Development of a human biomonitoring method for assessing the exposure to 2,4,7,9-Tetramethyl-5-decyne-4,7-diol (TMDD) in the general population

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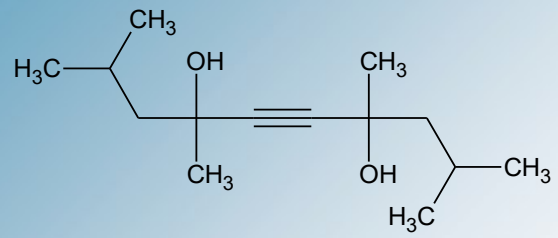
TMDD

- Non-ionic surfactant
  - Use in industry as an adjuvant in manufacture of inks, paper, etc.
- Effluents of wastewater plants as main source of emission
- Toxicity
  - No acute toxicity after oral or dermal exposure
  - No long-term data
  - ECHA: NOAEL 200 – 500 mg/kg body weight
  - Skin sensitizing and mildly irritating, causes severe eye damage
- High production chemical
  - Production of > 1000 t/a
  - Broad exposure of the general population expected

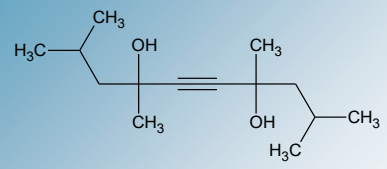


2,4,7,9-Tetramethyl-5-decyne-4,7-diol (TMDD)

MW: 226 g/mol

Human Biomonitoring

Workflow

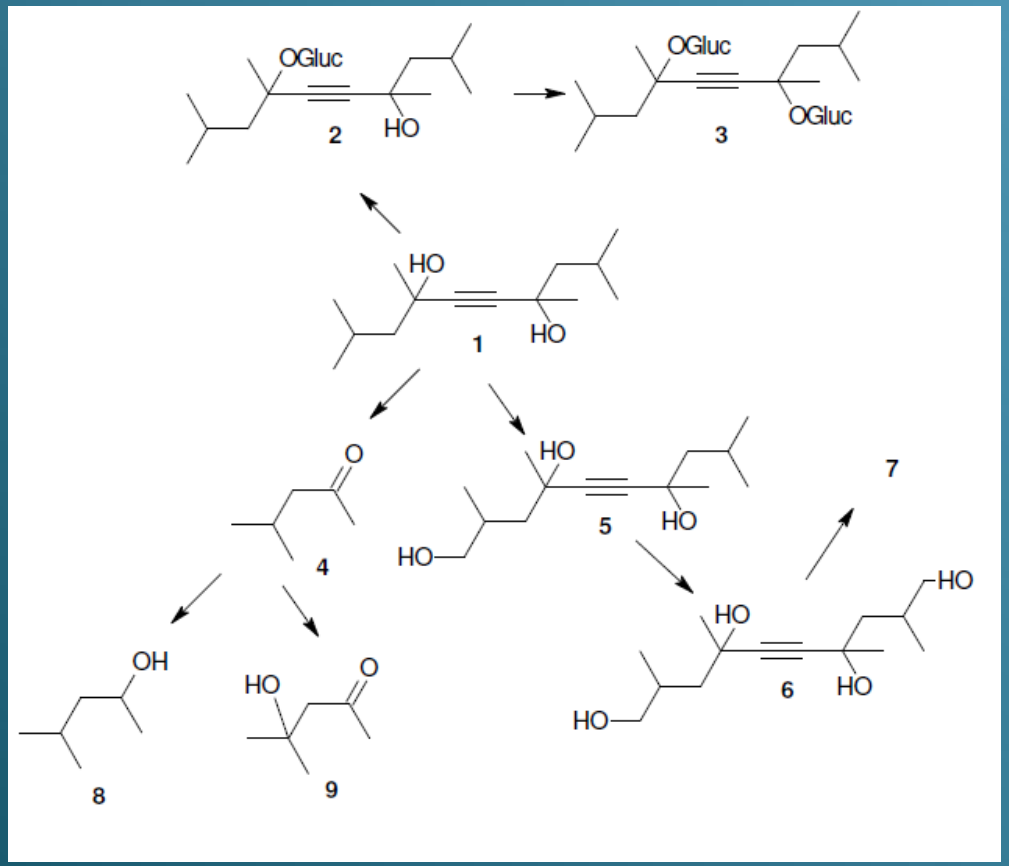


2,4,7,9-Tetramethyl-5-decyne-4,7-diol (TMDD)

