

Establishment of new human biomonitoring methods to unravel the exposure to emerging chemicals – showcased for the fragrances lysmeral and 7-hydroxycitronellal

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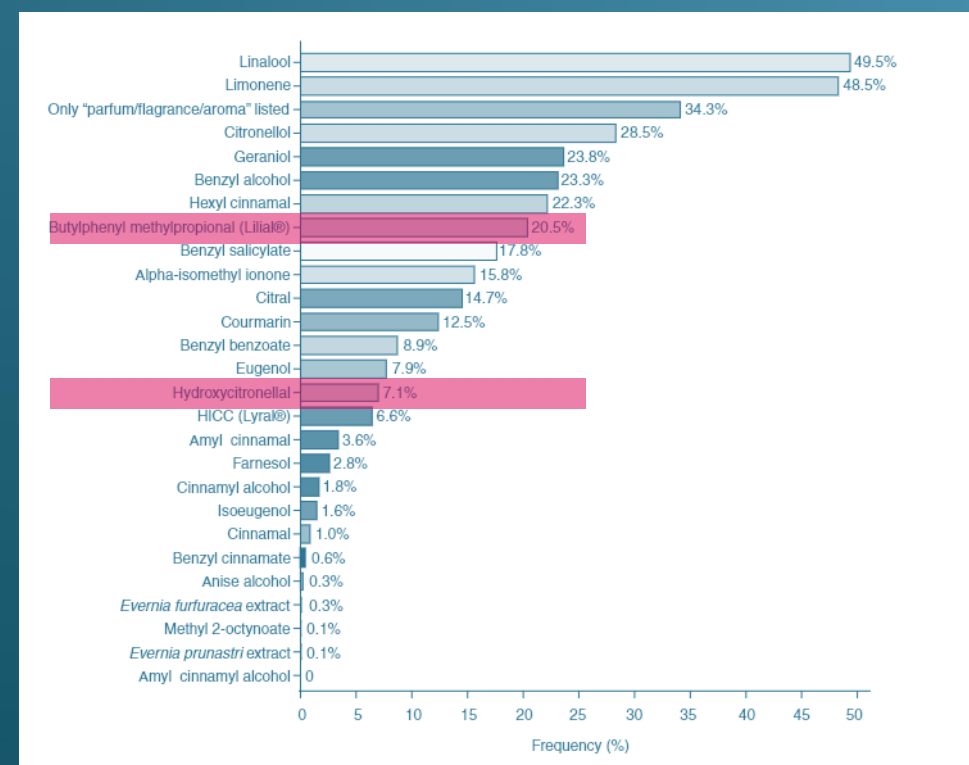
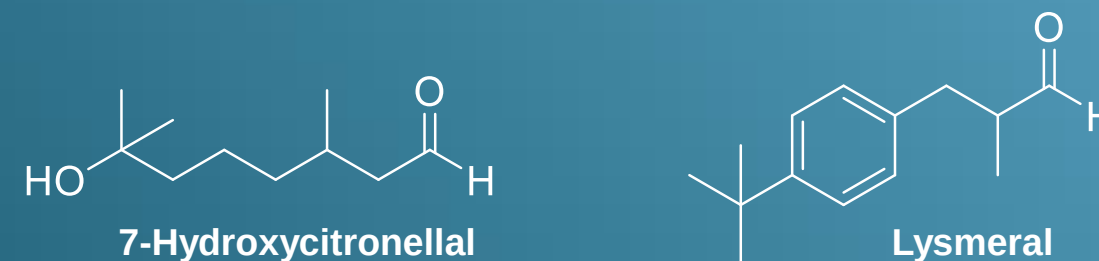
Fragrance chemicals lysmeral & 7-hydroxycitronellal

- Lysmeral: Lilial, Butylphenyl Methylpropional
- Aromatic aldehyde; (R)-form with strong floral odor of cyclamen and lily of the valleys
- 7-Hydroxycitronellal (7-HC): medium-chain aldehyde with sweet floral odor
- Vast range of applications as fragrance in different consumer products, *i.a.* cosmetics, deodorants, detergents, shampoos
- Production scales of 1,000-10,000 t/a in the EU
- In 2017: Lysmeral labelled in 21% and 7-HC labelled in 7% of EU fragranced cosmetic products¹

