predicting toxicity of chemicals and modeling of metabolism. LMC is a leader in software development for (eco)toxicity assessment and chemical databasing.'