Investigations on human biomonitoring for assessing the exposure to synthetic rose oxide in the general population

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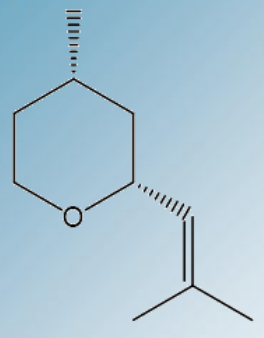
Rose Oxide (RO)



2-(2-Methyl-1-propenyl)-4-methyl-tetrahydropyran

MW: 154 g/mol

Use as a fragrance in cosmetic, cleaning agents, household products, etc.

NATURAL VS. SYNTHETIC

mainly (-)-cis-isomer

0.3 – 1.2 % natural rose oxide in roses ➔ time-consuming and expensive production

equal parts (-)- and (+)-cis-isomers & (-)- and (+)-trans isomers

(+)-isomers specific for synthetic rose oxide

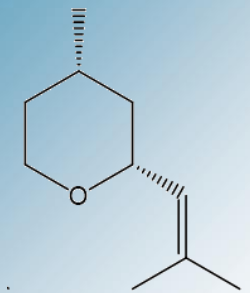
TOXICITY

- Low acute toxicity (LD_{50} > 2000 mg/kg body weight)
- ECHA: NOAEL 100 mg/kg bw/d (♂), 300 mg/kg bw/d (♀) (oral subacute dosage)
- Skin and eye irritating, not skin-sensitizing, no mutagenic effects, may impair fertility

