In silico prediction of metabolism as a tool to identify new metabolites of dietary monoterpenes

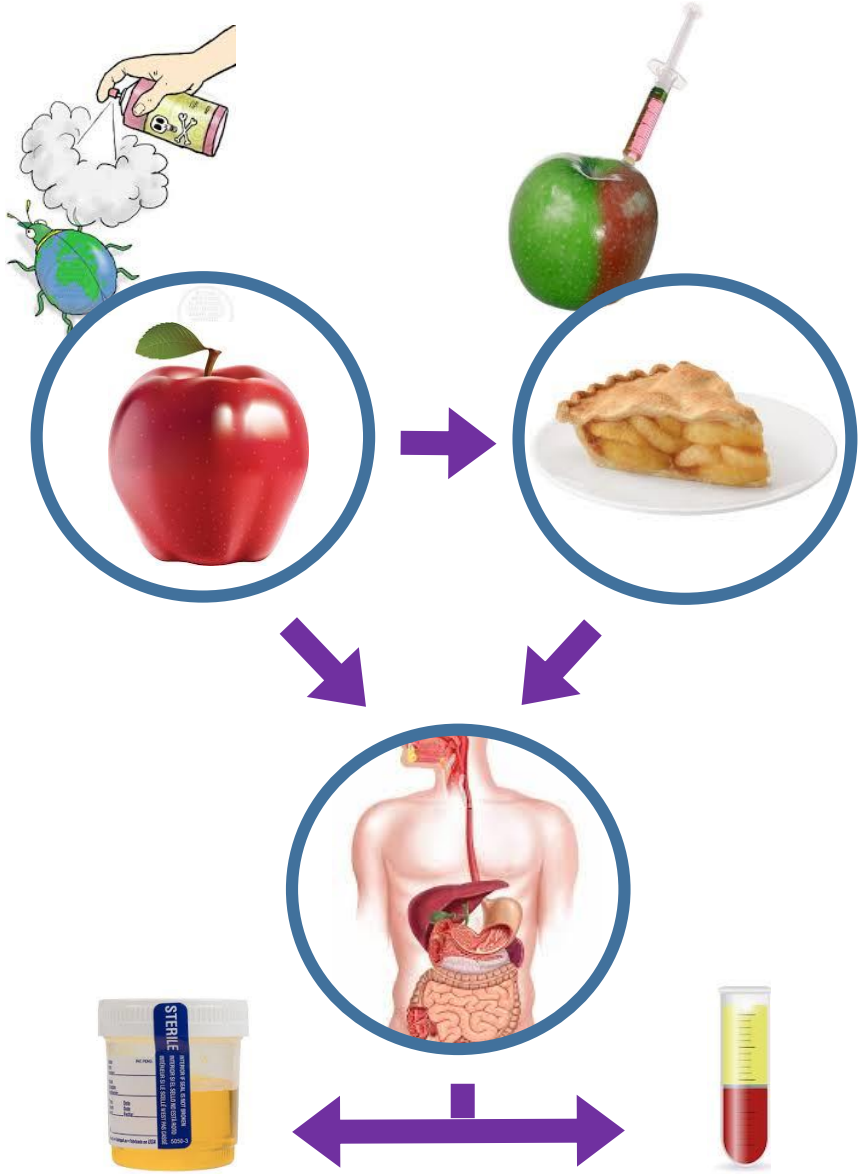
Jarlei Fiamoncini

Food Metabolome and the Metabolism of Food Compounds

Food metabolome is the part of the metabolome derived from the digestion and metabolism of food.

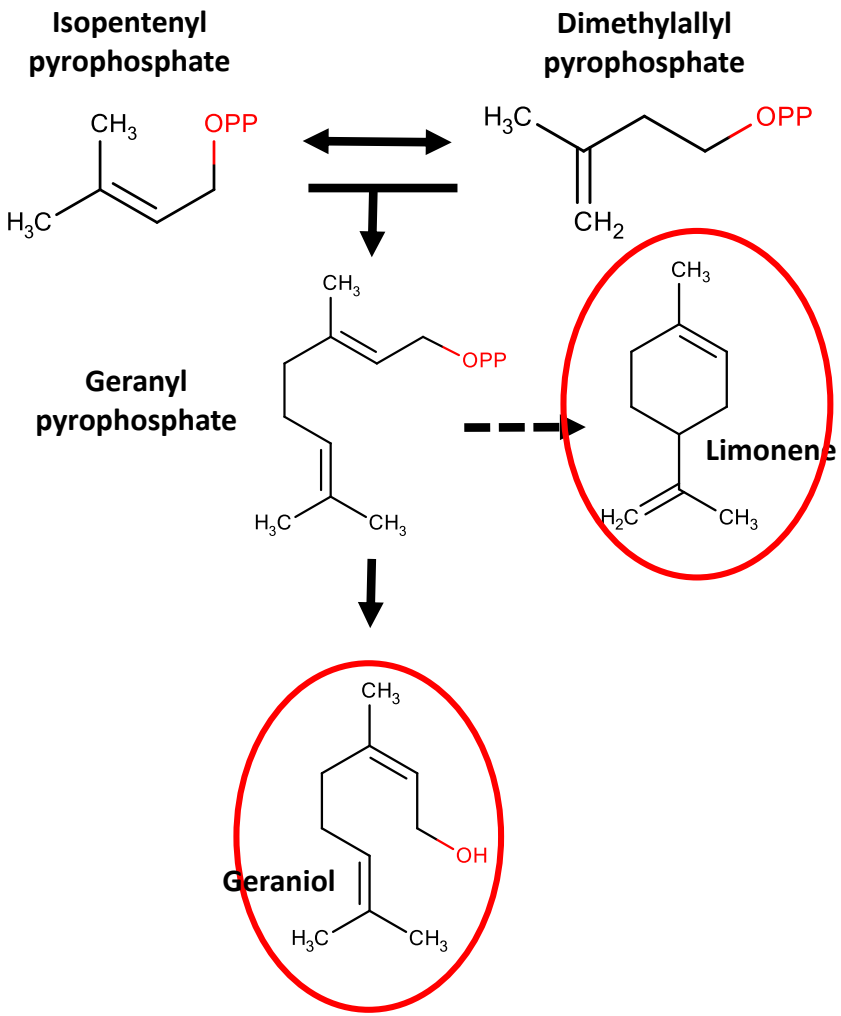
The more we know about **food compounds metabolism**, the better we can study the effects of **diet** in **health**.

Dietary monoterpenes are a part of the food metabolome that remains poorly studied.