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

Postulated metabolism

- Possible metabolites
  - Unmetabolised TMDD [1]
  - Conjugated hydroxy groups [2], [3]
  - Hydroxylation of alkyl moiety [5], [6], [7]
    - Conjugated hydroxy groups
  - Oxidation of hydroxy groups [4] and further metabolism products [8], [9]
  - Metabolization of triple bond is unlikely for TMDD



In-house metabolism study

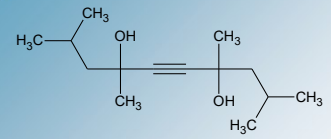
- 4 healthy volunteers dosed with TMDD (oral and dermal)

Subject	Age (years)	Gender	Number of urine fractions (oral)	Number of urine fractions (dermal)
1	71	male	21	20
2	37	male	24	26
3	37	male	25	29
4	32	female	22	31

- Urine collection for 72 h
  - Documentation of time points and urine volume
- Data evaluation – **metabolite screening**
  - Nontargeted analysis of selected urine fractions with UPLC-Q-Orbitrap-MS

oral
75 µg/kg bw

dermal
750 µg/kg bw



2,4,7,9-Tetramethyl-5-decyne-4,7-diol (TMDD)

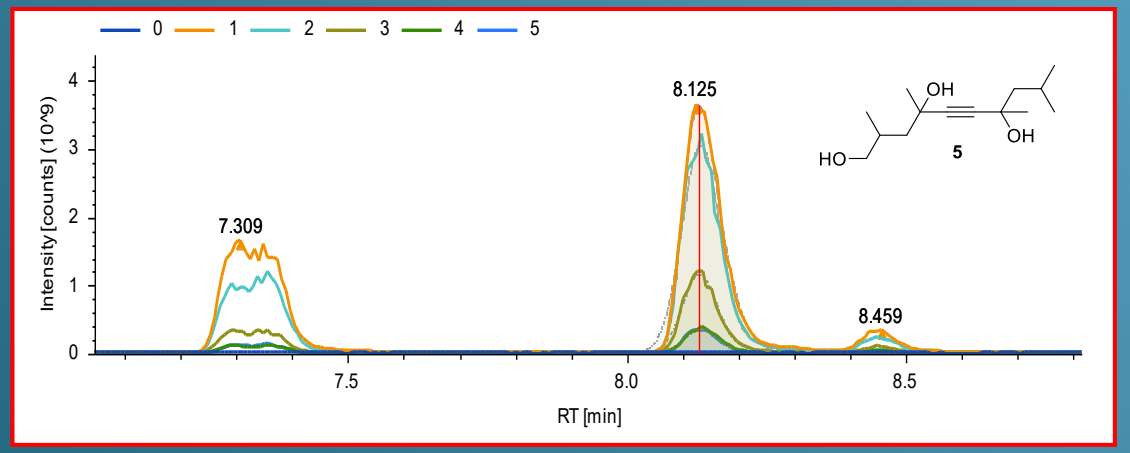
Metabolite screening – Full MS1 and MS2 spectrum of selected candidates