High production chemical: Broad exposure of the general population expected

➔ **HUMAN BIOMONITORING**

Workflow

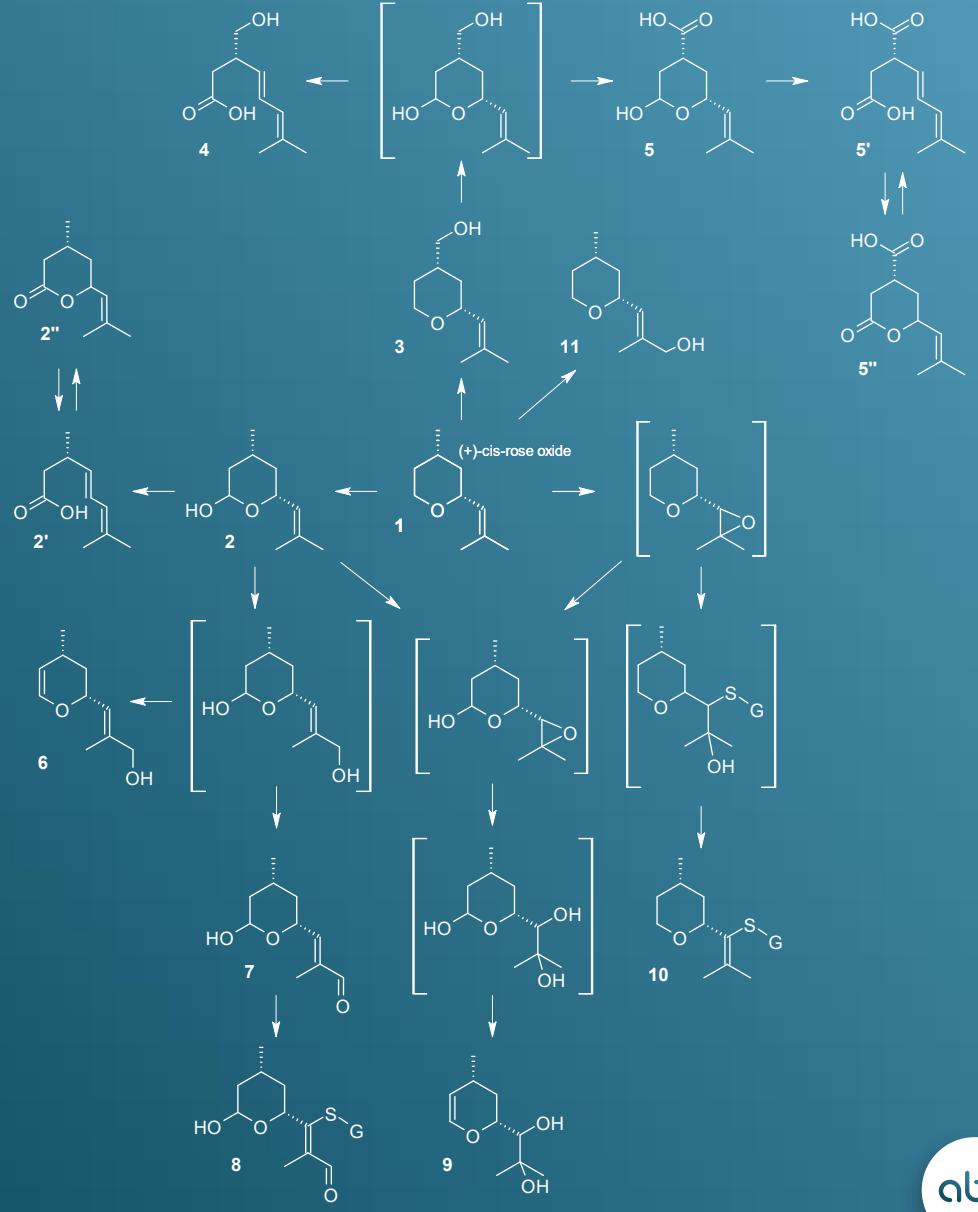


Rose oxide

- Identification of a suitable metabolite
- Method development and validation
- Excretion kinetics after single oral and dermal application
- Application to urine samples

Postulated Metabolism

- Postulated from in vitro experiments (microsomes from mouse, rat, rabbit & human)
- 10 postulated metabolites
  - Hydroxylation and oxidation reactions
  - Incubations with human microsomes:
    2, 3, 4, 5, 11
    ⇨ **2, 3, 5** and **11** retain both stereocenters



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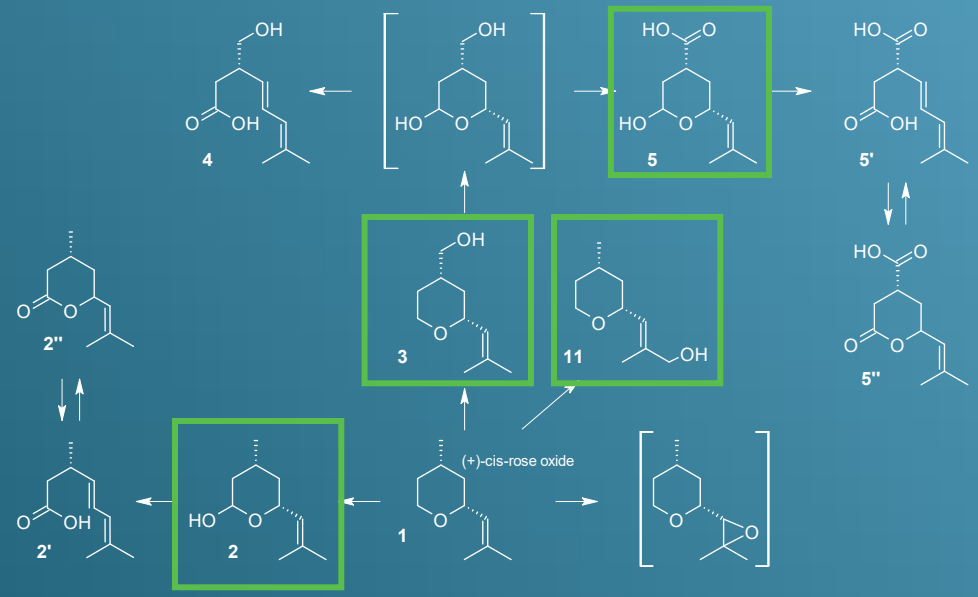


In-house Metabolism Study

- 5 healthy volunteers dosed with (+)-RO (oral and dermal)
- Urine collection for 48 h (oral) and 72 h (dermal)

  Documentation of time points and urine volume
- Non-targeted analysis of selected urine fractions with UPLC-HRMS
- Data evaluation – **Screening for metabolites**