Product labelling mandatory due to **contact allergenic** properties

Lysmeral: Toxic to reproduction and suspected endocrine disruptor

¹ Bennike, N.H., Oturai, N.B., Müller, S., Kirkeby, C.S., Jørgensen, C., Christensen, A.B., Zachariae, C. and Johansen, J.D. (2018), Fragrance contact allergens in 5588 cosmetic products identified through a novel smartphone application. J Eur Acad Dermatol Venereol, 32: 79-85. <https://doi.org/10.1111/jdv.14513>



Human Biomonitoring (HBM)

Collaboration between the BMUV and the VCI since 2010 ⇒ new HBM methods for chemicals of concern

OBJECTIVE

Improving knowledge about substances to which the population may be **increasingly exposed** and which are of **potential relevance to human health**

Elucidation of their metabolism in humans and **establishment of sensitive analytical methods** to detect the exposure in the general population

Establishment of new HBM methods for up to 50 chemicals

⇒ 2014: Selection of Lysmeral

⇒ 2015: Selection of 7-HC

Introduction

Study Design

Results & Discussion

Conclusion



Bundesministerium
für Umwelt, Naturschutz, nukleare Sicherheit
und Verbraucherschutz

Verband der
Chemischen Industrie e.V.
Wir gestalten Zukunft.



HBM

UV filter



Fragrances



Flame
retardants

Plasticizer

Development of the HBM methods



Human metabolism study:

Elucidation of the human metabolism in a study with 5 volunteers after defined oral administration

Establishment of LC-MS/MS methods for the quantification of the major metabolites

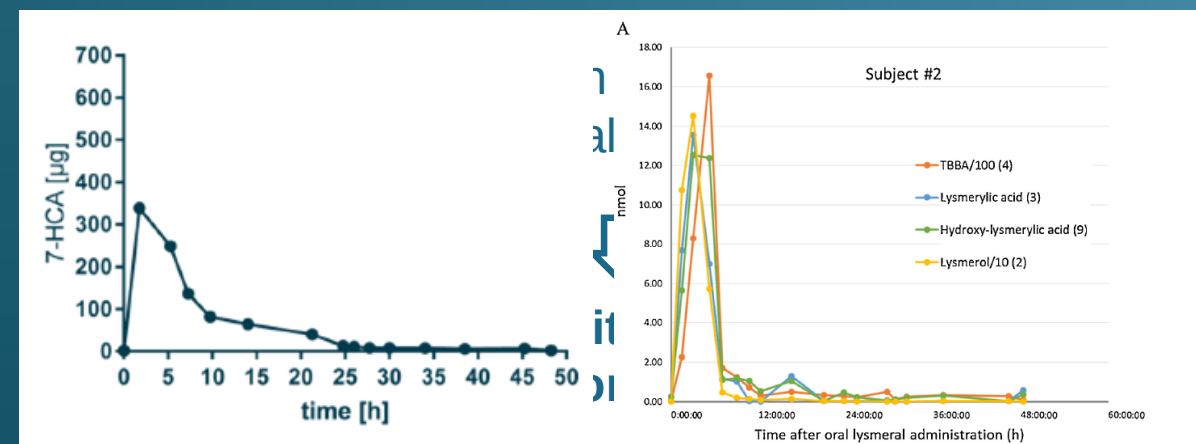
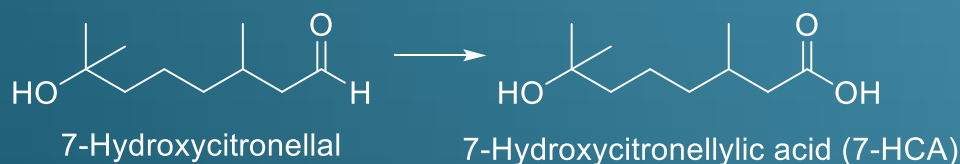
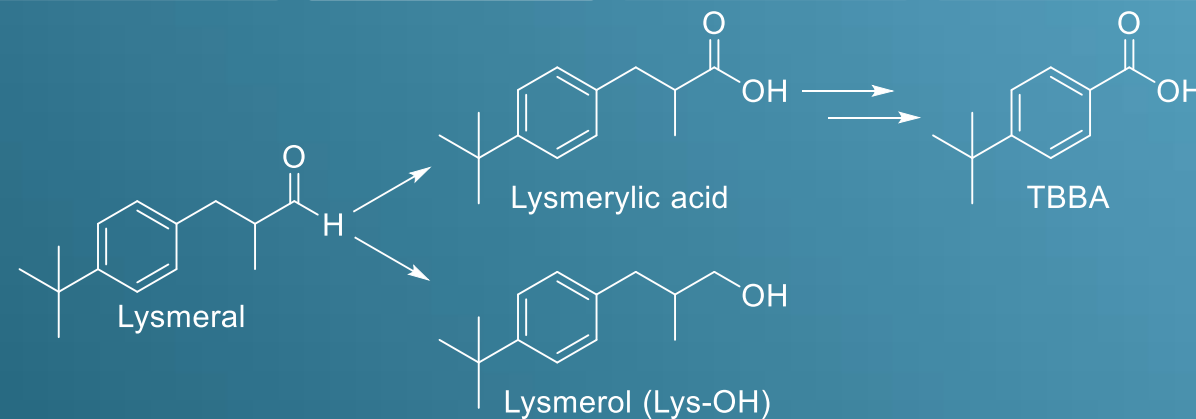
LLOQ = 0.2 µg/L