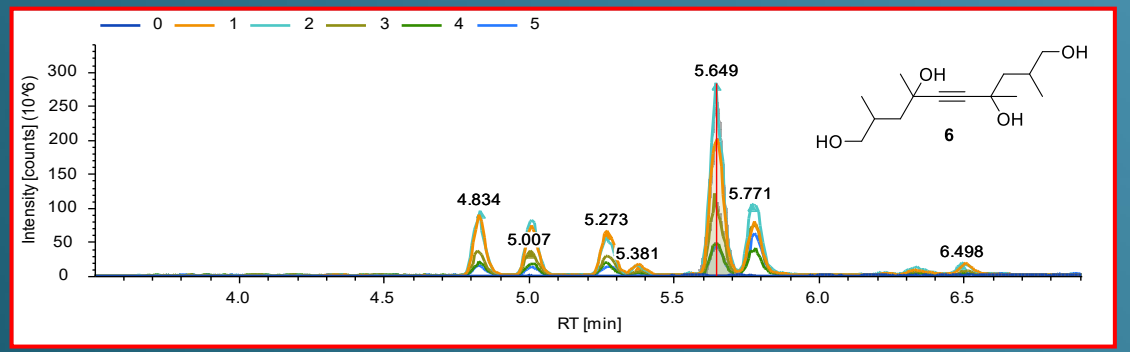
nontargeted analysis

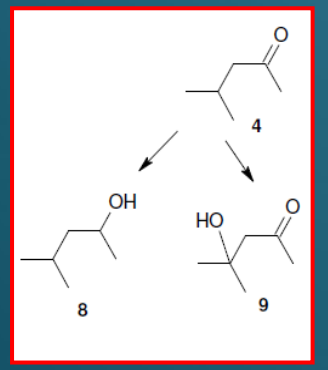
Q-Exactive-HF-X Orbitrap MS

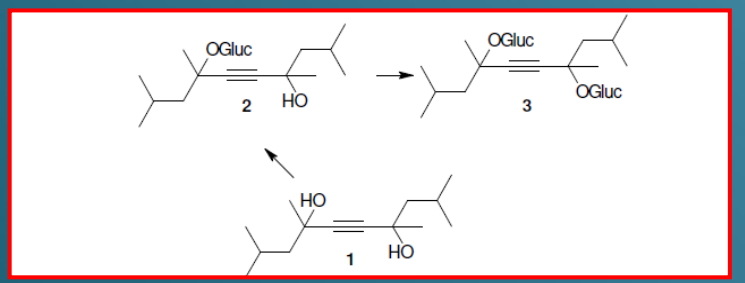
Results - nontargeted analysis

- Detected metabolites
  - Exact mass 206.1670
    - Monohydroxlated TMDD – $2H_2O$, [$C_{14}H_{22}O$], [5]
  - Exact mass 240.1725
    - Dihydroxylated TMDD – H_2O, [$C_{14}H_{24}O_3$] [6]
  - [1], [2], [3] not found in urine samples
  - [4], [8], [9] presumably not specific for TMDD
- Metabolite selection
  - Highest abundance: monohydroxylated TMDD
  - Comparison with reference standard
  - **1-OH-TMDD** confirmed as metabolite



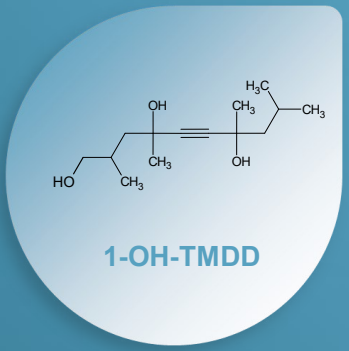






Quantitative LC-MS/MS method

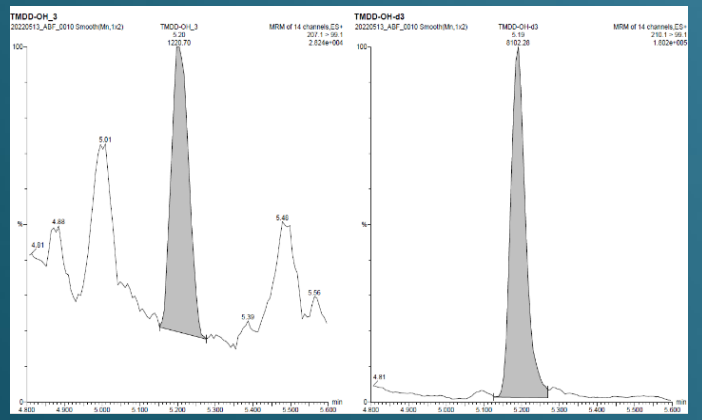
- Sample workup
  - **Enzymatic hydrolysis:** 1 mL urine + 10 µL IS (1-OH-TMDD-d_3) + 0.5 mL phosphate buffer (pH=6.4) + 10 µL β-glucuronidase (E. Coli), incubation 2h, 37 °C
  - **LLE:** + 2 mL MTBE, vortex and centrifugation, evaporation of organic phase, reconstitution in 100 µL ACN/H_2O 90/10 (v/v)
- LC-MS/MS
  - Waters Acquity I-class UPLC / Xevo TQ-S MS/MS, ESI positive, MRM
  - 1-OH-TMDD (Quan) m/z: 207/99
    1-OH-TMDD (Qual) m/z: 207/149
    1-OH-TMDD-d_3 m/z: 210/99