oral
0.1 mg/kg bw

dermal
0.5 mg/kg bw

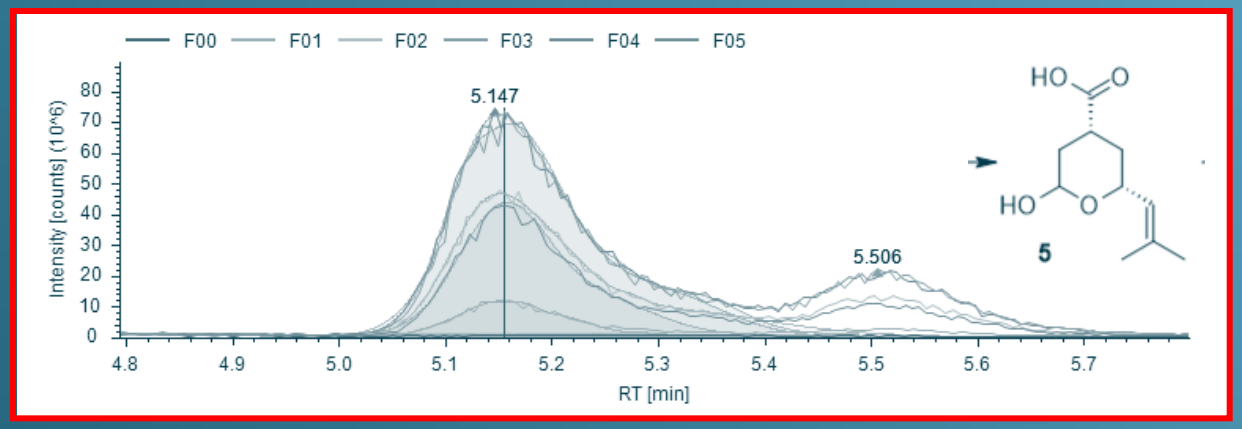
Non-targeted analysis

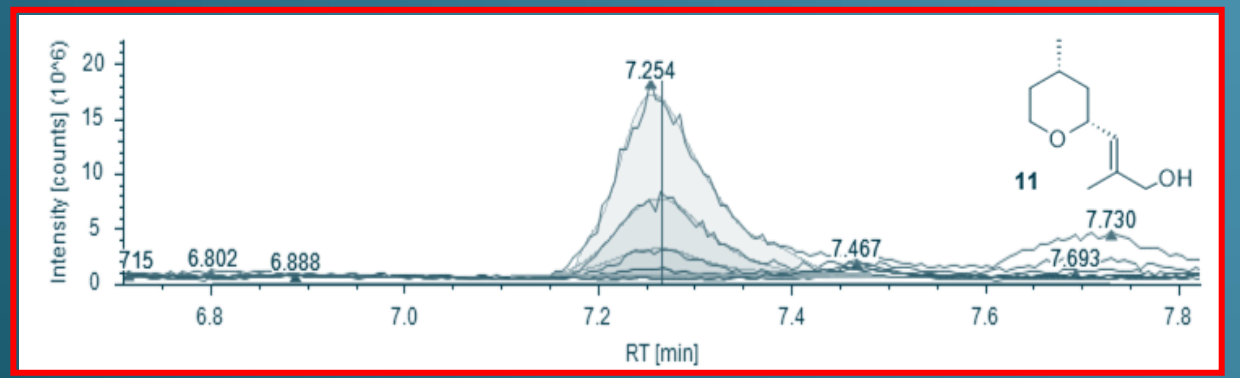
Q-Exactive-HF-X Orbitrap MS

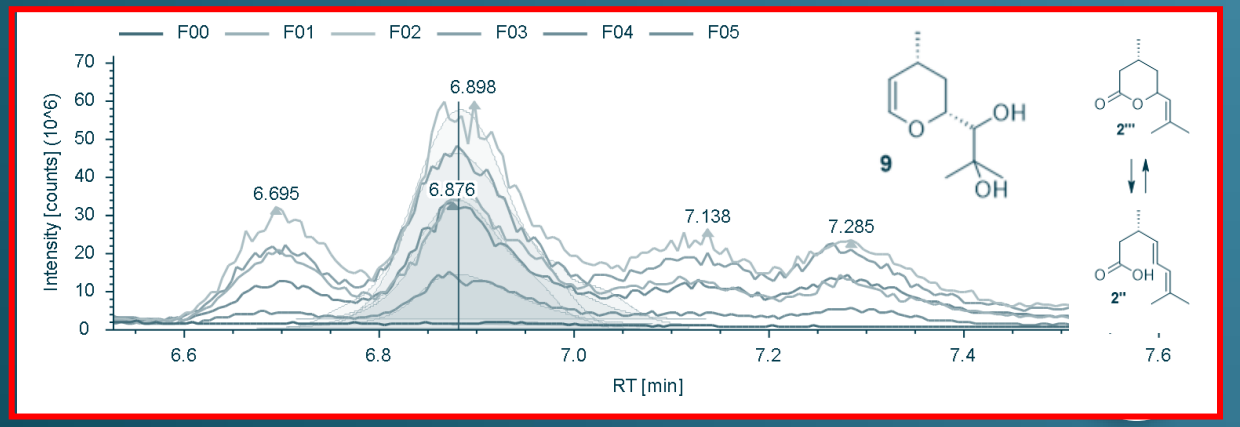
Screening for suitable metabolites
Full MS1 and MS2 spectrum of selected candidates