Dietary Monoterpenes

- Formed by the condensation of 2 isoprene units
- Low molecular weight and relatively high lipophilicity



Dietary Monoterpenes

- Formed by the condensation of 2 isoprene units
- Low molecular weight and relatively high lipophilicity

- Found in the essential oil of herbs and citrus fruits
- Daily intake up to 200 mg

Demonstrated effects

Antinociceptive
Antimicrobial
Hypotensive
Anti-inflammatory
Hypoglycemic (STZ diabetic mice)
Antioxidant
Antineoplasic
Modulators of the activity of ion channels
Toxic effects

Pharmacokinetics of Monoterpenes

- ✓ Both in humans and rats, dietary terpenes reach effective concentrations in plasma within 1 hour
- ✓ Their metabolites are detected in circulation up to 24 hours after intake
- ✓ Topic administration of terpenes is also effective to increase their concentration in plasm

ELSEVIER

Available online at www.sciencedirect.com
SCIENCE DIRECT®

Journal of Chromatography B, 799 (2004) 111–117

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Determination of menthol in plasma and urine of rats and humans by headspace solid phase microextraction and gas chromatography–mass spectrometry

Miriam Spichiger[a,b], Roman C. Mühlbauer[b], Rudolf Brenneisen[a,*]

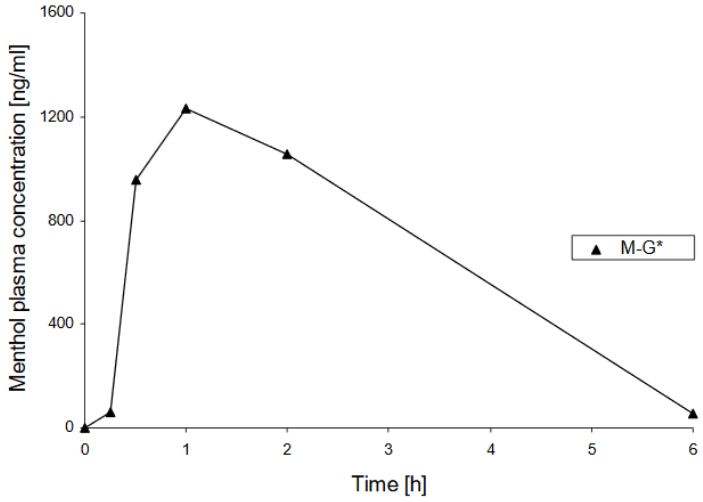


Fig. 4. Plasma levels of M-G (*expressed as menthol after enzymatic hydrolysis) in a human volunteer after ingestion of 100 mg menthol.

Systemic Availability and Pharmacokinetics of Thymol in Humans

Claudia Kohlert, PhD, Gernot Schindler, MD, Reinhard W. März, PhD, Gudrun Abel, PhD, Benno Brinkhaus, MD, Hartmut Derendorf, PhD, FCP, Eva-Ulrike Gräfe, PhD, and Markus Veit, PhD

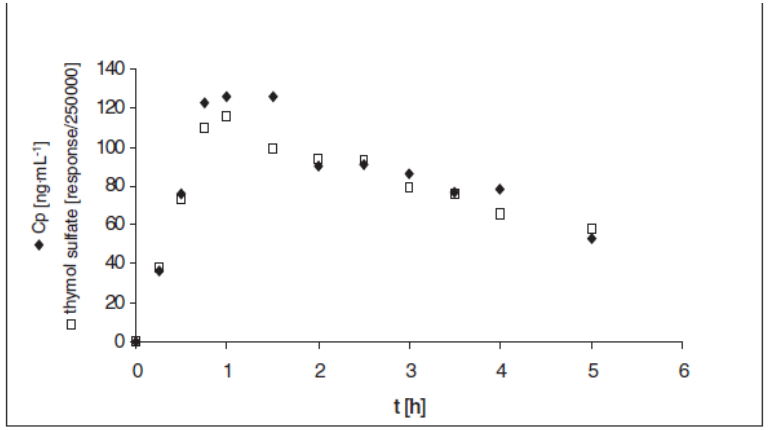


Figure 2. Thymol plasma concentration determined after enzymatic hydrolysis of plasma (◆) and thymol sulfate measured by LC-MS in 1 subject (□).

JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 637–642
© 2001 The Authors
Received October 5, 2000
Accepted January 30, 2001
ISSN 0022-3573

Percutaneous absorption of the montoterpene carvone: implication of stereoselective metabolism on blood levels