1-OH-TMDD

- Method validation
  - According to FDA Guideline on Bioanalytical Method Validation

LLOQ	0.05 ng/mL		
accuracy and precision	**level**	**accuracy [%]**	**precision (CV, [%])**
Inter-day	0.05 ng/mL	107.5	7.6
(N = 3x5)	0.1 ng/mL	111.9	5.5
	1 ng/mL	104.2	8.7
	40 ng/mL	105.5	4.0



1-OH-TMDD 1-OH-TMDD-d_3

urine sample, 0.14 ng/ml

In-house metabolism study

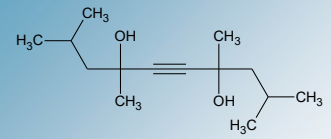
- 4 healthy volunteers dosed with TMDD (oral and dermal)

Subject	Age (years)	Gender	Number of urine fractions (oral)	Number of urine fractions (dermal)
1	71	male	21	20
2	37	male	24	26
3	37	male	25	29
4	32	female	22	31

- Urine collection for 72 h
  - Documentation of time points and urine volume
- Data evaluation – **excretion kinetics**
  - Analysis of all urine fractions with the validated quantitative LC-MS/MS method

oral
75 µg/kg bw

dermal
750 µg/kg bw



2,4,7,9-Tetramethyl-5-decyne-4,7-diol (TMDD)

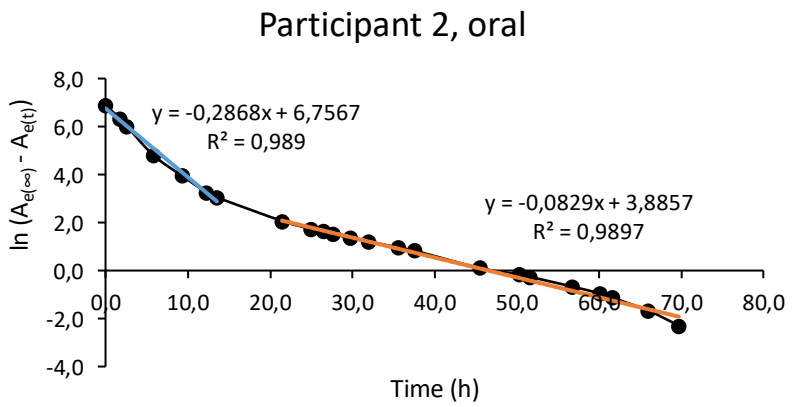
Evaluation of excretion kinetics and calculation of toxicokinetic parameters for each participant

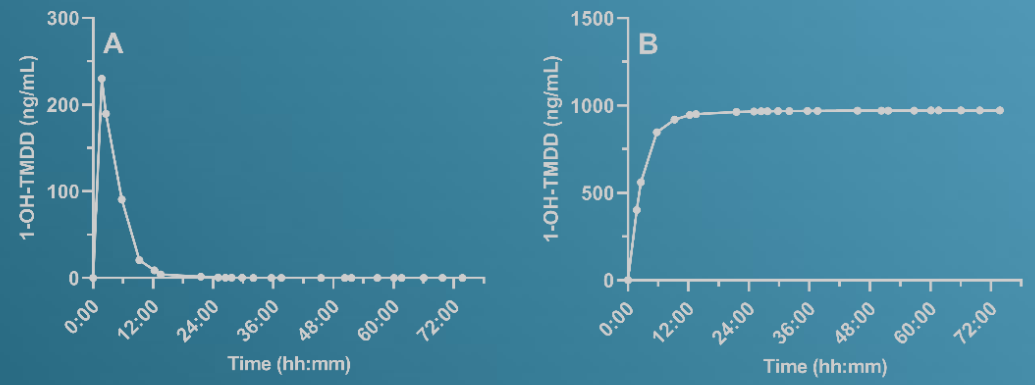
targeted analysis

Waters Xevo TQ-S LC-MS/MS

Excretion kinetics after oral application

- Low baseline levels of 1-OH-TMDD before TMDD application (0 – 0.76 ng/ml)
- Fast metabolism and elimination
  - Mean t_{max} 1.7 h
  - Almost complete elimination after 12 h
- Recovery of 18.5 % of TMDD as 1-OH-TMDD
- Bi-exponential decline with 2 elimination phases