Results – Non-targeted Analysis

- 18 features followed the typical excretion pattern – 4 metabolites
- m/z 201: “Hydroxy-Carboxy-RO”, [C10H16O4], [**5**]
- m/z 153: “9-OH-RO” –H_2O, [C10H16O], [**11**]
- m/z 169: “Di-OH-RO”/Lactone, [C10H16O2], [**9/2’’/2’**]
- m/z 229: not identified







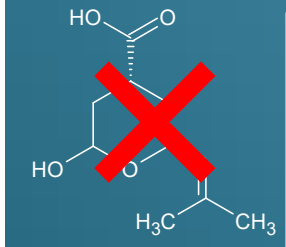
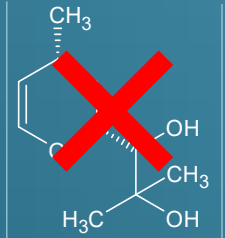
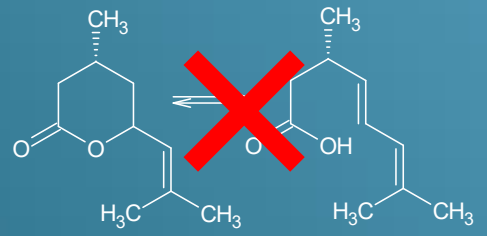
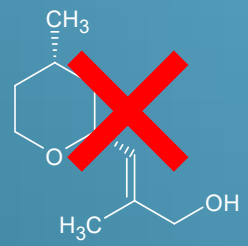
Metabolite Selection

Criteria for suitable metabolites

Clarified structure
Confirmed by reference standard

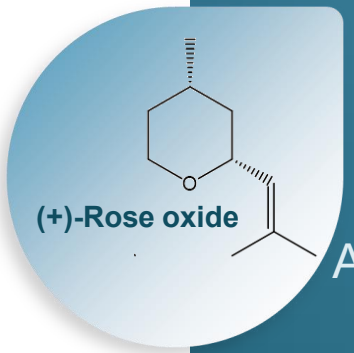
Both stereocenters remain intact
((+)-cis-isomer is specific for synthetic rose oxide)

Metabolite formation sufficient for analysis

Identification of a suitable metabolite	Method development and validation	Excretion kinetics	Application to urine samples
			
✗	✗	✗	✓
✓	✓	✗	✓
?	?	?	✗

Parent compound (+)-cis-RO

Quantitative GC-MS Method



Analytical Workflow

Sample workup

3 mL urine sample
+ 1 g KCl
+ IS (linalool-d_5)
(10 mL Headspace-Vial)

⬇

SPME-HS-GC-MS

EI, SIM

Rose oxide (Quan)	m/z: 139
Rose oxide (Qual)	m/z: 69
Linalool-d_5	m/z: 159

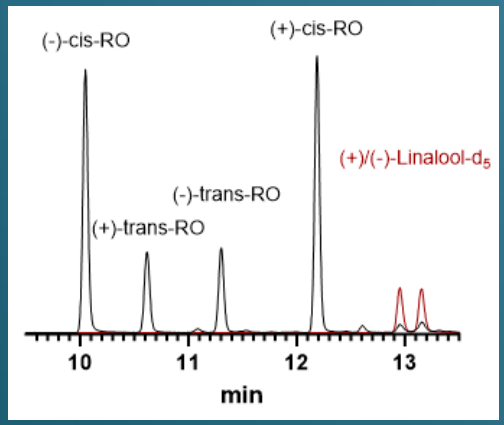
Method Validation

According to FDA Guideline on Bioanalytical Method Validation (2001)

Calibration range	1 – 200 ng/L		
LLOQ	1 ng/L		
Accuracy and precision	**level**	**accuracy [%]**	**precision (CV, [%])**
Inter-day (N = 3x5)	1 ng/L	99.7	11.2
	2 ng/L	91.2	8.7
	10 ng/L	96.2	5.6
	100 ng/L	99.9	4.2

Parameters: Linearity, accuracy & precision, selectivity, matrix effect, stability, carryover, dilution integrity