Walter Jäger, Marion Mayer, Gottfried Reznicek, and Gerhard Buchbauer

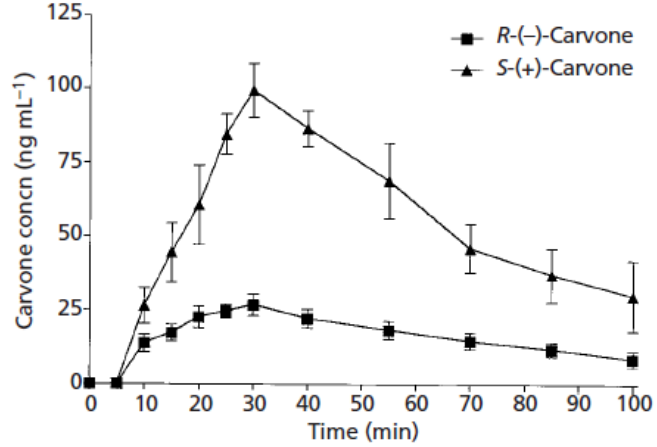


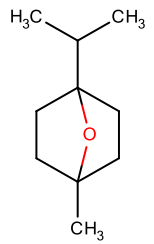
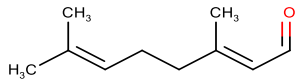
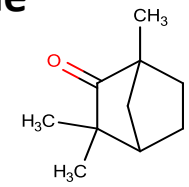
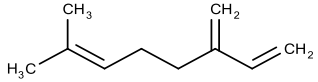
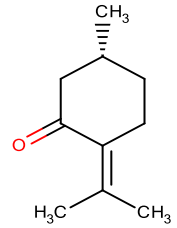
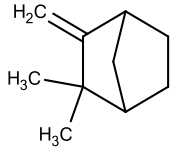
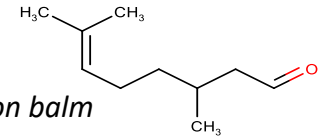
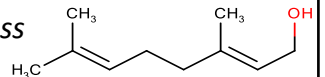
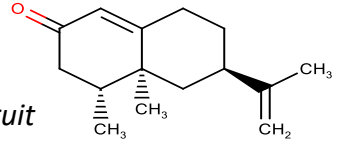
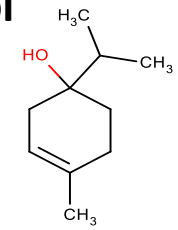
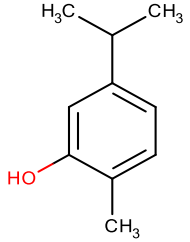
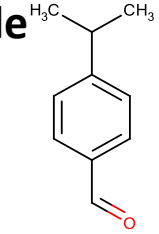
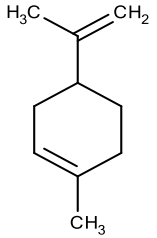
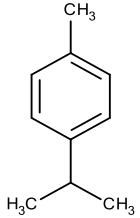
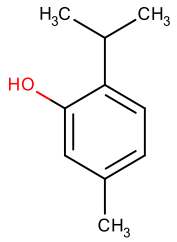
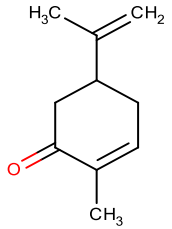
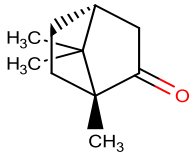
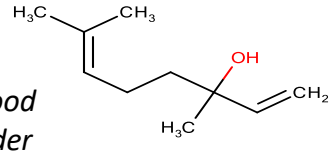
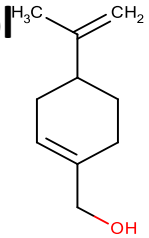
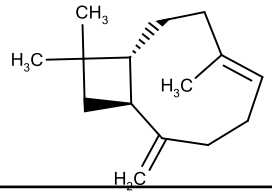
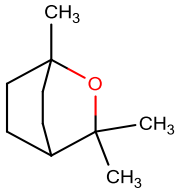
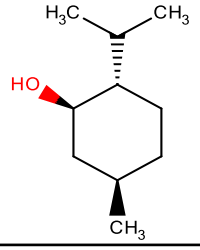
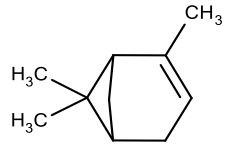
Figure 1 Plasma concentrations of *R*-(−)- and *S*-(+)-carvone in healthy subjects, administered as a single topical application of 300 mg. Data are mean ± s.d., n = 4.

Problems

- ✓ Despite recognized health effects, the metabolism of dietary terpenoids is poorly known
- ✓ Different isomers for each compound make terpenoids analysis very complex.

Aims of the study

- ✓ Identify enzymatic reactions involved in the metabolism of terpenoids
- ✓ Validate metabolism predictions
- ✓ Identify new metabolites of dietary terpenoids

1,4-Cineole *lime* *eucalyptus* 	**Citral**  *lemongrass* *lemon balm* *eucalyptus*	**Fenchone** fennel 	**Myrcene**  *hop*	**Pulegone** *mint* 
Camphene *thyme* 	**Citronellal**  *lemon balm*	**Geraniol**  *lemon grass* *citronella* *geranium*	**Nootkatone**  *grapefruit*	**Terpinen-4-ol**  *juniper*
Carvacrol  *thyme*	**Cuminaldehyde**  *eucalyptus* *myrrh*	**Limonene**  *orange*	**p-Cymene**  *cumin* *thyme*	**Thymol**  *thyme*
Carvone  caraway spearmint	**D-Camphor**  *camphor tree*	**Linalool**  *rosewood* *coriander*	**Perillyl alcohol**  *lavender* *sage*	**Tested dietary terpenoids**
Caryophyllene  *clove* *cannabis* *rosemary*	**1,8-Cineole**  *eucalyptus*	**Menthol**  *mint*	**Pinene**  *pine*	