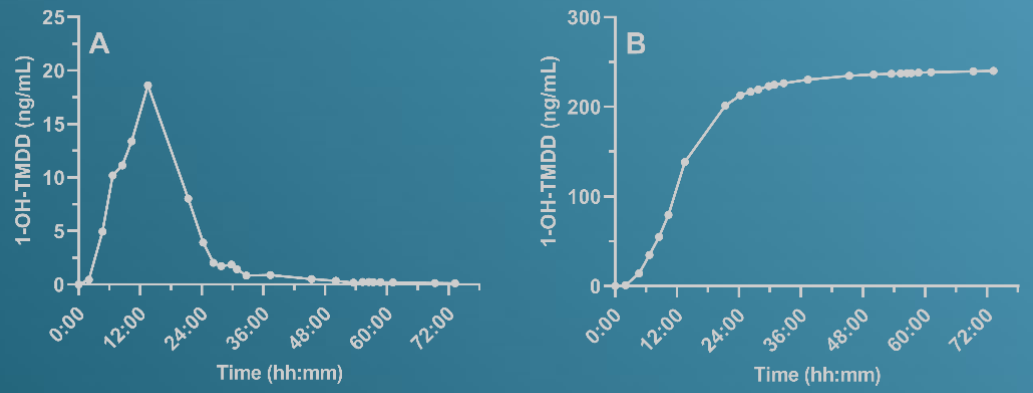


	Mean ± SD	Median	Min–Max
Amount excreted after 72 h [µmol] (A72h)	4.7 ± 0.8	4.6	3.9–5.9
tmax [h]	1.7 ± 0.6	1.9	0.7–2.3
Percent of total 1-OH-TMDD excreted after 3 h [%]	70.3 ± 13.9	72	52.3–85.0
Percent of total 1-OH-TMDD excreted after 6 h [%]	87.5 ± 9.8	90.5	73.0–96.0
Percent of total 1-OH-TMDD excreted after 12 h [%]	96.1 ± 3.5	97.8	90.1–98.6
Percent of total 1-OH-TMDD excreted after 24 h [%]	99.1 ± 0.8	99.4	97.7–99.8
Percent of total 1-OH-TMDD excreted after 48 h [%]	99.8 ± 0.2	99.9	99.6–100.0
Elimination constant λ1 [h−1]	0.73 ± 0.20	0.78	0.43–0.93
Elimination half-life t1/2 λ1 [h]	1.05 ± 0.35	0.91	0.75–1.61
Elimination constant λ2 [h−1]	0.20 ± 0.004	0.2	0.19–0.20
Elimination half-life t1/2 λ2 [h]	3.52 ± 0.07	3.51	3.42–3.63
Urinary excretion factor Fue (24 h) [%]	18.3 ± 2.7	18.3	14.5–22.0
Urinary excretion factor Fue (72 h) [%]	18.5 ± 2.7	18.6	14.6–22.1

Excretion kinetics after dermal application

- Low baseline levels of 1-OH-TMDD before TMDD application (0.13 – 0.82 ng/ml)
- Slower metabolism and elimination compared to oral application
  - Mean t_{max} 12h
  - Almost complete elimination after 48 h
- Recovery of 0.4 % of TMDD as 1-OH-TMDD
- Dermal resorption rate: 2.4 %