Urine sample, 200 ng/L



In-house Metabolism Study

- 5 healthy volunteers dosed with (+)-RO (oral and dermal)

- Urine collection for 48 h (oral) and 72 h (dermal)

  Documentation of time points and urine volume

- Targeted analysis of all urine fractions with SPME-HS-GC-MS
- Data evaluation – **Excretion kinetics**

oral
0.1 mg/kg bw

dermal
0.5 mg/kg bw

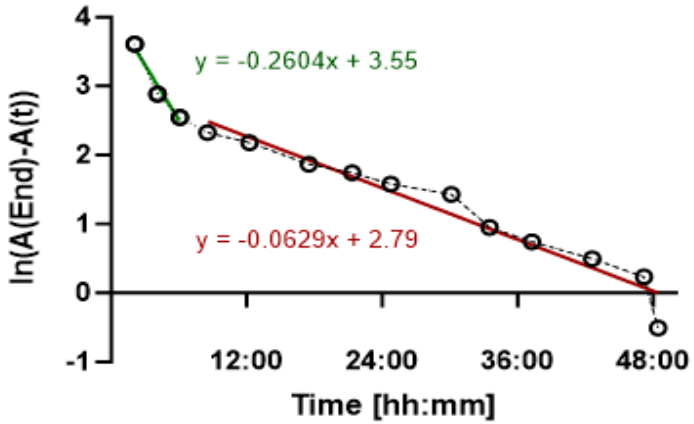
Targeted analysis

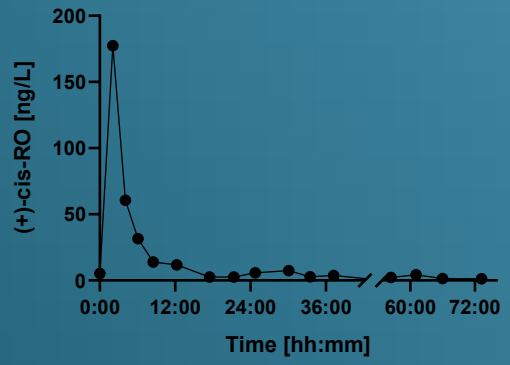
Agilent GCMS 5975

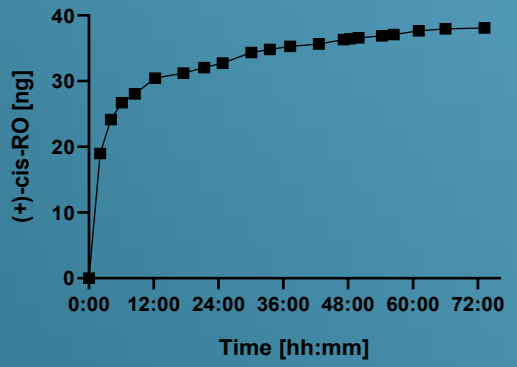
Evaluation of excretion kinetics and toxicokinetic parameters for each participant

Excretion Kinetics - **Oral Application**

- Baseline levels of (+)-cis-RO before application: 0 – 6.2 ng/L
- Fast elimination
  - Mean t_{max} = 1.6 h
  - Complete elimination after 48 h
- Recovery of 7 ppm
- Bi-exponential decline with 2 elimination phases