Investigation of Metabolism of Food Compounds

Training

defining the reactions involved in the the metabolism of dietary monoterpenes

Prediction

using selected reactions to predict the metabolites of monoterpenes

in vivo experiment

feeding monoterpenes to rats and collecting metabolites-rich urine

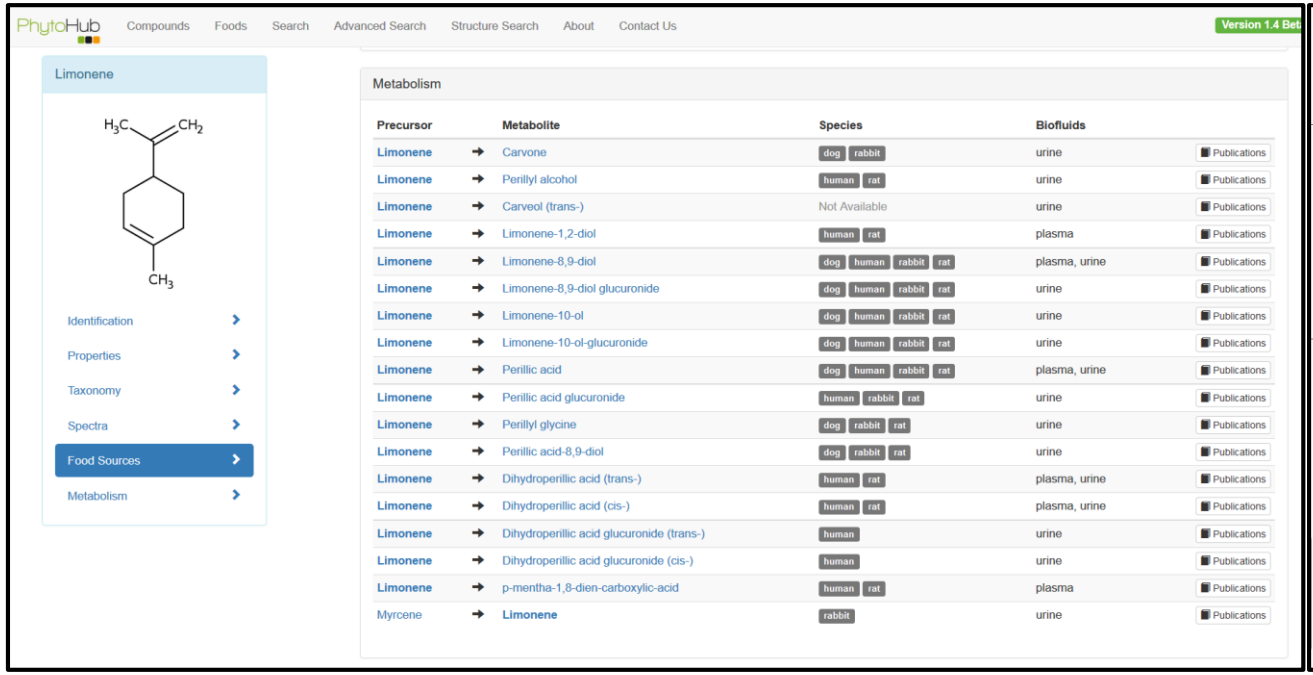
Analysis

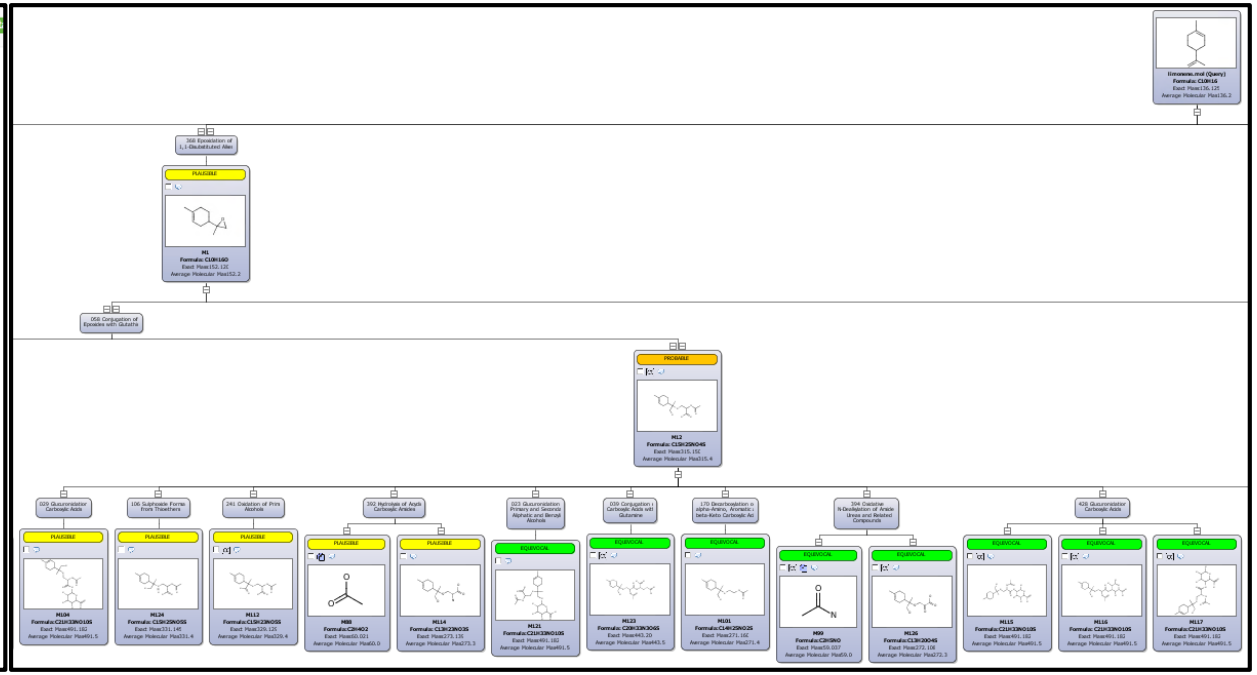
non-targeted high-resolution LC-MS analysis of urine in search of predicted metabolites