Pilot study with 40 volunteers:

Location: Bavaria, 23 ♂ / 17 ♀, 18-83 years

All urine samples with metabolite concentrations > LLOQ

- ⇒ Exposure to lysmeral and 7-HC
- ⇒ Sufficient sensitivity of the method



HBM of Lysmeral & 7-HC

24h-urine samples, stored in the Environmental Specimen Bank (**ESB**) and provided by the German Environmental Agency (Umweltbundesamt, **UBA**)

OBJECTIVES

Monitor **time trends**
Identification of possible **sex differences**
Estimation of the **daily intake**

Morning urine samples collected during the **German Environmental Survey for Children and Adolescents GerES V**, provided by the **UBA**

OBJECTIVES

Identification of **exposure sources**
Evaluate possible **differences** in **exposure** by
sex/age & socio-economic status
Basis for establishment of **HBM reference values**

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Study years

2000

2004

2008

2012

2015

2018

329 urine samples

Location: Halle (Germany)

Age: 20-29 years; HBM of lysmeral and 7-HC



Study years

2015

2016

2017

2133 urine samples

Population-representative sample; 167 locations in Germany

Age: 3-17 years; HBM of lysmeral

GerES V: HBM of lysmerral

100 % (TBBA) / 99 % (LysOH) of study samples quantified > LLOQ

Higher exposure in girls compared to boys

♀ 1.1-1.4x higher concentrations of LysOH/TBBA

Gradient along socioeconomic status (SES)

significantly higher concentrations of LysOH/TBBA in participants with lower SES
1.2-1.8x higher concentrations in children with low SES compared to high SES

LysOH [µg/L]

Sex	N	Median	P95	GM
male ♂	1108	1.43	6.51	1.430
female ♀	1025	1.64	7.19	1.641

$p < 0.001$

TBBA [µg/L]

Sex	N	Median	P95	GM
male ♂	1108	8.73	49.5	9.309
female ♀	1025	11.8	55.2	11.28

$p < 0.001$

LysOH [µg/L]

SES	N	Median	P95	GM
low	438	2.17	8.91	2.082
medium	1252	1.49	6.44	1.513
high	384	1.17	5.41	1.171

$p < 0.001$

TBBA [µg/L]

SES	N	Median	P95	GM
low	438	12.0	53.3	11.38
medium	1252	9.86	55.6	10.30
high	384	9.24	45.4	9.199

$p < 0.01$

significance test: one-way ANOVA

GerES V: HBM of lysmeral



100 % (TBBA) / 99 % (LysOH) of study samples quantified > LLOQ

Higher exposure in girls compared to boys

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Main exposure sources: use of personal care products and consumer goods

fabric softener, fragrances, air freshener, perfume, deodorant, body lotion, cream, shower gel

LysOH [µg/L]

Usage of air freshener	N	Median	P95	GM
yes	832	1.91	8.36	1.850
no	1299	1.33	6.32	1.353

p < 0.001

TBBA [µg/L]

Usage of air freshener	N	Median	P95	GM
yes	832	12.1	55.6	11.81
no	1299	8.89	49.6	9.309

p < 0.001

LysOH [µg/L]

Usage of perfume	N	Median	P95	GM
more than once/week	415	2.25	10.1	2.311
once/week or less	459	1.60	7.30	1.636
never	1257	1.31	5.87	1.300

p < 0.001

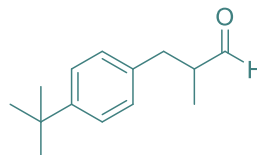
TBBA [µg/L]