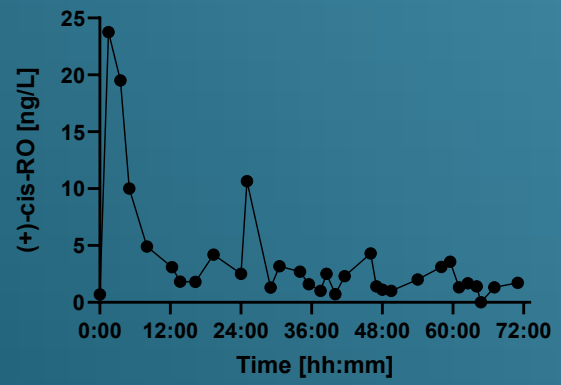


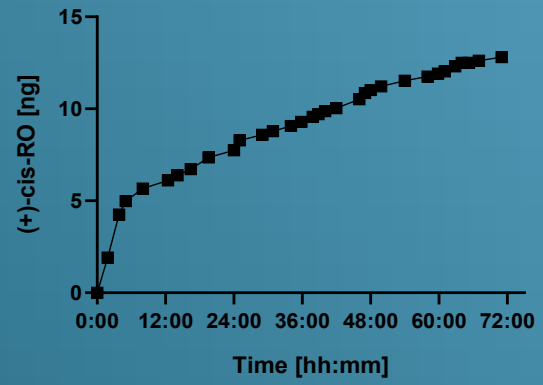
	Mean ± SD	Median	Min–Max
Cumulative excretion after 48 h [pmol] (A_{48h})	337 ± 196	296	71–657
t_{max} [h]	1.63 ± 0.98	1.25	0.73–3.33
Total (+)-cis-RO excreted after 3 h [%]	62 ± 23	67	22–89
Total (+)-cis-RO excreted after 6 h [%]	76 ± 17	86	44–92
Total (+)-cis-RO excreted after 12 h [%]	83 ± 1	91	55–95
Total (+)-cis-RO excreted after 24 h [%]	90 ± 8	94	77–98
Total (+)-cis-RO after 48 h [%]	99 ± 2	100	96-100
Elimination constant λ_1 [h^{-1}]	0.36 ± 0.19	0.35	0.10–0.69
Elimination half-life $t_{1/2\,\lambda1}$ [h]	4.10 ± 3.01	2.88	1.44–9.93
Elimination constant λ_2 [h^{-1}]	0.07 ± 0.02	0.06	0.05–0.09
Elimination half-life $t_{1/2\,\lambda2}$ [h]	14.7 ± 3.35	15.9	10.6–18.0
Urinary excretion factor F_{ue} (24 h) [‰]	0.007 ± 0.005	0.005	0.001–0.017
Urinary excretion factor F_{ue} (48 h) [‰]	0.007 ± 0.005	0.005	0.001–0.017

Excretion Kinetics - **Dermal Application**

- Baseline levels of (+)-cis-RO application: 0.7 – 4.8 ng/L
- Slower elimination compared to oral application
  - Mean t_{max} = 2.77 h
  - 82 % of (+)-cis-RO eliminated after 48 h
- Recovery of 0.3 ppm
- Dermal resorption rate: 18 %

Participant	Dermal (+)-RO dose [µmol]	Excreted amount after dermal application [nmol]	Excreted amount after oral application [%]	Resorption rate [%]
1	259.3	0.77	0.0008	37.1
2	259.3	0.19	0.0005	14.7
3	275.5	0.10	0.0005	7.3
4	201.0	0.08	0.0017	2.3
5	243.1	0.07	0.0001	28.8
Mean ± SD				18.0% ± 13.1%





	Mean ± SD	Median	Min–Max
Cumulative excretion after 72 h [pmol] (A_{72h})	103 ± 44.6	83.0	65–189
t_{max} [h]	2.77 ± 0.73	3.00	1.67–3.50
Total (+)-cis-RO excreted after 3 h [%]	29 ± 12	29	13–45
Total (+)-cis-RO excreted after 6 h [%]	35 ± 10	30	22–50
Total (+)-cis-RO excreted after 12 h [%]	50 ± 12	47	37–73
Total (+)-cis-RO excreted after 24 h [%]	63 ± 8	60	57–78
Total (+)-cis-RO excreted after 48 h [%]	82 ± 7	86	72–90
Urinary excretion factor F_{ue} (24 h) [‰]	0.0003 ± 0.0001	0.0002	0.0002-0.0004
Urinary excretion factor F_{ue} (48 h) [‰]	0.0003 ± 0.0001	0.0003	0.0002-0.0006

Application

- “General population”
  - 13 non-occupationally exposed participants
  - 1 participant intentionally exposed to rose oxide-containing perfume (positive control)

positive control

- Quantifiable amounts of (+)-cis-RO in 54 % of unexposed samples
  - 1.2 ng/L on average (LLOQ: 1 ng/L)

SPME-HS-GC-MS
Targeted analysis

Very low urinary excretion rate of 7 ppm (oral) / 0.3 ppm (dermal)

Summary and Conclusion

Identification of a suitable metabolite	No suitable metabolite – Parent compound used: **(+)-cis-RO**
Method development and validation	Successful development and validation of a quantitative GC-MS method
Excretion kinetics after single oral and dermal application of (+)-RO	Fast elimination, Low recovery rate of 7 ppm (oral)/ 0.3 ppm (dermal)
Application to urine samples of non-occupationally exposed adults	(+)-cis-RO quantifiable in 54 % of samples at LLOQ level

CHALLENGES FOR HBM

Low excretion rate of (+)-cis-RO
Possible **contamination** by environmental RO

Acknowledgment

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