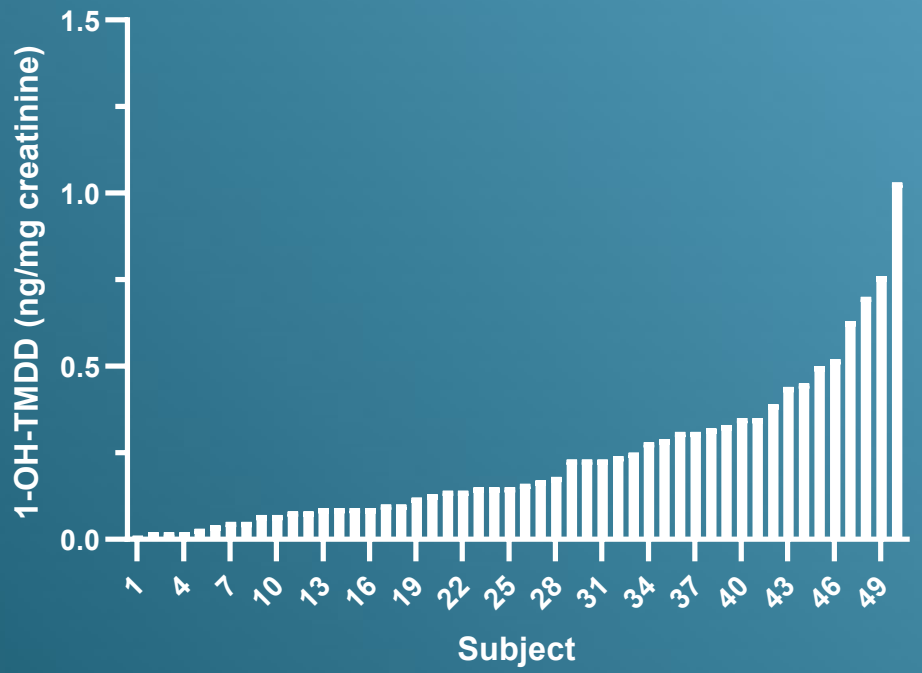
Participant	Dermal TMDD dose [µmol]	Excreted amount after dermal application [µmol]	Excreted amount after oral application [%]	Resorption rate [%]
1	281.6	1.21	18.3%	2.4%
2	275.0	0.99	14.6%	2.5%
3	265.1	0.85	22.1%	1.4%
4	208.7	1.36	18.9%	3.4%
Mean ± SD				2.4% ± 0.7%



	Mean ± SD	Median	Min–Max
Amount excreted after 72 h [µmol] (A72h)	1.1 ± 0.2	1.1	0.8–1.4
Proportion of TMDD dose PMxD [%]	0.4 ± 0.1	0.4	0.3–0.7
tmax [h]	12.0 ± 2.5	13.2	7.8–13.8
Percent of total 1-OH-TMDD excreted after 3 h [%]	2.5 ± 0.4	2.5	1.9–3.1
Percent of total 1-OH-TMDD excreted after 6 h [%]	11.3 ± 1.7	10.9	9.5–14.1
Percent of total 1-OH-TMDD excreted after 12 h [%]	39.8 ± 6.6	41.2	30.7–46.1
Percent of total 1-OH-TMDD excreted after 24 h [%]	82.3 ± 5.0	82.2	76.6–88.2
Percent of total 1-OH-TMDD excreted after 48 h [%]	96.5 ± 2.2	97.1	93.2–98.7

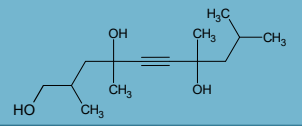
Application

- 50 healthy volunteers
  - 38 male, 12 female
  - 18 – 62 years
  - Spot-urine samples
- Quantifiable 1-OH-TMDD in 90 % of samples
  - 0.19 ng/ml (0.23 ng/mg creatinine) on average
- Estimated systemic intake dose: 1.7 µg/d
- Predicted exposure: 1.3 – 1.9 µg/d (EPA)



Excreted 1-OH-TMDD	ng/mL urine	Mean ± SD	0.19 ± 0.17
		Median (Min-Max)	0.14 (0.03–0.70)
	ng/mg creatinine	Mean ± SD	0.23 ± 0.21
		Median (Min-Max)	0.16 (0.01–1.03)
	nmol/g creatinine	Mean ± SD	0.97 ± 0.88
		Median (Min-Max)	0.65 (0.05–4.25)
	nmol/d	Mean ± SD	1.34 ± 1.14
		Median (Min-Max)	0.97 (0.07–5.10)
Estimated daily dose of TMDD	nmol/d	Mean ± SD	7.30 ± 6.22
		Median (Min-Max)	5.29 (0.41–27.87)
	µg/d	Mean ± SD	1.65 ± 1.41
		Median (Min-Max)	1.20 (0.09–6.31)