Usage of perfume	N	Median	P95	GM
more than once/week	415	13.4	59.6	13.50
once/week or less	459	10.8	40.5	10.34
never	1257	8.73	53.7	9.276

p < 0.001

significance test: one-way ANOVA

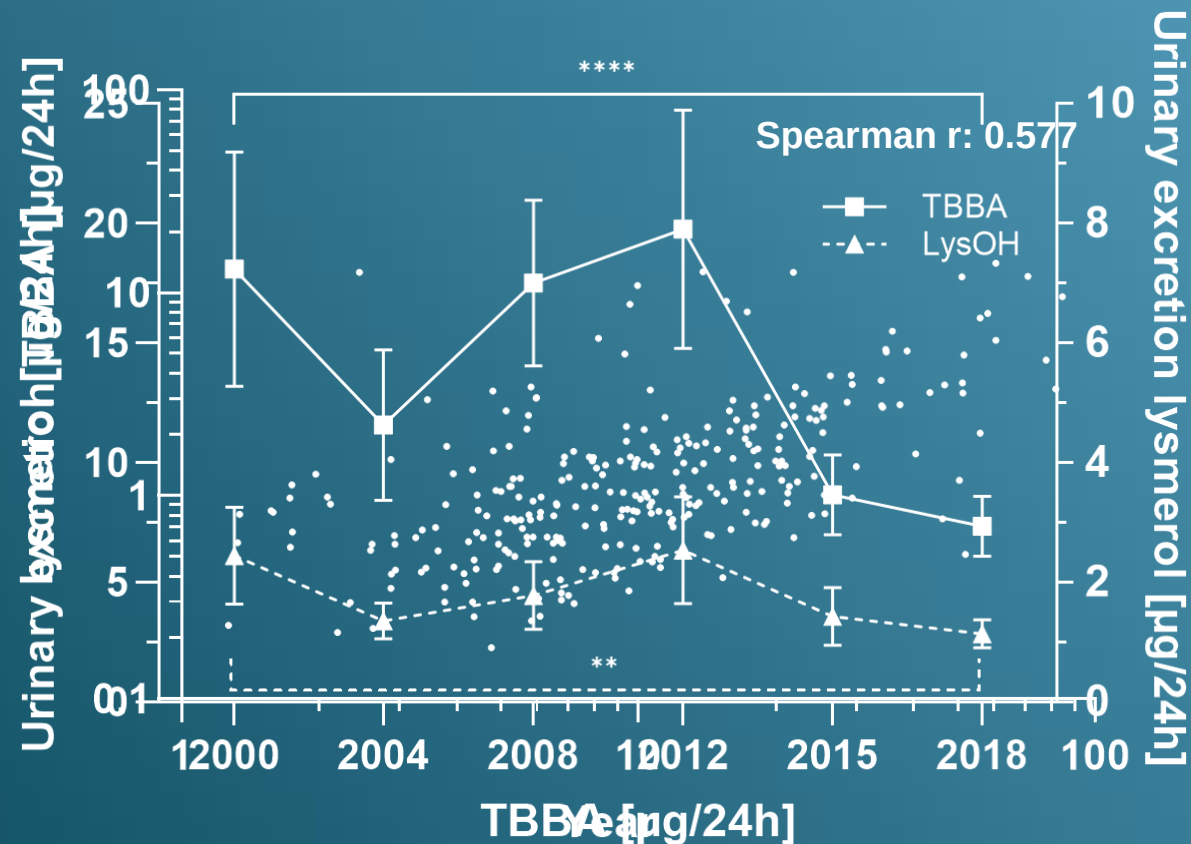
ESB: HBM of Lysmeral



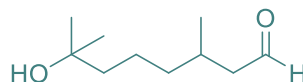
- 99 % (TBBA) / 93 % (LysOH) of study samples quantified > LLOQ
- Higher **excretion rate** of TBBA (~ 10-fold)
TBBA 9.47 µg/24h | LysOH 1.17 µg/24h
- Moderate correlation TBBA / LysOH
- No differences** in excretion rates between sexes
- Time trend:** Significant **decrease** between 2000 and 2018