1

Training

defining the reactions involved in the the metabolism of dietary monoterpenes





PhytoHub

http://phytohub.eu/

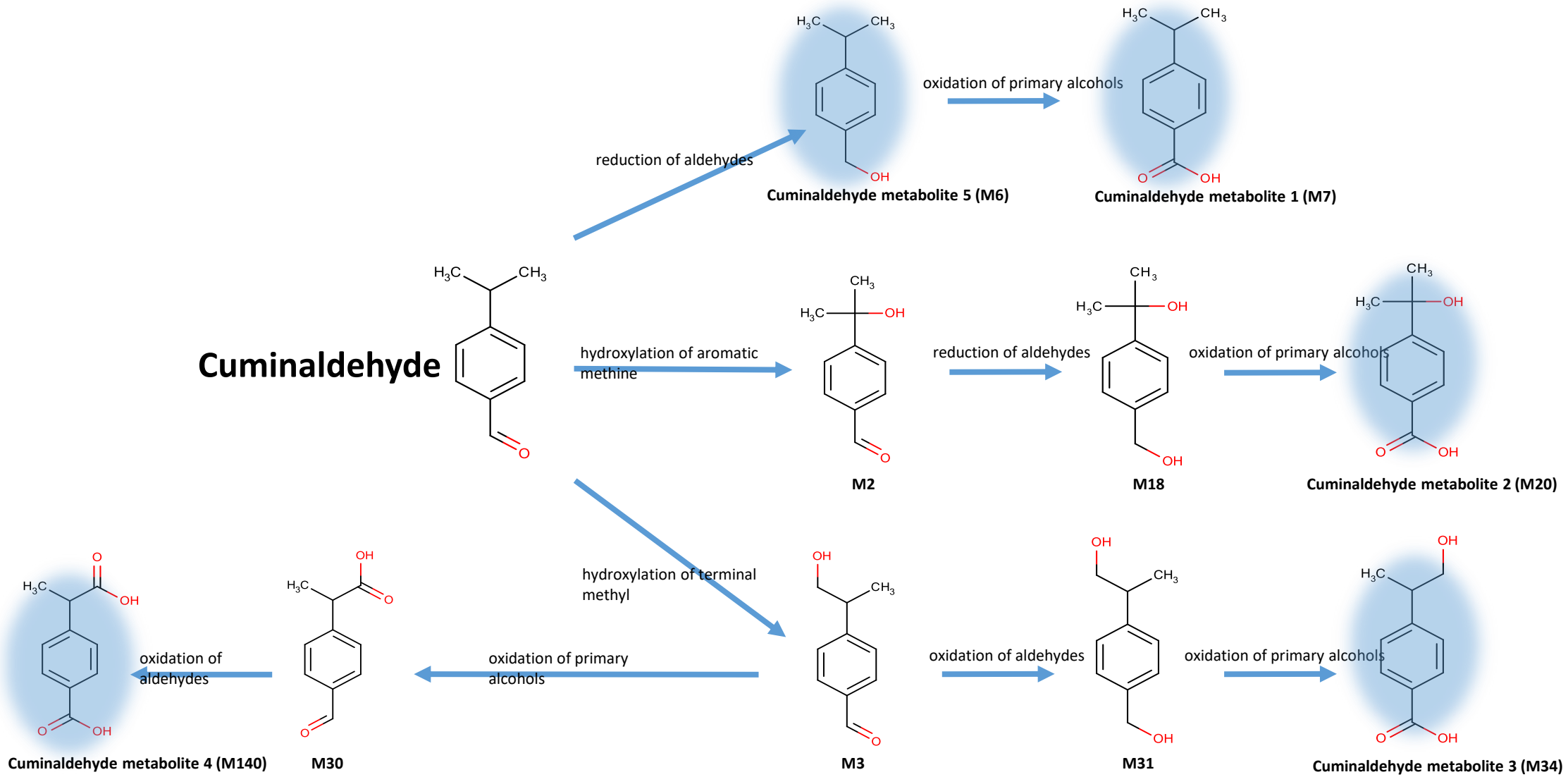
Meteor nexus

https://www.lhasalimited.org

1

Training

defining the reactions involved in the the metabolism of dietary monoterpenes



1

Training

defining the reactions involved in the the metabolism of dietary monoterpenes

Biotransformation Name	Phase	Enzyme	Compounds that undergo the specific reactions								
Allylic Hydroxylation	Phase I	CYP450	limonene	nootkatone	geraniol	terpinen-4-ol	perillyl alcohol	linalool			
Conjugation of Alkyl Carboxylic Acids with Glycine	Phase II	ACS, AANAT	geraniol	terpinen-4-ol	perillyl alcohol						
Conjugation of Carboxylic Acids with Glutamine	Phase II	ACS, AANAT	geraniol								
Epoxidation of 1,1,2-Trisubstituted Alkenes	Phase I	CYP450	limonene	geraniol	terpinen-4-ol	perillyl alcohol	linalool				
Epoxidation of 1,1-Disubstituted Alkenes	Phase I	CYP450	limonene	nootkatone	perillyl alcohol						
Epoxidation of Monosubstituted Alkenes	Phase I	CYP450	linalool								
Glucuronidation of Aromatic Alcohols	Phase II	UGT	thymol								
Glucuronidation of Carboxylic Acids	Phase II	UGT	thymol	limonene	nootkatone	geraniol	terpinen-4-ol	perillyl alcohol	cuminaldehyde	linalool	menthol
Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	Phase II	UGT	thymol	limonene	nootkatone	geraniol	terpinen-4-ol	perillyl alcohol	cuminaldehyde	linalool	menthol
Hydroxylation of Alkyl Methine	Phase I	CYP450	nootkatone	terpinen-4-ol	menthol						
Hydroxylation of Aromatic Methine	Phase I	CYP450	thymol	cuminaldehyde							
Hydroxylation of Methyl Carbon Adjacent to an Aliphatic Ring	Phase I	CYP450	nootkatone	menthol							
Hydroxylation of Methyl Carbon Next to an Aromatic Ring	Phase I	CYP450	thymol								
Hydroxylation of Terminal Methyl	Phase I	CYP450	thymol	terpinen-4-ol	cuminaldehyde	linalool	menthol				
Hydroxylation of Unfunctionalised Alicyclic Methylene	Phase I	CYP450	limonene	nootkatone	perillyl alcohol	menthol					
Oxidation of Aldehydes	Phase I	ALDH	cuminaldehyde								
Oxidation of Primary Alcohols	Phase I	ADH	thymol	limonene	nootkatone	geraniol	terpinen-4-ol	perillyl alcohol	cuminaldehyde	linalool	menthol
Oxidation of Secondary (Alicyclic) Alcohols	Phase I	ADH	limonene	nootkatone	geraniol	terpinen-4-ol	perillyl alcohol	menthol			
Reduction of Aldehydes	Phase I	ALDR	cuminaldehyde								
Reduction of Alicyclic Ketones	Phase I	ADH	menthol								
Reduction of alpha,beta-Unsaturated Compounds	Phase I	abKDBR	nootkatone								
Vicinal Diols from Epoxides	Phase I	EH	limonene	nootkatone	geraniol	perillyl alcohol	linalool				