Summary and Conclusion

Identification of a suitable metabolite	 1-OH-TMDD
Method development and validation	Fully validated quantitative LC-MS/MS method
Excretion kinetics after single oral and dermal application of TMDD	Effective metabolism as well as rapid oral and substantial dermal resorption of TMDD
Application to urine samples of non-occupationally exposed adults	Quantification of 1-OH-TMDD in 90 % of samples

1-OH-TMDD is a suitable biomarker to assess the exposure to TMDD in the general population

Acknowledgment

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Development of a human biomonitoring method for assessing the exposure to 2,4,7,9-Tetramethyl-5-decyne-4,7-diol (TMDD) in the general population

1-OH-TMDD as a suitable biomarker to assess the exposure to TMDD in the general population

Results of method validation

Parameter		1-OH-TMDD	
LOD		0.017 ng/mL	
LLOQ		0.05 ng/mL	
calibration range		0.05–100 ng/mL	
selectivity		no interferences; accuracy of spiked samples (1 ng/mL): 87.9–116.2%; verification with qualifier	
accuracy and precision	level	accuracy (acc., %)	precision (CV, %)
Intra-day day 1	0.05 ng/mL	107.6	5.4
(N = 5)	0.1 ng/mL	107.1	2.7
	1 ng/mL	110.2	4.4
	40 ng/mL	101.4	2.9
Intra-day day 2	0.05 ng/mL	111.0	8.2
(N = 5)	0.1 ng/mL	116.7	4.6
	1 ng/mL	93.7	5.3
	40 ng/mL	106.4	3.2
Intra-day day 3	0.05 ng/mL	104.0	8.9
(N = 5)	0.1 ng/mL	112.0	5.3
	1 ng/mL	108.8	4.7
	40 ng/mL	108.6	2.4
Inter-day	0.05 ng/mL	107.5	7.6
(N = 3x5)	0.1 ng/mL	111.9	5.5
	1 ng/mL	104.2	8.7
	40 ng/mL	105.5	4.0
recovery	low (0.1 ng/mL)	80.7%	
	medium (1 ng/mL)	80.1%	
	high (50 ng/mL)	83.80%	
matrix effect	low (0.1 ng/mL)	144–156%	
	high (40 ng/mL)	110–126%	
	IS (1 ng/mL)	107–118%	

Parameter		1-OH-TMDD		
carryover		low, <0.1% after high-concentrated samples		
accuracy after dilution		1/20	1/10	1/2
Matrix 1	acc. (%)	93.7	96.1	92.2
	CV (%)	4.6	14.4	6.1
Matrix 2	acc. (%)	94.4	96.2	103.9
	CV (%)	1.8	0.5	0.9
Matrix 3	acc. (%)	96.1	97.2	99.5
	CV (%)	1.1	0.9	1.5
reinjection, CV		QCL (0.2 ng/mL)	QCH (32.5 ng/mL)	
(N = 3 on 3 separate days)		4.60%	4.30%	
short-term stability		QCL (0.2 ng/mL)	QCH (32.5 ng/mL)	
(20 h, 21 °C)		97.60%	105.50%	
freeze-thaw stability		QCL (0.2 ng/mL)	QCH (32.5 ng/mL)	
(6 cycles)		92.50%	91.30%	
post-preparative stability		QCL (0.2 ng/mL)	QCH (32.5 ng/mL)	
(autosampler, 72 h, 10 °C)		95.20%	108.40%	
post-preparative stability		QCL (0.2 ng/mL)	QCH (32.5 ng/mL)	
(freezer, 8 days, -20 °C)		95.70%	103.60%	
working solution		AL 1 (1 µg/mL)	AL 4 (1 ng/mL)	
(4 months, -20 °C)		98.80%	111.20%	