2000-2018

↓ 44-51 %



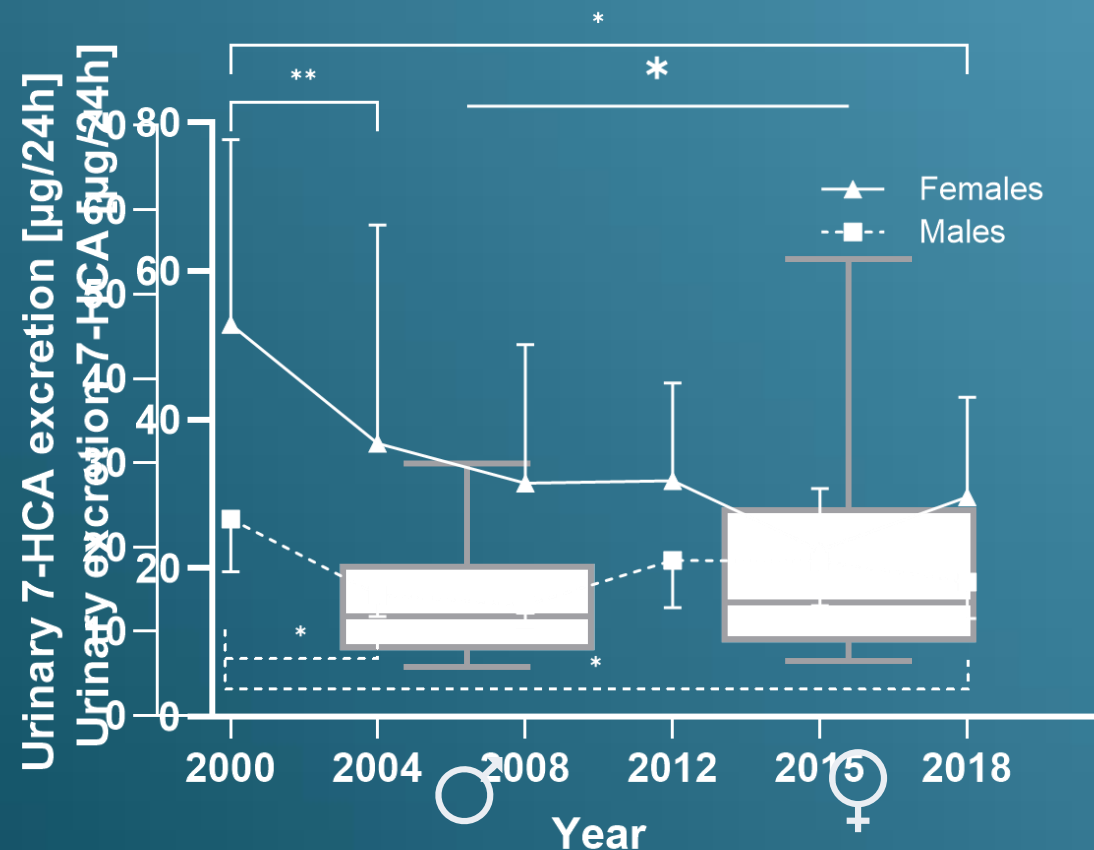
ESB: HBM of 7-HC



- 100 % of study samples quantified > LLOQ
- Sex-specific differences
♂ 14.1 µg/24h | ♀ 18.4 µg/24h
- Time trend:** Significant **decrease** between 2000 and 2018 for both sexes
- Most significant **decline** already between **2000 and 2004**

2000-2018

↓ 37 %



Daily Intake (DI)

Fractional urinary excretions (F_{ue}) of the metabolites determined in metabolism study

+

Consideration of the body weight (bw)



Calculation of daily intake $\Rightarrow$ DI [$\mu\text{g/kg bw/d}$]

- **No differences in DAILY INTAKE** in males vs females for lysmeral
- Significantly **higher DAILY INTAKE** in **females** compared to males for 7-HC
- Higher DI for 7-HC compared to lysmeral
- Comparison with DNEL and health based guidance values (HBM-I) for preliminary risk assessment

F_{ue} TBBA/LysOH: 15.5%

F_{ue} 7-HCA: 44.6%

Daily Intake of Lysmeral [$\mu\text{g/kg bw/d}$]

Parameter	Male ♂	Female ♀
GM	1.06	1.22
Median	1.04	1.22
P95	4.47	4.90

Daily Intake of 7-HC [$\mu\text{g/kg bw/d}$]

Parameter	Male ♂	Female ♀
GM	14.07	18.35
Median	13.50	15.36
P95	50.45	114.2

$p < 0.01$

Risk Assessment

DNEL (derived no-effect levels):
toxicological threshold value for chronic
exposure

(no health consequences below this value)

DNEL Lysmerral = 62.5 µg/kg bw/d

DNEL 7-HC = 600 µg/kg bw/d

HBM-I: health based guidance value: no
impact on health below this concentration

HBM-I 7-HCA = 9,000 µg/L

Daily intakes of the DNEL:

Lysmerral
2.6%

7-HC
0.07%

All concentrations of 7-HCA <<< HBM-I

Based on available data, risk is
considered rather low, especially
under consideration of decreasing
trend over time



Limitations

DNEL based on repeated dose toxicity animal tests

**HBM values more reliable but lacking for
lysmeral**

HBM-I value available for 7-HCA but **no
population-representative data for 7-HC** exposure
1st population-representative HBM of 7-HC currently
ongoing in adults in Germany in GerES VI

Isolated interpretation for each compound

**Possible underestimation of
the risk considering the
exposure to chemical mixtures**



Conclusion

- Elucidation of human metabolism of the fragrance chemicals lysmeral (lilial) and 7-HC
- Successful development of sensitive HBM methods monitoring the major urinary metabolites
- Application in GerES V: ⇒ major exposure sources
- HBM in ESB samples: ⇒ time trends and sex-specific differences

General exposure of adults and children in Germany with significant decrease from 2000 - 2018

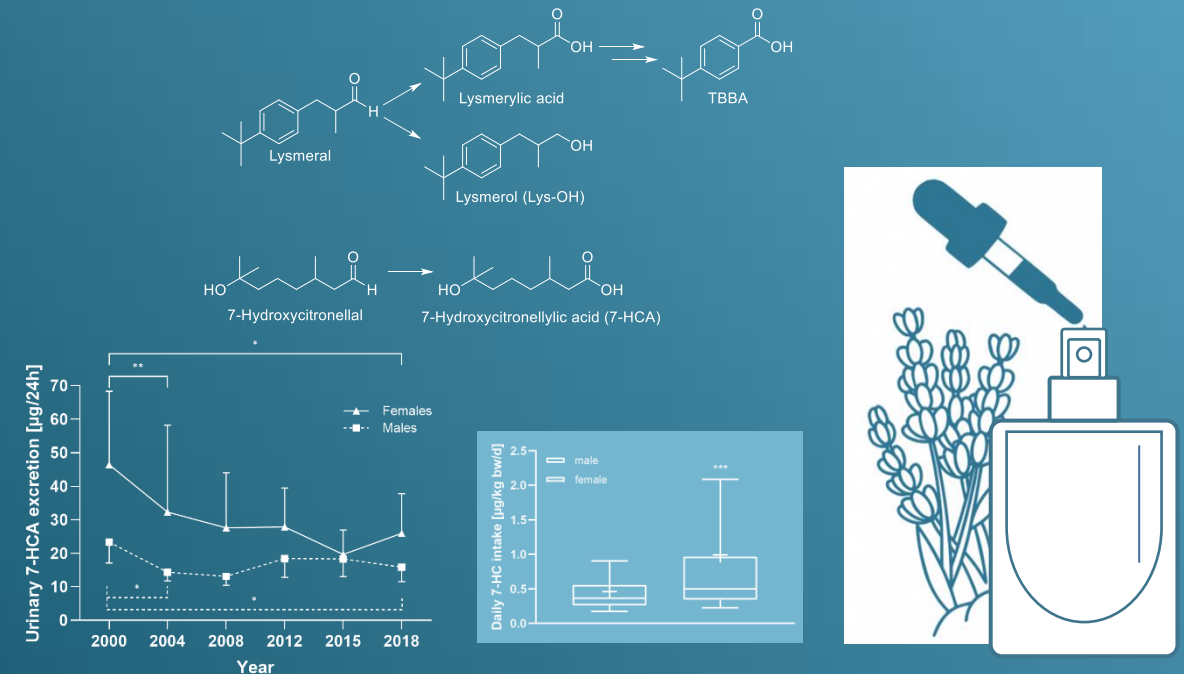
Further HBM ongoing (GerES VI, ESB, LANUV)
⇒ monitor **time trend** and **exposure sources**
⇒ Establish **reference** and **guidance values**
⇒ Observe **impact** of **regulatory measures** (ban of lysmeral March 2022 in cosmetics in the EU)

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Establishment of **new HBM methods** within the BMUV/VCI collaboration and subsequent analysis in **population-representative** (GerES) and **biobanked** samples (ESB) clarifies exposure to emerging chemicals of concern

HBM of emerging chemicals such as the contact allergens lysmeral and 7-HC offers new insights for **risk assessment** and the effects of **regulatory measures for evidence-based policymaking**

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