2

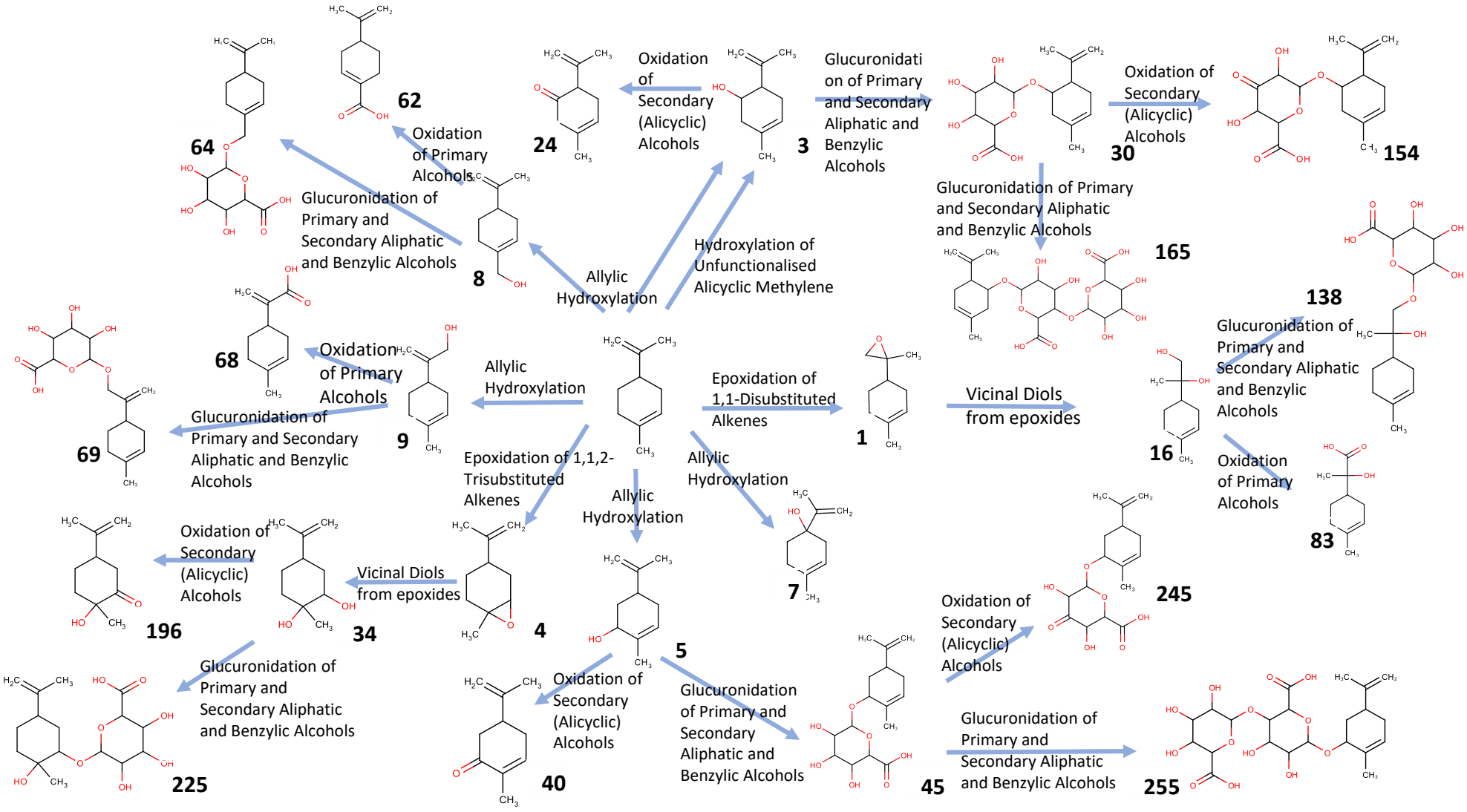
Prediction

using selected reactions to predict the metabolites of monoterpenes

Meteor nexus

BioTransformer

University of Alberta

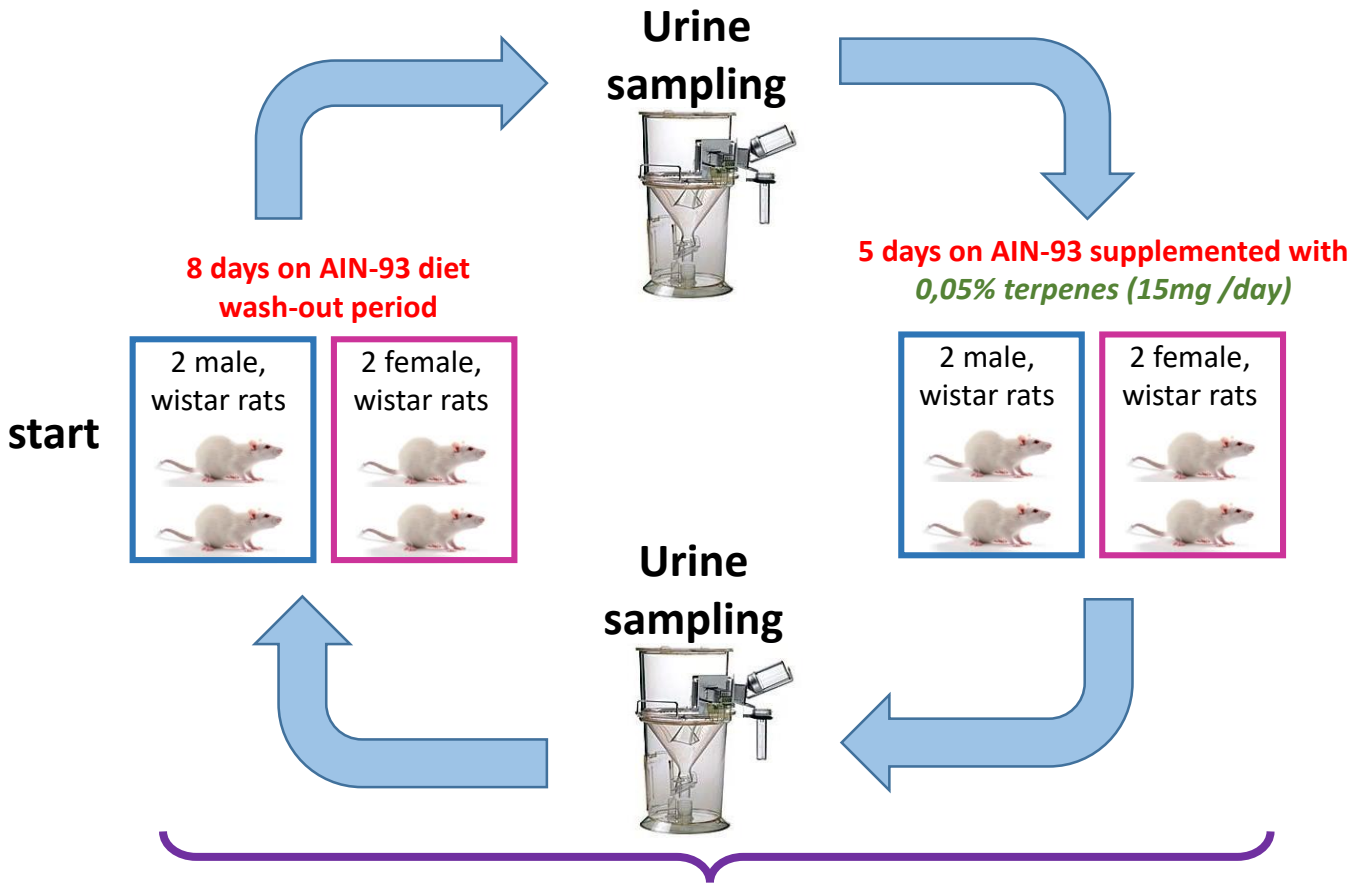


LIMONENE

3

in vivo experiment

feeding rats isolated monoterpenes and collecting metabolites-rich urine

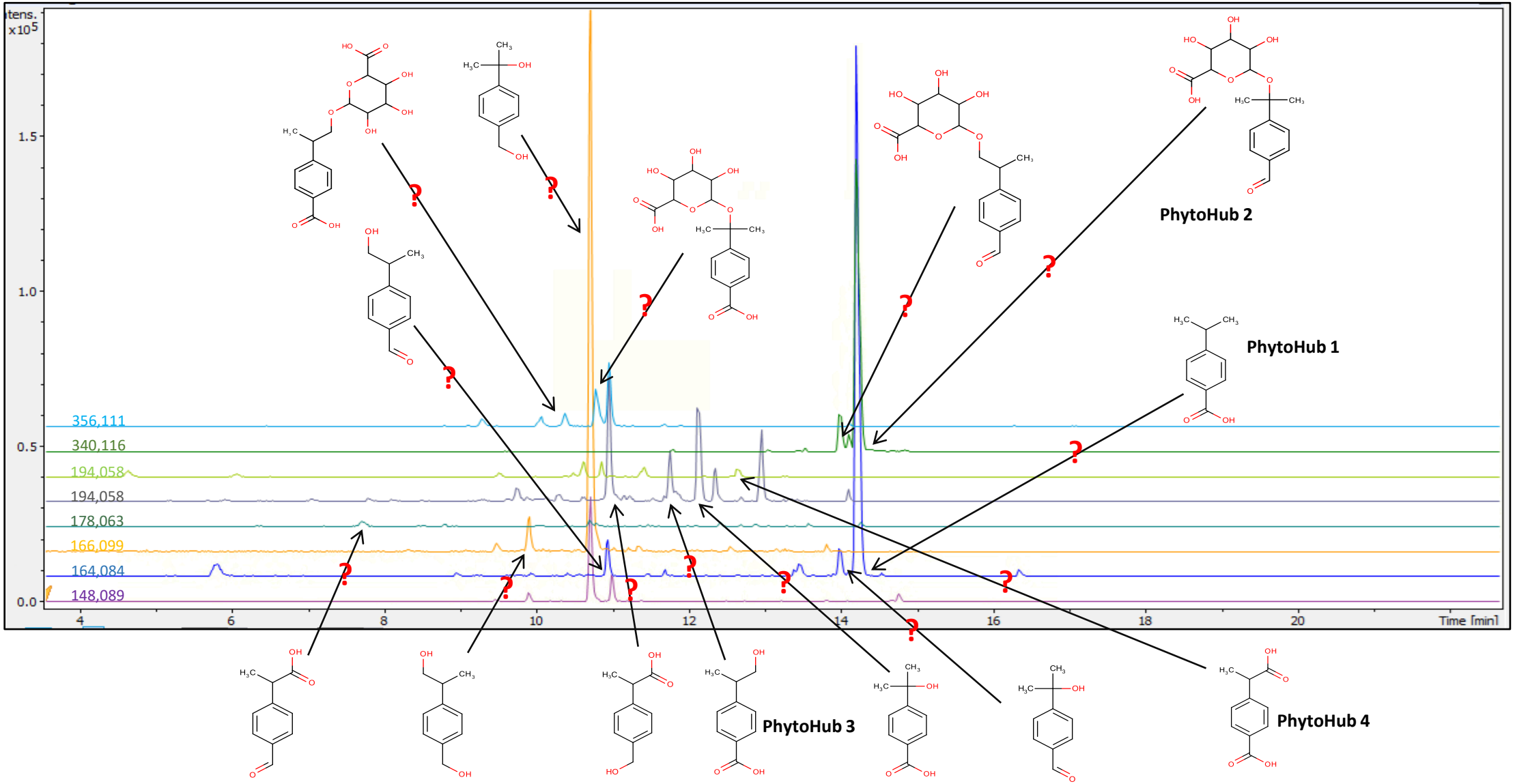


5 cycles – same rats were exposed to different food compounds

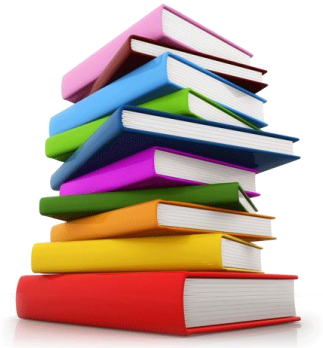
4

Analysis

non-targeted LC-MS analysis in search of predicted metabolites



Investigation of Metabolism of Dietary Terpenoids



Literature & Databases

1 Training

2 Prediction

3 *in vivo* experiment

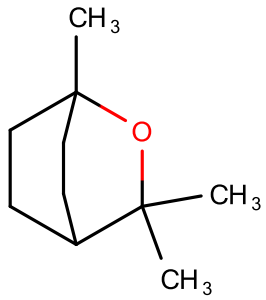
4 Analysis

Known metabolites ⟷ **Predicted metabolites** ⟷ **Experimental data on rat metabolites**

Validation of the predictions
Identification of new metabolites

Example:

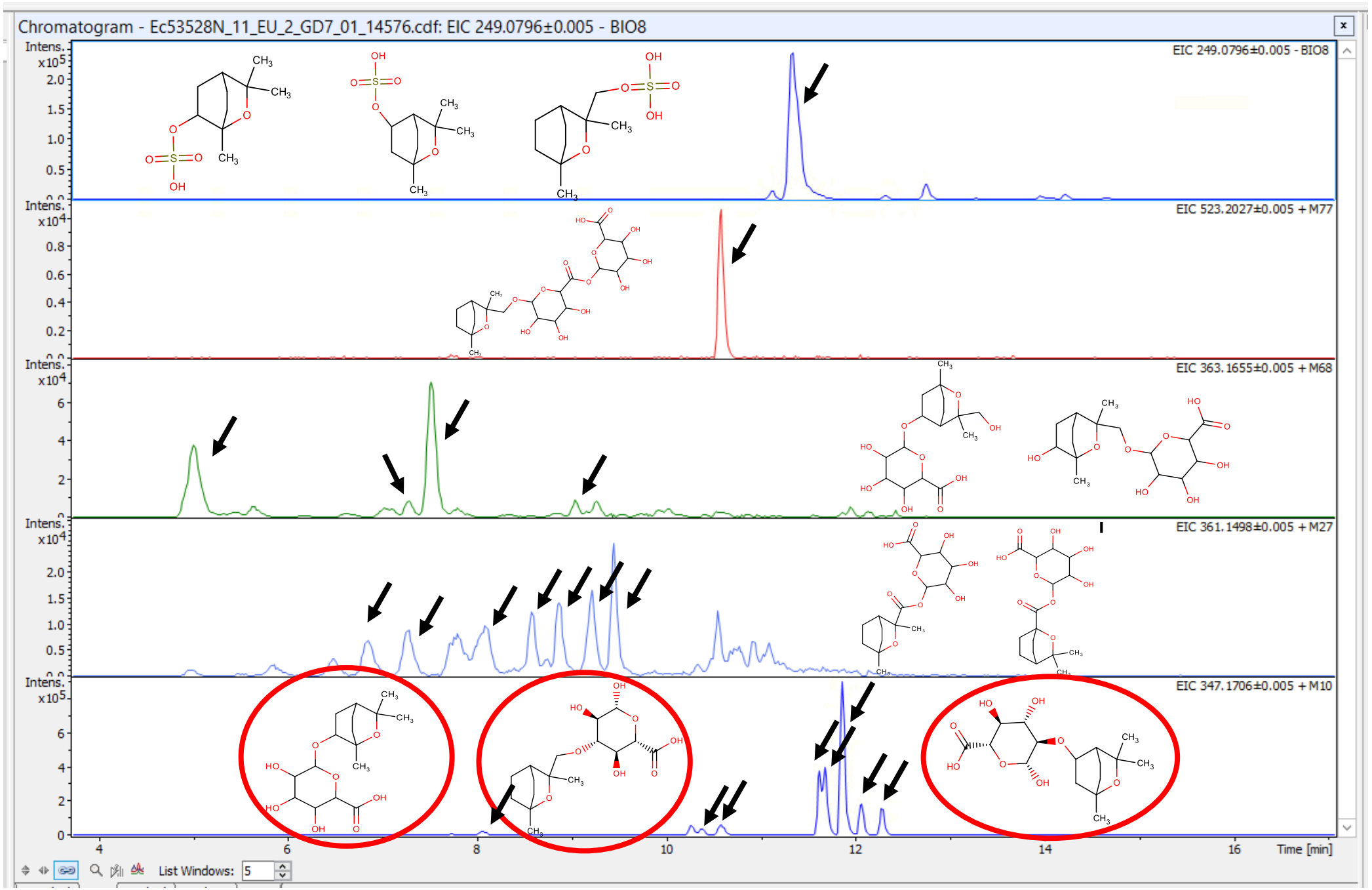
1,8-cineole



The structures in the chromatogram were not yet confirmed.

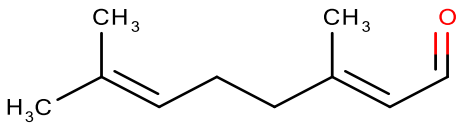
They have the same mass as the assigned peaks and are therefore used as examples.

There are other structures predicted with same molecular mass.



Example:

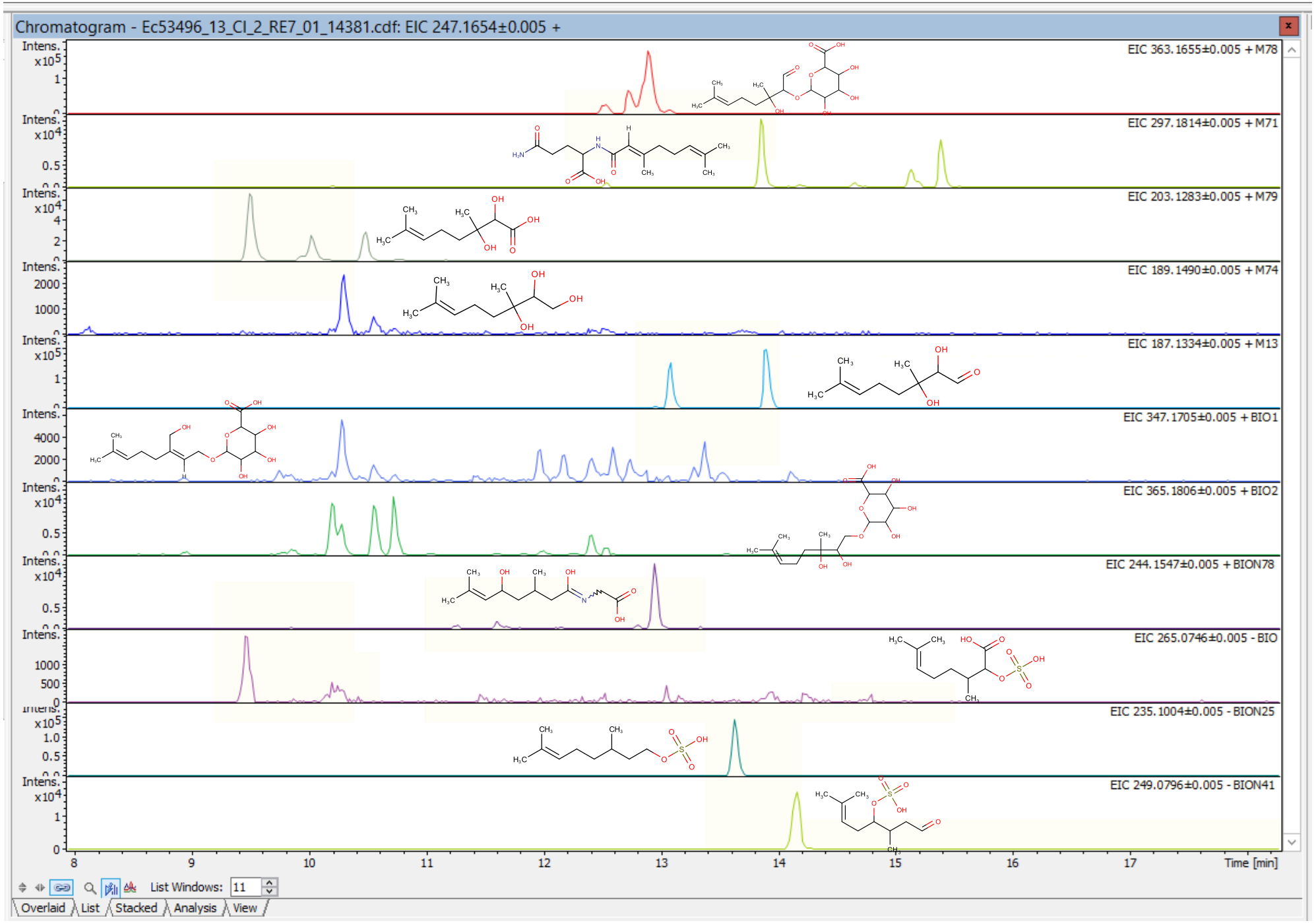
citral



The structures in the chromatogram were not yet confirmed.

They have the same mass as the assigned peaks and are therefore used as examples.

There are other structures predicted with same molecular mass.



Conclusions

- ✓ Considering the selected 22 biotransformations, more than 1500 metabolites were predicted from the 23 tested terpenoids.
- ✓ The predicted metabolites were helpful for the annotation of the peaks detected after the rats were exposed to the terpenoids.
- ✓ Next step is to validate the hypothetical structures of known and ***new*** metabolites using qToF MS/MS.
- ✓ The knowledge generated is being used to improve *in silico* prediction tools (BioTransformer)
- ✓ The generated data will be made available in food compounds databases (PhytoHub, HMDB)

Acknowledgements and financial support

- **Claudine Manach**
- Celine Dalle
- Marie-Anne Verny
- Stephanie Durand
- Delphine Centero
- Charlotte Joly
- Estelle Pujos
- Bernard Lyan

- David Wishart
- Yannick Djoumbou Feunang